Detection of Silent Myocardial Ischemia in Asymptomatic Diabetic Subjects

The DIAD study

FRANS J.TH. WACKERS, MD[1]
LAWRENCE H. YOUNG, MD[1]
SILVIO E. INZUCCHI, MD[2]
DEBORAH A. CHYUN, PHD[3]
JANICE A. DAVEY, MSN[1]
EUGENE J. BARRETT, MD[4]
RAYMOND TAILLEFER, MD[5]
STEVEN D. WITTLIN, MD[6]
GARY V. HELLER, MD[7]
NEIL FILIPCHUK, MD[8]
SAMUEL ENGEL, MD[9]
ROBERT E. RATNER, MD[10]
AMI E. ISKANDRIAN, MD[11]
FOR THE DETECTION OF ISCHEMIA IN ASYMPTOMATIC DIABETICS (DIAD) INVESTIGATORS*

OBJECTIVE — To assess the prevalence and clinical predictors of silent myocardial ischemia in asymptomatic patients with type 2 diabetes and to test the effectiveness of current American Diabetes Association screening guidelines.

RESEARCH DESIGN AND METHODS — In the Detection of Ischemia in Asymptomatic Diabetics (DIAD) study, 1,123 patients with type 2 diabetes, aged 50–75 years, with no known or suspected coronary artery disease, were randomly assigned to either stress testing and 5-year clinical follow-up or to follow-up only. The prevalence of ischemia in 522 patients randomized to stress testing was assessed by adenosine technetium-99m sestamibi single-photon emission–computed tomography myocardial perfusion imaging.

RESULTS — A total of 113 patients (22%) had silent ischemia, including 83 with regional myocardial perfusion abnormalities and 30 with normal perfusion but other abnormalities (i.e., adenosine-induced ST-segment depression, ventricular dilation, or rest ventricular dysfunction). Moderate or large perfusion defects were present in 33 patients. The strongest predictors for abnormal tests were abnormal Valsalva (odds ratio [OR] 5.6), male sex (2.5), and diabetes duration (5.2). Other traditional cardiac risk factors or inflammatory and prothrombotic markers were not predictive. Ischemic adenosine-induced ST-segment depression with normal perfusion ($n = 21$) was associated with women (OR 3.4). Selecting only patients who met American Diabetes Association guidelines would have failed to identify 41% of patients with silent ischemia.

CONCLUSIONS — Silent myocardial ischemia occurs in greater than one in five asymptomatic patients with type 2 diabetes. Traditional and emerging cardiac risk factors were not associated with abnormal stress tests, although cardiac autonomic dysfunction was a strong predictor of ischemia.

Diabetes Care 27:1954–1961, 2004

Coronary artery disease (CAD) is the leading cause of death in patients with diabetes (1). Myocardial ischemia in patients with diabetes is often asymptomatic and frequently in an advanced stage when it becomes clinically manifest (2,3). Once CAD is symptomatic in diabetes, morbidity and mortality are high and significantly worse than in patients without diabetes.

Patients with type 2 diabetes should be treated with aggressive risk factor modification to prevent the development and progression of CAD (4–7). Many physicians already perform screening by stress testing, as suggested by the American Diabetes Association (ADA) consensus guidelines, when two or more additional CAD risk factors are present (8). However, the yield of such screening in asymptomatic patients has been varied (9–12). The Detection of Ischemia in Asymptomatic Diabetics (DIAD) study was designed to determine the prevalence and severity of inducible myocardial ischemia in asymptomatic patients with type 2 diabetes, using adenosine-stress single-photon emission–computed tomography (SPECT) myocardial perfusion imaging as

From the [1]Section of Cardiovascular Medicine, Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut; the [2]Section of Endocrinology, Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut; the [3]Yale University School of Nursing, New Haven, Connecticut; the [4]Department of Medicine/Endocrinology, University of Virginia, Charlottesville, Virginia; the [5]Department of Nuclear Medicine, University of Montreal, Montreal, Canada; the [6]Department of Medicine/Endocrinology, University of Rochester, Rochester, New York; the [7]Hartford Hospital, Hartford, Connecticut; [8]Cardiology Consultants, Calgary, Canada; [9]Soundview Research Associates, Norwalk, Connecticut; the [10]MedStar Research Institute, Washington, DC; and the [11] Department of Medicine/Cardiovascular Disease, University of Alabama, Birmingham, Alabama.

Address correspondence and reprint requests to Frans J. Th. Wackers, MD, Yale University School of Medicine, Section of Cardiovascular Medicine, 333 Cedar St., Fitkin-3, New Haven, CT 06520. E-mail: frans.wackers@yale.edu.

Received for publication 9 February 2004 and accepted in revised form 5 May 2004.

*A complete list of the Detection of Ischemia in Asymptomatic Diabetes (DIAD) Investigators can be found in the APPENDIX.

F.J.Th.W. has received honoraria and consulting fees from Bristol-Myers Squibb Medical Imaging and Fujisawa; has received grant/research support from Bristol-Myers Squibb Medical Imaging, Fujisawa, AstraZeneca, and Mitsubishi; and has served on advisory boards for AstraZeneca and Mitsubishi. L.H.Y., D.A.C., J.A.D., and S.D.W. have received grant/research support from Bristol-Myers Squibb Medical Imaging and Fujisawa. S.E.I. has received honoraria and grant/research support from Bristol-Myers Squibb Medical Imaging. R.T. holds stock in and has received grant/research support from Bristol-Myers Squibb Medical Imaging. S.E. has received grant/research support from Bristol-Myers Squibb Medical Imaging. R.E.R. holds stock in and has received grant/research support from Bristol-Myers Squibb Medical Imaging. A.E.I. has received honoraria from Fujisawa and grant/research support from CV Therapeutics, Fujisawa, Berlex, and Amersham.

