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ORIGINAL RESEARCH

Association of Cardiac Autonomic Neuropathy With Subclinical Myocardial Dysfunction in Type 2 Diabetes

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OBJECTIVES The purpose of this study was to investigate the independent association between global cardiac autonomic neuropathy (CAN) and left ventricular (LV) dysfunction in addition to regional associations of LV dysinnervation and function, in patients with type 2 diabetes mellitus (T2DM).

BACKGROUND CAN represents a potential mechanism in the etiology of nonischemic diabetic cardiomyopathy.

METHODS Clinical measures of CAN based on total spectral power of heart rate variability and cardiac reflex testing and echocardiographic assessment of LV function were performed in 118 patients with type 2 diabetes mellitus. Systolic and diastolic function were defined at rest and peak exercise using peak systolic and peak early diastolic (Em) tissue velocities, calculated in 6 basal- and mid-segments using color tissue Doppler. Iodine 123-metaiodobenzylguanidine imaging was performed in 33 patients to directly quantify global (heart/mediastinum ratio) and regional LV sympathetic integrity.

RESULTS Patients with CAN demonstrated higher resting heart rate, systolic and mean blood pressures, aortic stiffness, hemoglobin A_{1c}, and urine albumin/creatinine ratio, in addition to lower peak heart rate, chronotropic index, and exercise capacity. Diastolic function (Em) was associated with CAN, evidenced by total spectral power ($r = 0.42$, $p < 0.001$) and heart/mediastinum ratio ($r = 0.41$, $p = 0.017$). Diastolic function (Em) at rest and systolic function (peak systolic tissue velocity) at rest and exercise were significantly reduced in patients with CAN. Furthermore, total spectral power was associated with Em independent of age, hypertension, metabolic factors, and other relevant contributors to LV dysfunction ($\beta = 0.20$, $p = 0.035$). Relative regional tracer deficits indicative of local denervation were predominant in the anterior and lateral walls ($p < 0.001$). Associations with regional Em, independent of global iodine 123-metaiodobenzylguanidine uptake, were identified exclusively in mid-anterior ($\beta = 0.45$, $p = 0.01$) and mid-lateral walls ($\beta = 0.34$, $p = 0.03$). However, no association was found between regional denervation and systolic or diastolic dyssynchrony.

CONCLUSIONS The diastolic dysfunction of type 2 diabetes mellitus shows associations with both regional markers of sympathetic integrity and clinical markers of autonomic neuropathy. (J Am Coll Cardiol Img 2010;3:1207–15) © 2010 by the American College of Cardiology Foundation

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Subclinical myocardial disease in patients with type 2 diabetes mellitus (T2DM) may progress to heart failure in the absence of ischemia and hypertension (1). The pathophysiology of this diabetic cardiomyopathy (DCM) has been attributed to a variety of mechanisms including cardiac autonomic neuropathy (CAN), which is related to adverse outcome

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(2–4) and may affect up to one-third of patients with T2DM (5). However, the independent association of a distinct autonomic cardiopathy with DCM has been difficult to establish due to the similar etiologies of these conditions (6). Moreover, the spectrum of left ventricular (LV) functional abnormalities characterizing this syndrome is not well defined. CAN has been related to altered LV filling patterns in the presence of preserved systolic function in patients with type 1 (7–11) as well as T2DM (12). However, these findings may simply reflect the parallel development of diabetic complications with poor metabolic control, and the independence of the association has not been investigated relative to factors with known involvement in DCM. In addition, the detection of concomitant systolic dysfunction of early DCM in CAN may require sensitive techniques such as tissue Doppler imaging and exercise stress responses (13).

Radionuclide imaging characterizes the direct effects of autonomic dysinnervation on the myocardium, which demonstrates marked regional heterogeneity in diabetic patients (14). However, associations of relative segmental denervation with regional functional deficits and mechanical dyssynchrony have not been investigated. In this study, we sought to: 1) identify the importance of CAN in the etiology of myocardial functional abnormalities in patients with T2DM, including rest and exercise diastolic and systolic dysfunction, relative to other contributors; and 2) determine whether the association reflects a distinct autonomic cardiopathy, describing a causative relationship evidenced by regional associations of myocardial sympathetic integrity with function. Myocardial scintigraphy with the post-synaptic tracer iodine 123-metaiodobenzylguanidine (^{123}I-MIBG) characterized the global and regional cardiac effects of CAN, which supplemented clinical assessment using heart rate (HR) variability and cardiac reflex testing. Associations were sought with sensitive markers of DCM at rest and during exercise.

