Cardiac Autonomic Function Is Associated With Myocardial Flow Reserve in Type 1 Diabetes

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The link between cardiac autonomic neuropathy and risk of cardiovascular disease is highlighted as an area in which research is needed. This study was undertaken to evaluate the association between measures of cardiac autonomic function and cardiac vascular function in type 1 diabetes using new and sensitive methods. This was a cross-sectional study in patients with type 1 diabetes, stratified by normoalbuminuria (n = 30) and macroalbuminuria (n = 30), and in healthy control subjects (n = 30). Cardiac autonomic function was evaluated using heart rate variability (HRV) indices, cardiovascular autonomic reflex tests (CARTs), and cardiac ^{123}I-metaiodobenzylguanidine (MIBG) imaging. Cardiac vascular function was assessed as myocardial flow reserve (MFR) measured by cardiac ^{82}Rb-positron emission tomography/computed tomography. The measures of cardiac autonomic function (except low frequency–to–high frequency ratio and the Valsalva test ratio) were positively correlated to MFR in unadjusted analysis. All the HRV indices lost significance after adjustment for age and heart rate. After further adjustment for relevant cardiovascular risk factors, the late heart-to-mediastinum ratio directly measuring the function of adrenergic receptors and sympathetic integrity (from the MIBG scintigraphy) and the 30-to-15 ratio (a CART), remained positively associated with MFR ($P \leq 0.04$). Cardiac autonomic dysfunction, including loss of cardiac sympathetic integrity in type 1 diabetes, is associated with and may contribute to impaired myocardial blood flow regulation.

Cardiac autonomic neuropathy (CAN) is defined as impairment of autonomic control of the cardiovascular system. CAN involves damage to the autonomic nerve fibers that innervate the heart and blood vessels, resulting in abnormalities in heart rate control and vascular dynamics (1).

The cardiac autonomic function can be evaluated with simple bedside tests using heart rate variability (HRV) indices or response in heart rate to standing, slow breathing, or the Valsalva maneuver (cardiovascular autonomic reflex tests [CARTs]). These indirect tests can reveal altered sympathetic and parasympathetic activity. Cardiac radionuclide imaging using the nonmetabolized norepinephrine analog metaiodobenzylguanidine (MIBG) allows direct assessment of cardiac sympathetic integrity and may reveal CAN in the early stages before it can be detected by the HRV indices and the CARTs (2).

CAN is a severe and often overlooked complication in diabetes associated with increased mortality and silent myocardial ischemia (2). In a recent scientific statement from the American Heart Association and the American Diabetes Association, the pathophysiology linking CAN to the risk of cardiovascular disease in type 1 diabetes was highlighted as an area in which research is needed (3). We have demonstrated an association between impaired cardiac autonomic function and reduced MFR in type 2 diabetes (4). In the current study, we examine whether this association extends to type 1 diabetes.

The myocardial flow reserve (MFR) reflects the function of the large epicardial arteries and the microcirculation of the myocardium. MFR is a powerful independent predictor of cardiac death and nonfatal myocardial infarction in diabetes (5), and can be calculated using cardiac

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^{82}Rb-positron emission tomography (PET)/computed tomography (CT) imaging.

Using MIBG imaging, HRV indices, and CARTs, this study was undertaken to evaluate the association between the cardiac autonomic function and cardiac vascular function assessed as the MFR measured by cardiac ^{82}Rb-PET/CT.

RESEARCH DESIGN AND METHODS

Study Population

We included 60 participants with type 1 diabetes stratified by albuminuria: 30 with normoalbuminuria (<30 mg/24 h or 30 mg/g creatinine and without a history of albuminuria) and 30 with the presence of or a history of macroalbuminuria (≥300 mg/24 h or 300 mg/g creatinine in two of three consecutive urine collections). History of macroalbuminuria was defined as albuminuria ≥300 mg/24 h or 300 mg/g creatinine in two of three consecutive measurements documented in the electronic medical files at any time point.

These participants were recruited from the Steno Diabetes Center Copenhagen among subjects participating in a cross-sectional study focusing on detailed phenotyping of patients with type 1 diabetes with or without progressive renal complications. We stratified participants according to normoalbuminuria or macroalbuminuria, as one of the overall aims of the study was to examine the prevalence of impaired MFR in *1*) patients with type 1 diabetes and normoalbuminuria compared with *2*) patients with type 1 diabetes and macroalbuminuria (6). We matched the persons with normoalbuminuria and macroalbuminuria by sex and age. Exclusion criteria included the following: *1*) nondiabetic kidney disease; *2*) renal failure (estimated glomerular filtration rate [eGFR] <15 mL/min/1.73 m^2), kidney transplantation, or dialysis; *3*) change in renin angiotensin aldosterone system blocking treatment 1 month prior to study participation; and *4*) contraindications for cardiac PET/CT.