Abbreviations: ADA, American Diabetes Association; CAD, coronary artery disease; DIAD, Detection of Ischemia in Asymptomatic Diabetics; ECG, electrocardiogram; SPECT, single-photon emission–computed tomography.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

well as clinical and laboratory predictors of abnormal test results. The results of myocardial perfusion imaging in the DIAD study are the subject of this report. The effectiveness of the ADA guidelines in identifying patients with silent ischemia was also evaluated.

RESEARCH DESIGN AND METHODS

— The DIAD study recruited patients from diabetes and primary care outpatient practices at 14 centers throughout the U.S. and Canada between July 2000 and August 2002 (see APPENDIX). Institutional review boards at each participating center approved the protocol and procedures. All patients underwent initial clinical and laboratory evaluation and are being followed for adverse cardiac events over a period of 5 years until 2007. At the time of enrollment, patients were assigned a sequential identification number at each site and a corresponding envelope was opened to determine whether the randomization included perfusion imaging. Random permuted blocks were used to assign the randomization sequence, one to one for each site. One-half of the patients were randomized to adenosine stress perfusion imaging and follow-up, whereas the other half were randomized to follow-up only.

Inclusion criteria were *1*) type 2 diabetes, with onset at age ≥30 years and no history of ketoacidosis and *2*) age 50–75 years. Exclusion criteria were *1*) angina pectoris or anginal equivalent symptoms; *2*) stress test or coronary angiography during the 3 years before entry into the study; *3*) history of myocardial infarction, heart failure, or coronary revascularization; *4*) electrocardiographic evidence of Q-wave myocardial infarction, ischemic ST-segment or T-wave changes, or complete left bundle branch block; *5*) any clinical indication for stress testing; *6*) active bronchospasm, precluding the use of adenosine; and *7*) limited life expectancy due to cancer or end-stage renal or liver disease.

Investigators evaluated potentially eligible patients and obtained written consent. A resting 12-lead electrocardiogram (ECG) was recorded, and the Rose questionnaire for angina was completed (13). Medical history and physical examination were performed. Patients were tested for diabetic neuropathy (sensation to touch by monofilament, vibration sensation by tuning fork, and Achilles tendon reflex by reflex hammer) and cardiac autonomic dysfunction (heart rate changes during deep breathing, the Valsalva maneuver, and standing) using Holter monitor recordings and a standardized technique (14) and, during the last year of recruitment, with the automated Anscore system (15). Blood and urine samples were obtained for laboratory testing. Recent fundoscopy reports by an eye-care professional were evaluated for the presence and stage of diabetic retinopathy (i.e., background diabetic retinopathy, preproliferative and proliferative lesions, macular edema, and history of prior laser treatment).

Vasodilator stress SPECT myocardial perfusion imaging

ECG-gated adenosine technetium-99m sestamibi SPECT imaging was performed in concordance with standards of the American Society of Nuclear Cardiology (16). Rest and stress imaging were performed on the same day if BMI was <30 kg/m^2 (n = 294); otherwise, stress imaging was done the next day (n = 228). Vasodilator stress (16) was performed by intravenous infusion of adenosine (140 $\mu g \cdot kg^{-1} \cdot min^{-1}$), with simultaneous very-low-level treadmill exercise (Bruce stage 1) when feasible. This approach was used since many patients with type 2 diabetes may be unable to complete a symptom-limited exercise test because of obesity, peripheral vascular disease, and peripheral neuropathy. Vasodilator stress can be applied to almost all patients and ensures a reproducible intervention. A 12-lead ECG was recorded each minute during the procedure.

Image analysis

Raw ECG-gated SPECT image data were sent to the Yale University radionuclide core laboratory for quantitative analysis (17). Myocardial perfusion defects were quantified as a percentage of the left ventricle in comparison to a normal reference database. The left ventricular ejection fraction was derived from the ECG-gated images (18).

After the completion of patient enrollment, a panel of expert readers (A.E.I., G.V.H., and F.J.Th.W.), blinded to the patient's identity, ECG responses, and symptoms during adenosine infusion, interpreted all perfusion images by consensus and confirmed the quantitative analysis. Images were presented in random order and mixed with an unknown number of non-DIAD images to prevent interpretation bias. Image quality was deemed excellent in 56 (11%), good in 452 (86%), and poor in 13 (2%) images and not interpretable in 1 image (<1%). Images were interpreted as normal or abnormal. A total of 54 (10%) images with obvious or probable diaphragmatic or breast attenuation artifacts were interpreted as normal. Stress and rest myocardial perfusion abnormalities were described as reversible (ischemia), fixed (scar), or mixed (scar and ischemia). Left ventricular perfusion abnormalities were categorized on the basis of quantification as small, moderate, or large (19). SPECT images revealing increased radiotracer lung uptake, left ventricular dilation after stress, and resting left ventricular dysfunction (left ventricular ejection fraction <0.45) were also categorized as abnormal. Flat or downsloping ST-segment depression ≥1 mm at 80 ms after the J-point in two or more leads on the ECG during adenosine infusion was also interpreted as an abnormal test result.