ABBREVIATIONS AND ACRONYMS

Am = late diastolic tissue velocity
BP = blood pressure
CAN = cardiac autonomic neuropathy
DCM = diabetic cardiomyopathy
Em = early diastolic tissue velocity
HbA_{1c} = hemoglobin A_{1c}
H/M ratio = ratio of ^{123}I-MIBG uptake in the heart and mediastinum
HR = heart rate
^{123}I-MIBG = iodine 123-metaiodobenzylguanidine
Sm = systolic tissue velocity
SPECT = single-photon emission computed tomography
T2DM = type 2 diabetes mellitus
TP = total spectral power of heart rate variability

METHODS

Study population. Patients with T2DM (n = 118) were enrolled for the study. Inclusion criteria were as follows: apparently healthy and asymptomatic, age 40 years and older, and no history of cardiovascular disease, cancer, psychiatric illness, or other severe illness. Patients with an abnormal electrocardiogram were excluded. All patients provided written informed consent, and the study was approved by hospital and university human research ethics committees. Biochemical analyses of fasting blood and urine samples were performed according to standard hospital pathology laboratory protocols. Blood pressure (BP) was recorded in the supine position using a calibrated automatic cuff. Pulse wave velocity (SphygmoCor, AtCor Medical, Sydney, Australia) between carotid and femoral sites was applied to measure aortic stiffness.

Resting LV function. Resting echocardiography was performed using a standard commercial ultrasound machine with a 2.5-MHz phased-array probe (Vivid 7, GE Vingmed, Horten, Norway). Conventional and color tissue Doppler apical views were acquired by standard methods (13). Mitral inflow velocities were recorded using pulsed-wave Doppler echocardiography. LV internal dimensions and wall thicknesses, measured from 2-dimensional targeted M-mode echocardiography, were used to calculate LV mass using Devereux's formula (15). LV end-diastolic and -systolic volumes, measured by a modified Simpson biplane method, were indexed to body surface area and used to derive stroke volume index, cardiac index (stroke volume index × HR), and ejection fraction. Color tissue Doppler images were analyzed offline using commercial software (Echopac PC, GE Vingmed). Regional myocardial function, based on peak systolic (Sm), peak early diastolic (Em), and peak late diastolic (Am) tissue velocity, was classified in 6 basal and 6 mid-LV segments (septal, lateral, anteroseptal, posterior, inferior, and anterior walls) by generating tissue velocity profiles using a 5 × 10-mm^2 sample volume. Regional Em and Sm were expressed as percentages of normal regional velocity (16) for investigation of associations with regional ^{123}I-MIBG uptake. SDs of times to peak Sm and Em from the onset of the QRS complex (measured in the 12 LV segments) were also recorded to define systolic and diastolic mechanical synchrony. Global systolic and

diastolic dysfunction were classified by mean basal Sm <5.3 cm/s and mean basal Em <5.5 cm/s, respectively, corresponding to 1 SD below mean normal values (16). Subsequent grading of diastolic dysfunction distinguished impaired relaxation (grade 1), pseudonormal (grade 2), and restrictive (grade 3) filling patterns based on current guidelines (17).

Exercise testing and echocardiography. Patients underwent maximal treadmill exercise testing according to the Bruce protocol with simultaneous measurement of $V_{O_{2peak}}$ by expired gas analysis. The HR response to exercise (chronotropic index) was calculated by HR reserve (peak HR − resting HR) as a percentage of age-predicted HR reserve (18).

Tissue velocities, ejection fraction and stroke volume and cardiac indexes were measured post-stress using the techniques employed at rest. Post-exercise images were compared with the resting echocardiogram to identify inducible wall motion abnormalities. Patients with evidence of ischemia were excluded. Systolic functional reserve was assessed by augmentation in Sm, ejection fraction and stroke volume and cardiac indexes. Diastolic functional reserve was defined by augmentation in Em. Tissue velocity reserve values were indexed to resting levels, as described by Ha et al. (19).