As control subjects, we used 30 healthy persons without diabetes recruited from a newspaper advertisement. These participants were studied at our center in 2013 using an identical protocol and the same equipment; results on these control subjects have previously been reported (4,7).

As in our study in type 2 diabetes, the sample size calculation was based on MFR (6,7). Data were not available in a comparable cohort of individuals with type 1 diabetes. Participants were grouped according to disease status (diabetes vs. control subjects) and albuminuria analogous to our previous study in type 2 diabetes; however, we allowed participants to have a history of cardiovascular disease in the current study. In our previous study in type 2 diabetes, the sample size sufficient to detect differences in MFR was also adequate to detect associations between MFR and cardiac autonomic function assessed by MIBG (4). We therefore anticipated the sample size to be sufficient to detect these associations in the current study as well.

The study was conducted in compliance with the Declaration of Helsinki, and the protocol was reviewed and approved by the appropriate independent ethics committee. All participants provided written informed consent.

Clinical Measurements

We used high-performance liquid chromatography to measure HbA_{1c}, an enzymatic method to measure plasma creatinine (Hitachi 912; Roche Diagnostics, Mannheim, Germany), and the CKD-EPI equation was used to calculate eGFR (8). An enzyme immunoassay was used to measure the urinary albumin creatinine rate (UACR) in three consecutive morning urine samples. A cuff device (model TM2430; Takeda, Tokyo, Japan) (9) measured 24-h blood pressure. Height and weight were measured and used to calculate BMI (weight in kilograms divided by the square of height in meters). We obtained a detailed medical history, including treatment, previous cardiovascular disease, and smoking status (current smoking was defined as one or more cigarettes/cigars/pipes a day). Information on physical activity during leisure time (hours/week) was obtained from a questionnaire. The medical history was cross-referenced with electronic patient records.

All clinical measurements for control subjects were assessed and defined as described above with the following two exceptions: *1*) urinary albumin excretion rate (UAER) was measured by an enzyme immunoassay in two 24-h urine collections and *2*) 24-h blood pressure was recorded using a tonometric wrist device (BPro; HealthStats, Singapore) (10).

Hybrid Cardiac PET/CT Imaging

All participants underwent a dynamic, gated cardiac PET/CT after administration of 1,100 MBq of ^{82}Rb (CardioGen-82; Bracco Diagnostics, Monroe Township, NJ). We used a hybrid PET/CT scanner in 3D mode (Siemens Biograph mCT 128; Siemens, Munich, Germany). Cardiac PET/CT was performed at rest and during stress. Stress was assessed by adenosine infusion at 140 μg/kg/min for 6 min to induce maximum myocardial hyperemia. Myocardial blood flow was automatically calculated as previously described in detail (7).

Cardiac Autonomic Function by ^{123}I-MIBG Scintigraphy

All 60 participants with diabetes and 14 of the control subjects (the first consecutively included) underwent cardiac ^{123}I-MIBG scintigraphy. One hour before intravenous tracer injection (200 MBq ^{123}I-MIBG), participants were given potassium iodine to block thyroid iodine uptake. Planar anterior-posterior images were taken after 15 min (early) and after 240 min (late). We used a Philips SKYLight Gamma Camera with JETstream software (Philips Medical Systems, Best, the Netherlands) with medium energy collimator, 256 × 256 matrix, and an acquisition time of 600 s. A 15% energy window set symmetrically over the 159 keV photo peak was used to image ^{123}I. One

experienced observer assessed the images using the Extended Brilliance Workspace NM Application Suite version 4.5.3.40140 (Philips Medical Systems). According to guidelines, a region of interest was drawn *1*) following the epicardial contour and *2*) as a rectangle above the mediastinum (11). The mean count within these two regions of interest was reported for early and late anterior images.

The myocardial washout rate from early to late images was calculated according to previously published guidelines (11). Evidence supports the use of the late heart-to-mediastinum ratio for assessment of symptomatic autonomic neuropathy (12).

Cardiac Autonomic Function by Heart Rate Analyses

We used the Vagus device (Medicus Engineering, Aarhus, Denmark) to measure HRV in time and frequency domains and during conventional CARTs. The Vagus device automatically measures electrocardiographic signals with a sampling frequency of 1,000 Hz. All tests were performed between 8:00 A.M. and 3:00 P.M. in a quiet examination room by trained staff. In accordance with recommendations, we used a standard protocol (13), and participants were fasted for 4 h and advised to abstain from hard physical activity for 24 h before the examination.

HRV Indices

Resting heart rate was calculated from 5-min measures of R-R interval. We calculated the SD of normal-to-normal (SDNN) intervals and the root mean square of successive differences (RMSSD) as time-domain HRV indices. SDNN is a measure of combined sympathetic and parasympathetic activity, and RMSSD is predominantly a measure of sympathetic activity.