Data analysis

Data were double entered into an electronic database at the clinical sites and transmitted to the data coordinating center at Yale University. All data were imported into SAS for analyses (20). Data are presented as mean ± SD or median with lower and upper ends of the interquartile range. Bivariate associations were tested using t tests, Wilcoxon's rank-sum test, χ^2 test, and Fisher's exact test. When required, continuous variables were either transformed into quadratic terms or categorized into quartiles for multivariate logistic regression analyses. The highest quartile was used as the referent for autonomic testing. When the acquisition of cardiac autonomic data was incomplete, due to contraindication for Valsalva due to proliferative retinopathy or technically inadequate tests, patients were categorized as having not-available values. Logistic regression analysis was used to assess the odds ratio (OR) in each quartile, as well as in those with not-available values. When appropriate, ORs were calculated comparing individuals in the lowest quartile with all other subjects (Valsalva ratio) or, with regard to duration, individuals with the shortest or longest duration of diabetes with those in the two intermediate categories. A backward selection strategy was used and in-

Table 1—*Patient characteristics*

Demographic characteristics	
Men/women	277 (53)/245 (47)
Age (years)	60.7 ± 6.8
Race	
Caucasian	419 (80)
African American	79 (15)
Other	24 (5)
Diabetes-related and cardiac risk factors	
Diabetes duration (years)	8.1 ± 7.1
Age at diabetes diagnosis (years)	52.5 ± 9.0
HbA_{1c} (%)	7.1 ± 1.5
<7%	280 (54)
Treatment	
Insulin	52 (10)
Insulin plus oral agent	67 (12)
Diet only	77 (15)
Oral agent	326 (63)
Retinopathy*	
Yes	70 (14)
Missing data	48 (9)
Peripheral vascular disease†	47 (9)
BMI (kg/m^2)	31.1 ± 6.3
Aspirin use	229 (44)
ADA consensus guidelines risk factors	
Lipid abnormality‡ or lipid-lowering treatment	303 (58)
Lipid abnormality‡	124 (24)
Lipid treatment	242 (47)
Blood pressure >140/90 mmHg or hypertension treatment	336 (65)
Blood pressure >140/90 mmHg	160 (31)
Hypertension treatment	291 (56)
ACE use	191 (37)
β-Blocker use	55 (11)
Calcium channel blocker use	61 (12)
Smoking	
Past	252 (48)
Current	51 (10)
Family history of CAD§	110 (21)
Albuminuria	
<30 μg/mg creatinine	400 (77)
30–299 μg/mg creatinine	95 (18)
≥300 μg/mg creatinine	17 (3)
Missing	10 (2)
Two or more ADA screening risk factors	306 (60)

Data are means ± SD or *n* (%). *Retinopathy (i.e., background, preproliferative, proliferative, macular edema, or history of laser treatment) assessed from ophtalmological exam within the past year. †Presence of symptoms of claudication or history of peripheral vascular surgery. ‡Total cholesterol ≥240 mg/dl, LDL ≥160 mg/dl, or HDL cholesterol <35 mg/dl. §Diagnosis of CAD in parents or siblings before age 50 years.

cluded all variables identified in the bivariate analysis with $P \leq 0.10$. Final logistic modeling was performed to identify factors associated with any test abnormality, adjusting for potentially confounding factors.

The primary analysis examined the statistical association of clinical and laboratory variables in patients with entirely normal stress tests compared with those with any stress test abnormality. Subsequently, three predesignated secondary analyses were performed: entirely normal stress tests versus those with *1*) small perfusion abnormalities, *2*) marked (moderate or large) perfusion abnormalities, or *3*) ischemic ST-segment changes on ECG but normal imaging. The study was powered to detect associations with an OR of at least 1.5, based on a predicted 15% prevalence of abnormal tests.

Patients were also categorized according to the presence of cardiovascular risk factors that might warrant cardiac stress testing, according to current ADA consensus screening guidelines (8). These included two or more of the following in addition to diabetes: *1*) total cholesterol ≥240 mg/dl, LDL cholesterol ≥160 mg/dl, or HDL cholesterol <35 mg/dl; *2*) blood pressure >140/90 mmHg; *3*) smoking; *4*) family history of premature CAD; or *5*) micro- or macroalbuminuria. Patients on lipid-lowering or antihypertensive treatment at study entry were treated as having the risk factor.

RESULTS — A total of 2,764 patients were screened; of these, 1,700 were eligible for the study. Of the 1,123 patients who consented to participate, 561 were randomized to additional testing with adenosine perfusion imaging and 562 were assigned to follow-up alone. Of the patients randomized to imaging, 22 ultimately refused to have the test, 16 were excluded because imaging was delayed beyond the mandated 3-month window, and 1 study was not interpretable because of poor image quality. The characteristics of the remaining 522 patients are shown in Table 1. Only 259 patients (50%) were capable of performing low-level exercise in conjunction with adenosine infusion.

Stress radionuclide imaging

Overall, 113 patients (22%) had abnormal studies (Table 2). Regional perfusion abnormalities were present in 83 studies (16%); of these, 73 were reversible, 3 were fixed, and 7 were partially reversible. Markedly abnormal perfusion images with moderate or large stress defects occurred in 33 patients (6%). Despite normal myocardial perfusion, 30 additional patients (6%) had other significant test abnormalities, including 21 patients with transient ST-segment depression during adenosine infusion, 5 patients with left ventricular dysfunction, and 4 patients with transient ischemic left ventricular dilation. One or more of these latter abnormalities were also present in 28 (34%) patients with perfusion abnormalities (Table 2).