Cardiovascular autonomic function testing. Clinical assessment of CAN comprised a battery of 7 indexes, including 3 HR variability parameters and 4 cardiac reflex tests (6,20). Examination of HR variability was performed according to a standard protocol (21) after a light meal and after withholding antihypertensive medications for 24 h. Spectral power was defined in very low- (<0.04 Hz), low- (0.04 to 0.15 Hz), and high- (0.15 to 0.4 Hz) frequency bands. Total spectral power of HR variability (TP) was also calculated as a global marker of cardiac autonomic function to be used in regression analyses. Cardiovascular reflex tests were performed according to standard protocols (22) and included R-R interval variation during deep breathing (expiration/inspiration ratio), Valsalva maneuver, and standing and the systolic BP response to standing. Abnormal results within the 7-test battery were defined by age- and sex-based normal values for HR variability (21) and cardiac reflex tests (22), with the total number of abnormal results defining the CAN score. The presence of CAN was determined by a CAN score of ≥2, encompassing previously described criteria for borderline and definite CAN (6,20).

^{123}I-MIBG imaging. Scintigraphic assessment of CAN using ^{123}I-MIBG was performed in a subgroup (n = 33) composed of patients with CAN (n = 7) and control patients matched by age and sex (n = 26). Patients were pre-medicated with 600 mg potassium perchlorate at 7 AM on the morning of the test to block thyroid uptake of radioiodine and instructed to abstain from drinking tea, coffee, and alcohol. Imaging (Symbia, Siemens, Erlangen, Germany) was performed using a low-energy, high-resolution collimator. Anterior planar and single-photon emission computed tomography (SPECT) (32 projections for 50 s each) myocardial images were acquired 4 h after injection of 150 MBq of ^{123}I-MIBG. Tracer activity (mean count per pixel) was calculated in specific regions of interest. Global uptake was defined by the ratio of ^{123}I-MIBG uptake in the heart and mediastinum (H/M ratio). Regional uptake was measured in 12 LV segments (anterior, lateral, inferior, and septal walls in basal, mid-, and apical segments), and expressed relative to maximal regional tracer activity. To account for typical regional heterogeneity in ^{123}I-MIBG uptake, a percentage of normal relative regional uptake was then calculated based on previously reported data in healthy subjects (23).

Statistical analysis. Data were analyzed using SPSS version 16.0 (SPSS, Inc., Chicago, Illinois) and expressed as mean ± SD or median (interquartile range) where appropriate, after Kolmogorov-Smirnov assessment of normality of distribution. HR variability parameters achieved normality after $\log_{10}$ transformation. Categorical and normally distributed continuous variables were compared using the χ^2 test and independent t test, respectively. Skewed and ordinal variables were compared by the Mann-Whitney U test. Analysis of covariance was used to adjust for relevant group differences at baseline. A 2-way between-groups analysis of variance was applied to compare regional ^{123}I-MIBG uptake and LV function in myocardial segments and walls and their interaction, with Bonferroni adjustment for multiple post hoc comparisons. Linear associations were assessed by Pearson correlation coefficient or Spearman rank correlation coefficient (r), where appropriate. Regional relationships of ^{123}I-MIBG uptake and synchrony were assessed by partial correlation to remove influences of confounding variables. Multiple linear regression was performed using the enter method. Statistical significance was defined by $p < 0.05$.

RESULTS

Inducible wall motion abnormalities were absent in all patients; however, 4 patients were excluded due

to an abnormal electrocardiogram, leaving a study population of 114 patients. CAN was identified in 16 patients (14%). Clinical and echocardiographic data are displayed in Tables 1 and 2. Patients with CAN demonstrated higher resting HR, systolic and mean BPs, and aortic stiffness, in addition to lower peak HR, chronotropic index, and exercise capacity. Higher hemoglobin A_{1c} (HbA_{1c}), indicating poorer glycemic control, and higher urine albumin/creatinine ratio, consistent with microvascular dysfunction, were also observed in patients with CAN. To remove the potentially confounding influence of hypertension, group comparisons of myocardial function were corrected for mean arterial pressure.