Table 2—*Adenosine-sestamibi SPECT results* (n = 522)

	n	Percentage
Normal stress test	409	78.4
Abnormal stress test	113	21.6
Abnormal myocardial perfusion	83	15.9
Reversibility		
Ischemia	73	88
Ischemia and scar	7	8
Scar	3	4
Defect size (percent of left ventricle)		
Small (<5%)	50	60
Moderate (≥5 and <10%)	29	35
Large (≥10%)	4	5
Anatomic location		
Anterior	39	47
Inferior	31	37
Anterior and inferior	3	4
Lateral	10	12
Other associated abnormality*		
Left ventricular dysfunction	8	28
TID	8	28
TID and left ventricular dysfunction	3	11
Adenosine-induced ST-segment depression	9	33
Normal myocardial perfusion	439	84.1
Other associated abnormality†		
Left ventricular dysfunction	4	13
TID	4	13
TID and left ventricular dysfunction	1	4
Adenosine-induced ST-segment depression	21	70

TID, transient ischemic dilation. *n = 28; †n = 30.

Variables associated with abnormal test results

Any abnormal stress test result was not significantly associated with demographic characteristics, traditional cardiac risk factors, or laboratory variables (P = NS). However, lower Valsalva heart rate ratio, as measured by either automated or nonautomated techniques, was a strong predictor. Patients in the lowest quartile of heart rate responses to Valsalva were more likely to have an abnormal stress test (OR 2.6 [95% CI 1.6–4.1], $P = 0.0001$). Patients whose Valsalva data were not available were not at increased risk. Adjustment for age, sex, smoking status, and hypertension did not alter the increased risk associated with lower Valsalva heart rate ratio (2.4 [1.5–3.9], $P = 0.0005$).

There were no significant predictors found for small perfusion abnormalities (Table 3). Moderate or large perfusion abnormalities were again strongly associated with a diminished Valsalva response ($P = 0.001$), as well as with male sex ($P = 0.05$) and symptoms of autonomic neuropathy ($P = 0.04$) (Table 3). A trend toward significance was also noted for the association between moderate-to-large defects and BMI ($P = 0.09$), waist circumference ($P = 0.07$), and younger age at the time of diabetes diagnosis ($P = 0.07$). A multivariate analysis included the following variables: abnormal Valsalva maneuver, BMI, smoking, antihypertensive medication, duration of diabetes, sex, and age. In multivariate logistic regression, the lowest quartile of heart rate ratios during the Valsalva maneuver (OR 5.6 [95% CI 2.6–12.4], $P = 0.0001$) and male sex (2.5 [1.1–5.7], $P = 0.03$) remained independent predictors of moderate or large perfusion abnormalities.

The relationship between diabetes duration and moderate-to-large perfusion defects was complex. Patients with a short duration (lowest quartile, <2.8 years) of diabetes had a higher prevalence (11%) of moderate-to-large perfusion abnormalities than those with an intermediate duration (middle quartiles, 2.8–11.7 years) of diabetes (2–4%, $P = 0.002$). The risk associated with short duration was similar to that of patients with the longest duration (highest quartile, >11.7 years) of diabetes (14%). This interesting relationship persisted in the multivariate analysis, where diabetes duration (<2.8 years, OR 5.2 [95% CI 1.8–14.9], $P = 0.002$; >11.7 years, 5.6 [2.0–15.3], $P = 0.001$) was an independent predictor of moderate-to-large defects.

ST-segment depression during adenosine infusion was present in 21 patients with normal perfusion imaging. As shown in Table 3, female sex ($P = 0.01$) and having a higher HDL level ($P = 0.04$) were bivariate predictors of ST depression. By multivariate analysis, female sex (OR 3.4 [95% CI 1.2–9.5], $P = 0.02$) was independently associated with an abnormal ECG response.

Categorization of the DIAD population according to the presence or absence of criteria for screening as defined in the ADA consensus screening guidelines (8) revealed that 306 patients (60%) had two or more risk factors and were candidates for screening by stress testing, whereas 204 patients had less than two risk factors. Twelve patients could not be categorized because not all laboratory data were available. Of 306 patients with two or more risk factors, 66 (22%) had abnormal test results, whereas of 204 patients with less than two risk factors, 45 (22%) had abnormal test results (P = NS). The latter constitutes 41% of all 83 abnormal test results. Markedly abnormal myocardial perfusion results were equally distributed among patients with two or more and less than two risk factors.

CONCLUSIONS — The patients enrolled in the DIAD study were truly asymptomatic patients with type 2 diabetes without any clinical reason to suspect CAD. Angina was ruled out by the administration of the Rose questionnaire at the time of recruitment into the study. The patients were recruited from diabetes and primary care clinics, and some patients were self-referred. The study included a representative proportion (15%) of African Americans, who have not been adequately included in prior screening studies (9–11). The DIAD patients were on contemporary medical treatment and were under reasonable metabolic control (mean HbA_{1c} 7.1 ± 1.5%, LDL 113 ± 32 mg/dl). Yet, 113 patients (22%) had evidence of silent myocardial ischemia, in-

Table 3 —*Bivariate comparisons between patients with normal stress tests and those with stress abnormalities*

	Stress test normal	Small stress perfusion abnormality	Moderate-to-large stress perfusion abnormality	ST-segment depression
n	409	50	33	21
Demographics				
Age	60 (55–65)	61 (56–66)	59 (53–67)	64 (60–68)
Sex				
Men	211 (52)	31 (62)	23 (70)*	5 (24)†
Women	198 (48)	19 (38)	10 (30)	16 (76)
Diabetes related				
Duration (years)	6 (3–11)	5 (3–10)	9 (3–15)	11 (4–16)
Age at diagnosis (years)	52 (48–58)	55 (49–62)	51 (48–54)	50 (45–62)
Insulin use	89 (22)	14 (28)	9 (27)	3 (14)
HbA_{1c} (%)	6.8 (6.1–7.8)	6.7 (6.1–7.7)	6.7 (6.2–7.7)	6.9 (6–7.8)
Retinopathy‡	56 (15)	5 (10)	3 (9)	3 (14)
Albuminuria				
Microalbuminuria (30–299 μg/mg) creatinine	72 (18)	8 (16)	7 (21)	7 (33)
Macroalbuminuria(≥300 μg/mg) creatinine	12 (3)	1 (2)	2 (6)	0 (0)
Peripheral neuropathy§	153 (37)	18 (36)	17 (52)	10 (48)
Autonomic neuropathy‖	49 (12)	4 (18)	8 (24)*	1 (5)
Cardiac related				
BMI (kg/m^2)	29.6 (26.0–34.3)	32.5 (27.2–36.5)	31.5 (27.6–35.5)	30 (26–32)
Waist circumference (in)	41 (37–45)	42.8 (38.0–47.3)	42 (39–45.5)	38 (34.4–42.1)
Never smoked	167 (41)	20 (40)	16 (49)	13 (62)
Past/current smoker	242 (59)	30 (60)	17 (52)	8 (38)
Family history of CAD	88 (22)	8 (16)	8 (24)	3 (14)
Blood pressure >140/90 mmHg or hypertension treatment	261 (64)	50 (66)	23 (70)	12 (57)
Blood pressure >140/90 mmHg	118 (29)	19 (38)	11 (33)	8 (38)
Peripheral vascular disease¶	38 (9)	1 (2)	4 (12)	4 (19)
Autonomic function testing				
E/I heart rate ratio deep breathing				
Anscore system#	1.12 (1.07–1.20)	1.10 (1.08–1.18)	1.09 (1.08–1.14)	1.15 (1.11–1.18)
Holter monitor**	1.11 (1.08–1.17)	1.10 (1.07–1.21)	1.08 (1.04–1.15)	1.12 (1.09–1.17)
Valsalva ratio				
Anscore system††	1.53 (1.40- 1.69)	1.41 (1.34–1.57)	1.36 (1.34–1.43)†	1.70 (1.46–1.95)
Holter monitor‡‡	1.31 (1.20–1.47)	1.25 (1.34–1.51)	1.17 (1.13–1.36)†	1.42 (1.19–1.60)
Standing heart rate ratio				
Anscore system§§	1.22 (1.14–1.32)	1.22 (1.12–1.32)	1.19 (1.11–1.30)	1.26 (1.19–1.39)
Holter monitor‖‖	1.20 (1.11–1.32)	1.25 (1.12–1.36)	1.19 (1.08–1.27)	1.25 (1.12–1.34)
Laboratory analyses				
Lipid abnormality or lipid-lowering treatment¶¶	238 (58)	25 (50)	20 (61)	14 (67)
Lipid abnormality§	100 (25)	7 (14)	11 (34)	4 (19)
Total–to–HDL cholesterol ratio	4.0 (3.3–5.0)	3.7 (3.2–4.8)	4.0 (3.5–5.5)	3.8 (2.7–4.6)
High-sensitivity CRP (mg/l)	2.7 (1.3–5.7)	2.6 (1.4–5.9)	4.7 (1.4–7.1)	3.3 (1.2–7.1)
Plasminogen activator inhibitor 1 (ng/ml)	27 (16–43)	29 (19–43)	30 (21–43)	33 (22–50)
Homocysteine (mcmol/l)	7.0 (5.7–8.8)	6.9 (5.9–8.2)	6.9 (5.7–8.5)	7.1 (5.6–10.2)

Data are median (interquartile range) and *n* (%). *$P = 0.05$. †$P = 0.01$. ‡Background diabetic retinopathy, preproliferative and proliferative lesions, macular edema, and history of laser treatment. §Two or more signs or symptoms of peripheral neuropathy: numbness, pain, or tingling in extremities; absent deep tendon reflexes; diminished vibration sensation by tuning fork; or diminished touch sensation with monofilament testing. ‖Two or more autonomic neuropathy symptoms: postprandial bloating, orthostatic dizziness, or erectile dysfunction. ¶Symptoms of claudication or history of peripheral vascular surgery. #$n = 150$. **$n = 444$. ††$n = 123$. ‡‡$n = 268$. §§$n = 182$. ‖‖$n = 182$. ¶¶Total cholesterol ≥240 mg/dl, LDL ≥160 mg/dl, or HDL <35 mg/dl (ADA screening cut points). E/I, expiration/inspiration.

cluding 33 with moderate-to-large myocardial perfusion abnormalities and 30 with adenosine-induced ST-segment changes.