Table 1. Clinical Data for the Study Population by CAN Status

	Patients With CAN (n = 16)	Patients Without CAN (n = 98)	p Value
Clinical parameters			
Age, yrs	63 ± 9	59 ± 9	0.12
Male, n (%)	6 (38)	62 (63)	0.055
Body mass index, kg/m^2	30 ± 4	30 ± 5	0.68
Duration of diabetes, yrs	8 (5–17)	6 (5–9)	0.21
Heart rate, beats/min	76 ± 9	66 ± 9	<0.001
Systolic blood pressure, mm Hg	141 ± 19	125 ± 15	<0.001
Diastolic blood pressure, mm Hg	75 ± 12	72 ± 9	0.19
Mean arterial pressure, mm Hg	97 ± 13	89 ± 10	0.007
History of hypertension	14 (88)	66 (67)	0.10
Aortic pulse wave velocity, m/s	11.1 ± 3.0	9.1 ± 1.9	<0.001
Biochemistry			
Hemoglobin A_{1c}, %	8.3 ± 1.2	7.3 ± 1.6	0.021
Glucose, mmol/l	10.4 ± 4.2	8.5 ± 3.2	0.039
Total cholesterol, mmol/l	4.7 ± 1.1	4.5 ± 1.1	0.48
LDL cholesterol, mmol/l	2.8 ± 0.8	2.7 ± 1.0	0.83
HDL cholesterol, mmol/l	1.0 ± 0.3	1.1 ± 0.3	0.43
Triglycerides, mmol/l	1.7 ± 0.8	1.6 ± 0.9	0.57
Creatinine, μmol/l	75.0 ± 17.0	73.0 ± 16.0	0.72
Urea, mmol/l	5.5 ± 2.2	5.2 ± 1.7	0.60
Urine albumin/creatinine ratio, g/mol	2.5 (1.1–9.7)	1.2 (0.5–2.6)	0.047
Diabetic complications			
Peripheral vascular disease	0	0	—
Renal impairment	6 (38)	21 (21)	0.16
Retinopathy	1 (6)	10 (10)	0.62
Peripheral neuropathy	1 (6)	13 (13)	0.43
Treatment			
Metformin	11 (69)	63 (64)	0.73
Insulin	5 (31)	13 (13)	0.067
Sulfonylureas	5 (31)	30 (31)	0.96
ACE inhibitors	7 (44)	26 (27)	0.16
β-blockers	3 (19)	6 (6)	0.082
Calcium channel blockers	2 (13)	8 (8)	0.57
Statins	11 (69)	52 (53)	0.24
Exercise hemodynamics			
Peak heart rate, beats/min	143 ± 18	158 ± 16	0.001
Chronotropic index, %	85 ± 20	96 ± 14	0.004
Peak systolic blood pressure, mm Hg	191 ± 18	200 ± 25	0.15
Peak diastolic blood pressure, mm Hg	87 ± 18	87 ± 11	0.89
Vo_2peak, ml/kg/min	25 ± 7	30 ± 7	0.016

Data are mean ± SD, median (interquartile range), or n (%) when appropriate.
ACE = angiotensin-converting enzyme; CAN = cardiac autonomic neuropathy; HDL = high-density lipoprotein; LDL = low-density lipoprotein; Vo_2peak = peak exercise oxygen uptake.

In the subgroup assessed by ^{123}I-MIBG scintigraphy, global myocardial sympathetic dysinnervation in the 7 patients with CAN was evidenced by a decreased H/M ratio (1.6 ± 0.2 vs. 1.8 ± 0.2 in controls, p = 0.01). In all patients in the subgroup, clinical markers of CAN correlated with global, but not regional, markers of sympathetic integrity, evidenced by TP (r = 0.47, p = 0.007 vs. H/M ratio) and CAN score (r = −0.52, p = 0.002 vs. H/M ratio).

Global CAN and LV diastolic function. Patients with CAN demonstrated significantly decreased resting Em and Em/Am ratio compared with patients without CAN, despite a similar mitral inflow pattern (Table 2). Moreover, patients with CAN demonstrated more adverse diastolic dysfunction grade (Table 3; grades 1 and 2 only). No differences were observed in exercise Em or Em/Am ratio. In all patients, positive associations between cardiac autonomic function and resting diastolic function were evidenced by correlations of TP with Em (Fig. 1), Em/Am ratio (r = 0.35, p < 0.001), and the ratio of early-to-late diastolic filling velocity (E/A ratio) (r = 0.29, p = 0.002). A higher CAN score was also associated with reduced Em (r = −0.38, p < 0.001), Em/Am ratio (r = −0.33, p < 0.001,) and E/A ratio (r = −0.21, p = 0.02). Similarly to clinical markers of CAN, the H/M ratio was positively associated with Em (Fig. 1) and the Em/Am ratio (r = 0.37, p = 0.04).