The prevalence of perfusion abnormalities in our study is somewhat higher than the 6–9% observed in the two large-scale screening studies performed in Italy and a smaller study in France (9–11). However, the lower prevalence reported in these earlier studies likely reflects differences in patient selection. Subjects

were younger and able to complete a full exercise stress test, and those with retinopathy or nephropathy were excluded. Also, in earlier studies perfusion was assessed visually with somewhat less-sensitive techniques, such as planar rather than SPECT imaging. Moreover, imaging was performed only if the initial exercise ECG stress test was abnormal or equivocal. Thus, patients who would have had perfusion abnormalities were likely missed. On the other hand, it should be noted that the prevalence of perfusion abnormalities in DIAD is considerably lower than that in several retrospective database reports, reflecting the effect of referral bias in those studies (21,22). Although we use the term "silent ischemia" to indicate the observed abnormalities, in some instances this may be imprecise, as in the case of isolated left ventricular dysfunction, which may instead represent diabetic or hypertensive cardiomyopathy.

Our second goal was to identify predictors of test abnormalities. Demographics, traditional cardiac risk factors, diabetes complications, and biomarkers of inflammation and thrombosis were analyzed. The strongest overall predictor was the heart rate response to Valsalva, performed as part of cardiac autonomic function testing. A decreased Valsalva heart rate response was also the strongest factor associated with moderate-to-large perfusion defects, image abnormalities that raise substantial clinical concern. An association between autonomic neuropathy and asymptomatic ischemia has been suggested by prior studies (9,23–28).

Men were more likely to have moderate or large perfusion defects during adenosine perfusion imaging, an association that was not attributable to other confounding factors and remained an independent predictor in the multivariate model. A greater prevalence of CAD in men than in women with diabetes has also been observed in other screening studies (9–12), although CAD is also recognized as a major problem in women with type 2 diabetes (29–30). Retinopathy and microalbuminuria, previously reported as risk factors for CAD and poor outcome (9–11), were not significantly associated in our population with either any test abnormality or marked perfusion defects.

In addition, traditional cardiac risk factors (hypertension, smoking, family history, or dyslipidemia), as well as novel biomarkers (including high-sensitivity CRP, homocysteine, lipid subfractions, and plasminogen activator inhibitor 1), did not emerge as significantly predictive of abnormal tests. This may be due to the generally increased levels of these markers in our study population or might reflect the impact of treatment with statins, ACE inhibitors, and thiazolidinediones, as well as generally aggressive blood pressure and glucose control.

Adenosine-induced ST depression was more common in women, both with and without perfusion abnormalities. This observation is of interest in view of recent evidence that adenosine-induced ST depression is a predictor of significant CAD and poor cardiac outcomes in symptomatic patients referred for perfusion imaging (31,32). ST-segment depression without perfusion abnormalities was present in 4% of all women studied.

The DIAD study is the first investigation to utilize quantitative myocardial perfusion imaging for screening in patients with type 2 diabetes. The extent of perfusion abnormality is known to correlate significantly with adverse cardiac outcomes in patients with diabetes referred for testing because of known or suspected CAD (33). Overall, the majority of abnormalities found in our study were small and involved <5% of the left ventricle, which is consistent with the impression that silent ischemia might be associated with less extensive CAD (34). However, 33 patients in the DIAD study had moderate or large perfusion abnormalities.

Clinical implications

The DIAD patient population may be considered representative of asymptomatic patients with type 2 diabetes seen in everyday diabetes practice. At the time of enrollment, great care was given to ensure that patients had no symptoms or signs suggestive of CAD. The findings of the DIAD study suggest that greater than one in five asymptomatic patients with type 2 diabetes, aged 50–75 years, have silent myocardial ischemia. More importantly, 1 in 16 patients overall and 1 in 12 men have markedly abnormal (moderate-to-large) myocardial perfusion abnormalities. Thus, this study indicates that totally asymptomatic patients with diabetes have at least an intermediate probability of CAD, a prevalence that may justify screening by noninvasive testing such as stress myocardial perfusion imaging (35).

The DIAD results also provide insight into the effectiveness of the 1998 ADA screening guidelines (8). The two-or-more-risk-factor recommendation for screening did not accurately identify a large number of patients with test abnormalities. Markedly abnormal myocardial perfusion results occurred with equal frequency among patients with two or more and less than two risk factors. When patients on antihypertensive or antilipid medications were excluded, similar results were observed. However, the present study suggests that patients with cardiac autonomic dysfunction warrant careful attention. Cardiac autonomic function testing may have an important role in the CAD risk assessment of patients with type 2 diabetes because of the important association shown in the present study and because of previously demonstrated increased risk for adverse events (24,36,37). The present data address only the prevalence, severity, and predictors of silent ischemia at the time of enrollment in the DIAD study. In 2007, all patients will have at least 5 years of follow-up evaluation. This should allow for defining the relationship between abnormal perfusion imaging and cardiac events in asymptomatic patients with diabetes.