Independent associations with diastolic function were sought by entering TP into a model of determinants of Em (adjusted R^2 = 0.37, p < 0.001) with significant clinical and biochemical correlates (age, mean BP, HbA_{1c}, aortic pulse wave velocity), in addition to factors with known influence (sex, body mass index, history of hypertension, and metformin and insulin therapy) (24). Independent predictors of Em included TP (β = 0.20, p = 0.035), age (β = −0.46, p < 0.001) and mean BP (β = −0.25, p = 0.013).

Global CAN and LV systolic function. Despite similar resting ejection fraction and stroke volume and cardiac indexes, patients with CAN had reduced Sm (Table 2) and a higher proportion of systolic dysfunction compared with patients without CAN (56% vs. 21%; p = 0.003). Furthermore, peak exercise and reserve indexes of LV contractile func-

tion, including Sm, ejection fraction, and cardiac index, were significantly reduced in patients with CAN. Despite the absence of associations with resting Sm, autonomic function markers correlated with measures of systolic functional reserve in all patients. TP was positively associated with peak Sm ($r = 0.29$, $p = 0.002$), peak cardiac index ($r = 0.22$, $p = 0.02$), Sm reserve index ($r = 0.30$, $p = 0.001$), and cardiac index reserve ($r = 0.26$, $p = 0.008$). Higher CAN score was associated with reductions in peak Sm ($r = -0.25$, $p = 0.008$), peak stroke volume index ($r = -0.23$, $p = 0.02$), peak cardiac index ($r = -0.35$, $p < 0.001$), peak ejection fraction ($r = -0.19$, $p = 0.049$), Sm reserve index ($r = -0.25$, $p = 0.009$), and cardiac index reserve ($r = -0.38$, $p < 0.001$). H/M ratio was associated with peak ejection fraction ($r = 0.38$, $p = 0.03$), but no other systolic functional parameters.

To determine independent associations with systolic functional reserve, TP was entered into a model of determinants of Sm reserve index (adjusted $R^2 = 0.39$, $p < 0.001$), into which HbA_{1c} was forced alongside clinical and biochemical correlates (age, sex, mean BP, resting HR, history of hypertension, aortic pulse wave velocity, LV mass index, chronotropic index, triglycerides, creatinine, albumin, and metformin, angiotensin-converting enzyme inhibitor, and β-blocker therapy). Independent predictors of Sm reserve index included age ($\beta = -0.23$, $p = 0.035$), history of hypertension ($\beta = -0.20$, $p = 0.048$), LV mass index ($\beta = 0.19$, $p = 0.024$), and chronotropic index ($\beta = 0.19$, $p = 0.042$).

Regional ^{123}I-MIBG-SPECT and LV function. Due to a high incidence of artifact secondary to mesenteric uptake of ^{123}I-MIBG, regional data for inferior wall segments was excluded. Moreover, 5 of 33 patients in whom the H/M ratio was measured required exclusion from regional analyses due to poor-quality tomograms. Based on 2-way analysis of variance using segment type (basal or mid) and myocardial wall classification (septal, anterior, or lateral), ^{123}I-MIBG uptake was not significantly different in basal and mid-segments, but varied across myocardial walls ($p < 0.001$). This variation was analyzed separately in basal and mid-segments due to an interaction effect ($p < 0.001$). Post hoc comparisons in basal segments revealed significantly greater tracer activity in the septal wall (114 ± 14% of normal) compared with both the anterior (85 ± 10%, $p < 0.001$) and lateral (83 ± 10%, $p < 0.001$) walls. Similarly in mid-segments, ^{123}I-MIBG uptake was greater in septal (105 ± 10%) compared with both anterior (92 ± 9%, $p < 0.001$) and lateral (88 ± 9%, $p < 0.001$) walls. Regional LV function demonstrated a different heterogeneous pattern, characterized by greater deficits of Em in mid- (68 ± 26% of normal) compared with basal segments (86 ± 22%, $p < 0.001$), but no differences between myocardial walls. Sm was also lower in mid- (75 ± 28%) compared with basal segments (95 ± 23%, $p < 0.001$), but similar in the 3 walls.

Borderline correlations of regional ^{123}I-MIBG uptake with regional Em were observed only in the mid-anterior ($r = 0.34$, $p = 0.08$) and

Table 2. Echocardiography Data for the Study Population by CAN Status

	Patients With CAN (n = 16)	Patients Without CAN (n = 98)	p Value
Resting echocardiography			
EDVI, ml/m^2	33 ± 9	35 ± 9	0.35
ESVI, ml/m^2	12 ± 6	12 ± 4	0.91
Stroke volume index, ml/m^2	21 ± 4	22 ± 6	0.21
Cardiac index, l/min/m^2	1.6 ± 0.3	1.5 ± 0.3	0.39
Ejection fraction, %	64 ± 8	65 ± 6	0.69
LV mass index, g/m^2	72 ± 16	83 ± 21	0.051
Doppler			
E, m/s	0.8 ± 0.1	0.7 ± 0.1	0.010
A, m/s	0.9 ± 0.2	0.7 ± 0.2	<0.001
E/A ratio	0.9 ± 0.2	1.0 ± 0.2	0.27
DT, ms	225 ± 50	217 ± 54	0.47
Tissue Doppler			
Sm, cm/s	5.3 ± 0.5	6.1 ± 1.0	0.001
Em, cm/s	4.7 ± 1.0	6.3 ± 1.3	<0.001
Am, cm/s	7.2 ± 1.0	7.1 ± 1.4	0.94
Em/Am ratio	0.7 ± 0.2	0.9 ± 0.3	0.011
Exercise echocardiography			
Peak EDVI, ml/m^2	33 ± 8	34 ± 9	0.70
Peak ESVI, ml/m^2	10 ± 4	8 ± 3	0.087
Peak stroke volume index, ml/m^2	23 ± 4	26 ± 6	0.20
Peak cardiac index, l/min/m^2	3.3 ± 0.7	4.1 ± 1.0	0.016
Peak ejection fraction, %	72 ± 5	76 ± 6	0.006
Tissue Doppler			
Peak Sm, cm/s	8.6 ± 1.9	10.8 ± 2.0	0.001
Peak Em, cm/s	9.9 ± 2.1	10.2 ± 2.0	0.59
Peak Am, cm/s	10.5 ± 2.0	10.8 ± 2.1	0.62
Peak Em/Am ratio	0.9 ± 0.2	0.9 ± 0.2	0.38
Em reserve index, cm/s	3.9 ± 1.8	3.2 ± 1.6	0.32
LV contractile reserve			
Stroke volume index reserve, ml/m^2	3 ± 4	4 ± 6	0.50
Cardiac index reserve, l/min/m^2	1.8 ± 0.7	2.6 ± 0.9	0.005
Ejection fraction reserve, %	7 ± 5	11 ± 7	0.045
Sm reserve index, cm/s	2.7 ± 1.5	3.9 ± 1.4	0.022

Data are mean ± SD. Doppler, tissue Doppler, and exercise echocardiographic variables were corrected for mean arterial pressure by analysis of covariance.

A = late diastolic filling velocity; Am = late diastolic tissue velocity; DT = deceleration time; E = early diastolic filling velocity; EDVI = end-diastolic volume index; Em = early diastolic tissue velocity; ESVI = end-systolic volume index; LV = left ventricular; Sm = systolic tissue velocity; other abbreviation as in Table 1.