APPENDIX

DIAD study investigators

Principal investigators: Frans J.Th. Wackers, MD; Lawrence H. Young, MD; Silvio E. Inzucchi, MD (Yale University School of Medicine, New Haven, CT); Deborah A. Chyun, RN, MSN, PhD (Yale University School of Nursing, New Haven, CT). Study coordinator: Janice A. Davey, APRN, MSN (Yale University School of Medicine, New Haven, CT). Clinical centers: University of Montreal: Raymond Taillefer, MD; Sylvain Prévost, MD; Carole Benjamin, CNMT; Andre Gagnon CNMT. MedStar Research Institute: Robert Ratner, MD; Maureen Passaro, MD; Evelyn Robinson, RN. Hartford Hospital: Gary V. Heller, MD; Neil Gray, MD; Deborah Katten, RN, MPH. Tulane University: Vivian Fonseca, MD; Richard Campeau, MD; Rhonda Fontenot, RN; Sunil Asnani, MD. University of Alabama: Ami E. Iskandrian, MD; Fernando Ovalle, MD; Mary Beth Hall, RN; Linda Blackmon, RN. University of Virginia: Eugene Barrett, MD; George Beller, MD; Wendie Price, RN; Denny Watson, PhD. Sound-

view Research Associates: Samuel Engel, MD; Mindy Sotsky, MD; Stella Carolan, RN. University of Rochester: Steven Wittlin, MD; Ronald Schwartz, MD; Mary Kelly, RN. University of North Carolina: Elisabeth Fasy, MD; Jean Dostou, MD; Leigh Gosnell, RD; Joe Largay, PA-C; John Buse, MD; Steven Falen, MD. Midwest Cardiology: Dennis Calnon, MD; Connie Zimmerman, ARRT. Maine Cardiology Associates: Mylan Cohen, MD; Jennifer Powers, RN. Cardiology Consultants: Neil Filipchuk, MD, and Melina Krulc, RN. Kansas City Cardiology: Timothy Blackburn, MD; Eric Hockstad, MD; Terry Plesser, RN. Yale University Radionuclide Core Laboratory and Data Coordinating Center: Donna Natale, CNMT; Laurie Finta, RN. Statistical Consultation: George Knafl, PhD; Kristopher Fennie, PhD (Yale School of Nursing). Advisory Board and Data Safety and Monitoring Board: Barry L. Zaret, MD; Robert S. Sherwin, MD; Ralph I. Horwitz, MD.

Acknowledgments— This work was supported by grants from Bristol-Myers Squibb Medical Imaging (N. Billerica, MA) and Fujisawa HealthCare (Deerfield, MI), who also provided technetium-99m sestamibi (Cardiolite) and adenosine (Adenoscan) for study patients. Boston Medical Technologies (Wakefield, MA) provided Anscore devices and logistical support. This work was performed with the support of the Yale General Clinical Research Center (NIH M01-RR-00125).

DIAD is an investigator-initiated study. The sponsors had no role in the design or conduct of the study; in the collection, analysis, or interpretation of data; or in the preparation of the manuscript.

References

1. Young LH, Chyun DA: Heart disease in patients with diabetes. In *Ellenberg and Rifkin's Diabetes Mellitus: Theory and Practice*. 6th ed. Porte DJ, Baron A, Sherwin RS, Eds. New York, McGraw-Hill, 2002, p. 823–844
2. BARI Investigators: Influence of diabetes on 5-year mortality and morbidity in a randomized trial comparing CABG and PTCA in patients with multivessel disease: the Bypass Angioplasty Revascularization Investigation (BARI). *Circulation* 96: 1761–1769, 1997
3. BARI Investigators: Seven-year outcome in the Bypass Angioplasty Revascularization Investigation (BARI) by treatment and diabetic status. *J Am Coll Cardiol* 35: 1122–1129, 2000
4. American Diabetes Association: Management of dyslipidemia in adults with diabetes (Position Statement). *Diabetes Care* 26 (Suppl. 1):S83–S86, 2003
5. American Diabetes Association: Aspirin therapy in diabetes (Position Statement). *Diabetes Care* 26 (Suppl. 1):S87–S88, 2003
6. American Diabetes Association: Treatment of hypertension in adults with diabetes (Position Statement). *Diabetes Care* 26 (Suppl. 1):S80–S82, 2003
7. American Heart Association: Prevention Conference VI: diabetes and cardiovascular disease. *Circulation* 105:2231–2239, 2002
8. American Diabetes Association: Consensus development conference on the diagnosis of coronary heart disease in people with diabetes. *Diabetes Care* 21:1551–1559, 1998
9. Milan Study on Atherosclerosis and Diabetes Group: Prevalence of unrecognized silent myocardial ischemia and its association with atherosclerotic factors in non-insulin-dependent diabetes mellitus. *Am J Cardiol* 79:134–139, 1997
10. Janand-Delenne B, Savin B, Habib G, Bory M, Vague P, Lassmann-Vague V: Silent myocardial ischemia in patients with diabetes. *Diabetes Care* 22:1396–1400, 1999
11. Gazzaruso C, Garzaniti A, Giordanetti S, Falcone C, De Amici E, Geroldi D, Fratino P: Assessment of asymptomatic coronary artery disease in apparently uncomplicated type 2 diabetic patients. *Diabetes Care* 25:1418–1424, 2002
12. De Lorenzo A, Lima RS, Siqueira-Filho AG, Pantoja MR: Prevalence and prognostic value of perfusion defects detected by stress technetium-99m sestamibi myocardial perfusion single-photon emission computed tomography in asymptomatic patients with diabetes mellitus and no known coronary artery disease. *Am J Cardiol* 90:827–832, 2002
13. Rose G, McCartney P, Reid DD: Self-administration of a questionnaire on chest pain and intermittent claudication. *Br J Prev Med* 31:42–48, 1977
14. Ewing DJ, Borsey DQ, Bellavere F, Clarke BF: Cardiac autonomic neuropathy in diabetes: comparison of measures of R-R interval variation. *Diabetologia* 21:18–24, 1981
15. Risk M, Bril V, Broadbridge C, Cohen A: Heart rate variability measurement in diabetic neuropathy: review of methods. *Diabetes Technol Ther* 3:63–76, 2001
16. DePuey EG, Garcia EV: Updated imaging guidelines for nuclear cardiology procedures: part 1. *J Nucl Cardiol* 8:G1–G58, 2001
17. Liu YH, Sinusas AJ, DeMan P, Zaret BL, Wackers FJ: Quantification of SPECT myocardial perfusion images: methodology and validation of the Yale-CG method. *J Nucl Cardiol* 2:190–204, 1999
18. Lam PT, Wackers FJT, Liu YH: Validation of a new method for quantification of left ventricular function from ECG-gated SPECT (Abstract). *J Nucl Med* 42:93P, 2001
19. Iskandrian AE: Risk assessment of stable patients (Panel II): proceedings of the 4th Invitational Wintergreen Conference Wintergreen panel summaries. *J Nucl Cardiol* 6:93–155, 1999
20. SAS Institute: *SAS Version 6.12*. Cary, NC, SAS Institute, 1998
21. Giri S, Shaw LJ, Murthy DR, Travin MI, Miller DD, Hachamovitch R, Borges-Neto S, Berman DS, Waters DD, Heller GV: Impact of diabetes on the risk stratification using stress single-photon emission computed tomography myocardial perfusion imaging in patients with symptoms suggestive of coronary artery disease. *Circulation* 105:32–40, 2002
22. Kang X, Berman DS, Lewin H, Miranda R, Erel J, Friedman JD, Amanullah AM: Comparative ability of myocardial perfusion single-photon emission computed tomography to detect coronary artery disease in patients with and without diabetes mellitus. *Am Heart J* 137:949–957, 1999
23. Langer A, Freeman MR, Josse RG, Steiner G, Armstrong PW: Detection of silent myocardial ischemia in diabetes mellitus. *Am J Cardiol* 67:1073–1078, 1991
24. Vinik AI, Mitchell BD, Maser RE, Freeman R: Diabetic autonomic neuropathy (Technical Review). *Diabetes Care* 26:1553–1579, 2003
25. Valensi P, Sachs RN, Harfouche B, Lormeau B, Paries J, Cosson E, Paycha F, Leutenegger M, Attali JR: Predictive value of cardiac autonomic neuropathy in diabetic patients with or without silent myocardial ischemia. *Diabetes Care* 24:339–343, 2001
26. Valensi P, Paries J, Attali JR, the French Group for Research and Study of Diabetic Neuropathy: Cardiac autonomic neuropathy in diabetic patients: influence of diabetes duration, obesity, and the microangiopathic complications: the French Multicenter study. *Metabolism* 52:815–820, 2003
27. Carnethon MR, Golden SH, Folsom AR, Haskell W, Liao D: Prospective investigation of autonomic nervous system function and the development of type 2 diabetes: the Atherosclerosis Risk in Communities study, 1987–1998. *Circulation* 107:2190–2195, 2003
28. Lee K, Jang H, Kim Y, Lee EJ, Choe YS, Choi Y, Lee MG, Lee SH, Kim B: Prognostic value of cardiac autonomic neuropathy independent and incremental to perfusion defects in patients with diabetes and

suspected coronary artery disease. *Am J Cardiol* 92:1458–1461, 2003
29. Kannel WB, Wilson PWF: Risk factors that attenuate the female coronary disease advantage. *Arch Intern Med* 155:57–61, 1995
30. Howard BV, Cowan LD, Go O, Welty TK, Robbins DC, Lee ET: Adverse effects of diabetes on multiple cardiovascular risk factors in women: the Strong Heart Study. *Diabetes Care* 21:1258–1265, 1998
31. Klodas E, Miller TD, Christian TF, Hodge DO, Gibbons RJ: Prognostic significance of ischemic changes during vasodilator stress testing in patients with normal SPECT images. *J Nucl Cardiol* 10:4–8, 2003
32. Abbott BG, Afshar M, Berger AK, Wackers FJT: Prognostic significance of ischemic changes during adenosine infusion in patients with normal myocardial perfusion imaging. *J Nucl Cardiol* 10:9–16, 2003
33. Kang X, Berman DS, Lewin HC, Cohen I, Friedman JD, Germano G, Hachamovitch R, Shaw LJ: Incremental prognostic value of myocardial perfusion single photon emission computed tomography in patients with diabetes mellitus. *Am Heart J* 138:1025–1032, 1999
34. Nesto RW: Screening for asymptomatic coronary artery disease in diabetes (Editorial). *Diabetes Care* 22:1393–1395, 1999
35. Berman DS, Kiat H, Friedman JD, Diamond G: Clinical applications for exercise nuclear cardiology studies in the era of healthcare reform. *Am J Cardiol* 75:3D–13D, 1995
36. Maser RE, Vinik AI, Mitchell BD, Freeman R: The association between cardiovascular autonomic neuropathy and mortality in individuals with diabetes. *Diabetes Care* 26:1895–1901, 2003
37. Gerritsen J, Heine RJ, Dekker JM, Bouter LM, TenVoorde BJ, Heethaar RM, Kostense PJ, Stehouwer CDA: Impaired autonomic function is associated with increased mortality, especially in subjects with diabetes, hypertension, or a history of cardiovascular disease. *Diabetes Care* 24:1793–1798, 2001