Table 3. Prevalence of Diastolic Dysfunction by CAN Status

	Patients With CAN	Patients Without CAN	Total
Normal diastolic function	1 (6)	66 (67)	67
Grade 1 diastolic dysfunction	6 (38)	16 (16)	22
Grade 2 diastolic dysfunction	9 (56)	16 (16)	25
Total	16 (100)	98 (100)	114

Data indicate n (%) of patients with various classifications of diastolic function according to CAN status. Patients with CAN demonstrated higher diastolic dysfunction grade (Mann-Whitney *U* test; $p < 0.001$).
Abbreviation as in Table 1.

mid-lateral walls ($r = 0.33$, $p = 0.09$). Independent associations of regional tracer activity with Em were sought using a multiple linear regression model including H/M ratio (to control for global ^{123}I-MIBG uptake) and age, which represented the strongest predictor of global diastolic function. Regional ^{123}I-MIBG uptake was independently associated with regional Em exclusively in the mid-anterior ($\beta = 0.45$, $p = 0.01$, adjusted $R^2 = 0.32$) and mid-lateral walls ($\beta = 0.34$, $p = 0.03$, adjusted $R^2 = 0.43$). No associations were found between regional ^{123}I-MIBG uptake and regional Sm.

Synchrony analyses did not reveal associations of regional ^{123}I-MIBG uptake with times to peak Sm or Em (after correcting for HR) in any segment. To determine whether regional denervation triggered global systolic or diastolic dyssynchrony, we compared SDs of times to peak Sm and Em across quartiles of ^{123}I-MIBG uptake in each wall; however, no group differences were identified ($p > 0.10$ for all).

DISCUSSION

This study demonstrated that the global relationships of CAN and LV dysfunction remain independent of known factors in the pathogenesis of myocardial disease and reflect underlying regional relationships of sympathetic integrity and function. These findings support the existence of a distinct autonomic cardiopathy in the setting of DCM, albeit a difficult syndrome to characterize in isolation. Our data indicate that resting diastolic dysfunction is a key feature, based on independent associations of CAN markers with resting Em, but not Sm or LV functional reserve parameters. Nonetheless, patients with fully evolved CAN demonstrate a variety of LV functional deficits, including systolic and diastolic dysfunction at rest and reduced systolic functional reserve. The divergent findings may be attributed to an association of CAN with additional mechanisms of DCM, whereby several factors contribute to heterogeneous LV functional abnormalities in patients with advanced disease.

DCM and CAN. The parallel association of reduced Em with global CAN based on clinical assessment and myocardial uptake of ^{123}I-MIBG, in addition to the independent regional association in the free

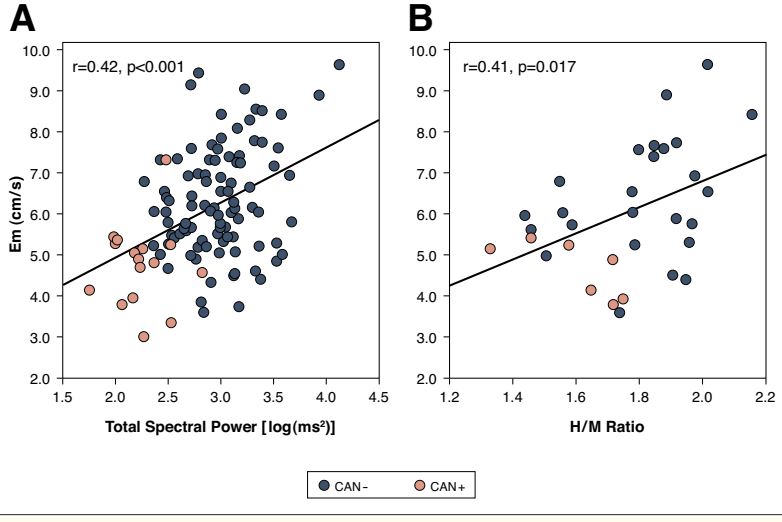

Figure 1. Association of CAN and Diastolic Function

Correlations of mean early diastolic tissue velocity (Em), measured in 6 basal left ventricular segments using color tissue Doppler imaging, with total spectral power of heart rate variability **(A)** and ratio of delayed (4-h) uptake of iodine 123-metaiodobenzylguanidine in the heart and mediastinum (H/M ratio) **(B)**. Patients with cardiac autonomic neuropathy (CAN) are shown in **orange**.

wall (mid-anterior and lateral walls) suggests that the diastolic impairment central to autonomic cardiopathy is linked to cardiac sympathetic denervation. These results concur with previously reported correlations between CAN and altered LV filling patterns, mainly in type 1 diabetes mellitus (7–11) but also in T2DM (12), in addition to associations of global sympathetic denervation with diastolic dysfunction in type 1 diabetes mellitus (25,26). The present study builds on previous findings by defining regional associations accompanying the independent global association in T2DM, for which the pathophysiology of LV dysfunction may be markedly different compared with type 1 diabetes mellitus due to increased contributions from insulin resistance, obesity, and hypertension.

CAN represents one of numerous mechanisms in the multifactorial etiology of DCM, which may include contributions from metabolic abnormalities, interstitial fibrosis, and microvascular disease (1). Indeed, we observed diastolic dysfunction in ~30% of patients without CAN. Potential heterogeneity in manifestations of diastolic dysfunction is highlighted in the present study, evidenced by preserved diastolic functional reserve with CAN. This unexpected result may be attributed to differential relationships of myocardial functional abnormalities with the spectrum of contributing pathologies.

The regional heterogeneity of the association of DCM and CAN likely reflects the variable pattern of sympathetic denervation, showing regional associations exclusively in the denervated walls (anterior and lateral). However, we found no evidence that these regional differences cause mechanical dyssynchrony. We were unable to determine potential associations in the inferior wall in the present study, although its involvement is possible in light of previous reports of abnormal tracer kinetics in this region in diabetic patients (14). The finding of regional associations in mid-, but not basal segments also deserves comment and may be related to the base-to-apex gradient of sympathetic dysinnervation shown in diabetic patients using ^{11}C-labeled hydroxyephedrine positron emission tomography (14). This is characterized by proximal hyperinnervation accompanying distal denervation, which would explain the association of mid-segmental sympathetic denervation with comparatively greater reductions in Em. Although ^{123}I-MIBG uptake was reduced in some basal segments in the current study, these findings must be considered in the context of the limitations of ^{123}I-MIBG SPECT, which, in contrast to positron emission tomography, precludes absolute quantification of regional sympathetic integrity.

Systolic dysfunction with reduced contractile reserve was also observed in patients with CAN; however, the lack of an independent global or regional association with markers of autonomic function indicates that these abnormalities are not direct manifestations of an autonomic cardiopathy. Previous reports in type 1 diabetes mellitus have also identified evidence of impaired contractile reserve with CAN, characterized by reduced exercise ejection fraction (27,28) and end-systolic pressure-volume ratio (27). The current finding of an independent association between Sm reserve and chronotropic index indicates that these results may be attributed to chronotropic incompetence in patients with CAN.

Study limitations. Fully evolved CAN was identified in a relatively small proportion of patients (14%). This was lower than 34% identified in a similarly unselected population using equivalent diagnostic criteria (5) and may reflect a more favorable risk factor profile for microvascular complications in our study population. This may have limited our capacity to detect differences in various myocardial functional parameters. However, preclinical abnormalities in autonomic function occur progressively with evolution of T2DM and before manifestation as overt neuropathy; indeed, myocardial sympathetic dysinnervation has been identified in 40% of diabetic patients not meeting clinical criteria for CAN (14). We assessed cardiac autonomic function with sufficient sensitivity to demonstrate a relationship with LV function across a wide spectrum of disease severity, in addition to the association of reduced function with definite CAN. However, considering the sizable number of covariates, a larger study population may have been beneficial; likewise, the performance of myocardial scintigraphy in only a relatively small subgroup is also recognized. Finally, we acknowledge that the more adverse clinical status of patients with CAN presented difficulties in isolating the direct effects of this complication on LV function, even after statistical adjustment. Worse metabolic control with CAN may represent a particularly important mechanism underlying associations with DCM, despite the lack of a relationship of HbA_{1c} with LV function. This might be clarified using more sensitive constructs of metabolic milieu based on longitudinal markers of disease severity.

Clinical relevance. This study has confirmed the presence of an autonomic cardiopathy in apparently

healthy patients with T2DM and an independent association with resting diastolic dysfunction, a key component of DCM. Moreover, the independent associations of regional sympathetic integrity with regional function suggest a closer link than one mediated by common etiology alone. Whether this is a specific attribute of DCM or might be seen in other subclinical heart disease warrants further study. In the absence of definitive evidence supporting direct treatment, the current management of diabetic CAN relies on aggressive strategies to improve glycemic control. Angiotensin-converting enzyme inhibitor and/or angiotensin receptor blocker therapy (29) have shown promising initial results, although further clinical trials are required to determine their efficacy and potential for translation of benefits to LV function.

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