The following frequency-domain HRV indices were calculated using fast Fourier transformation: low-frequency (LF) power (0.04–0.15 Hz), high-frequency (HF) power (0.15–0.4 Hz), and total power (≤0.4 Hz). We also calculated the ratio of LF-to-HF power. HF power predominantly reflects parasympathetic activity, whereas LF power is influenced by sympathetic and parasympathetic tone and baroreflex sensitivity, and LF power mainly contributes to total power (2,14,15).

CARTs

The CARTs included the following: *1*) response to standing (30-to-15 ratio), calculated as the ratio between longest (around the 30th heartbeat after the rise) and the shortest R-R interval (around the 15th heartbeat after the rise) during and shortly after standing from a supine position; *2*) deep breathing test for 1 min (expiration-to-inspiration [E-to-I] ratio) while sitting (the E-to-I ratio is calculated as the mean of the longest R-R interval divided by the mean of the shortest R-R interval during deep breathing during a deep breathing respiratory cycle; and *3*) the Valsalva test ratio, conducted as a 15-s exhalation through a 40 mmHg resistance mouthpiece while sitting. The Valsalva test ratio is calculated as the ratio of the longest and shortest R-R intervals during and immediately after the Valsalva maneuver. The 30-to-15 ratio and the E-to-I ratio are predominantly measures of parasympathetic tonus, whereas the Valsalva test ratio is influenced by sympathetic and parasympathetic tone (1,2). The CARTs were evaluated according to age-related reference intervals (16). CAN was defined using the American Diabetes Association criteria (17), and we defined CAN as *1*) "no CAN" when no pathological CARTs were detected or if only one abnormal CART was detected and *2*) definite CAN if two or three abnormal CARTs were detected. A diagnosis of CAN based on the CARTs was not evaluated in the participants with fewer than two CART results (n = 7) or in participants with two discrepant CART results (one normal and one pathological) (n = 6).

Sudomotor Function

In the 60 participants with diabetes, we assessed the function of small autonomic fibers using an electrochemical skin conduction test on the hands and feet (Sudoscan; Impeto Medical, Paris, France) (18). We applied age- and sex-stratified electrochemical skin conduction thresholds for hands and feet (19). Sudomotor dysfunction contributes to detection of autonomic dysfunction in peripheral diabetic neuropathy (2).

Statistical Analysis

The distribution of UACR, UAER, alcohol intake, and the time and frequency domain HRV indices was skewed, and these variables were $\log_2$ transformed for analyses and presented as the median with interquartile range (IQR). Continuous data with approximately normal distributions are given as the mean and SD. Categorical variables are provided as total numbers and percentages. Independent-samples t test was applied when comparing differences in continuous variables between two groups (e.g., control subjects vs. normoalbuminuric participants and normoalbuminuric vs. macroalbuminuric participants) and the χ^2 test or Fisher exact test, as appropriate, when analyzing categorical variables. We pooled all participants in the linear regression analyses and applied stepwise adjustment. Unadjusted models were used to determine whether an association existed between each of the measures of cardiac autonomic function and MFR (model 1). Subsequent adjustment included age (model 2); age and heart rate (model 3); age, heart rate, sex, 24-h systolic blood pressure, BMI, HbA_{1c}, UACR or UAER, and smoking (model 4); and additional adjustment of model 4 for physical activity and the prescribed drugs that could influence HRV or organ uptake of MIBG (β-blocker, amlodipine, tramadol, tricyclic antidepressants, and chlorpromazine) in a final model, model 5. The CARTs were not adjusted for heart rate since heart rate can influence these tests (13). Due to the risk of bias by indication, we did not adjust for total cholesterol, since it was highest in the healthy control subjects, most likely due to medical treatment of the participants with diabetes. Standardized regression coefficients are reported.

Pearson correlations were used to explore associations of the measures of cardiac autonomic function with each other, and ANCOVA was applied to determine if these correlations persisted after adjustment.

A two-tailed $P < 0.05$ was interpreted as significant. Statistical analyses were performed using SAS software (version 9.4; SAS Institute).

RESULTS

Clinical Characteristics

In the total cohort, the mean ± SD age was 59.3 ± 9.5 years and 41% were female. Clinical characteristics for the participants in the three groups are summarized in Table 1.

Among medications influencing HRV or organ uptake of MIBG (Table 1) (11), treatment with amlodipine was prescribed in 11 participants (18%), β-blockers in 9 participants (15%), tramadol in 3 participants (5%), and both a tricyclic antidepressant and chlorpromazine in 1 participant.

Seven participants with macroalbuminuria had a history of coronary artery disease (two had coronary artery bypass graft and five had percutaneous coronary intervention). Quantified by the PET/CT, 10 participants (9 with macroalbuminuria [of which 7 had a history of coronary artery disease] and 1 healthy control subject) had reversible ischemia. The median (IQR) extent was 24% (14–29%). In five of these participants, irreversible ischemia (fixed perfusions defects) was also observed (all five had macroalbuminuria). Median extent was 19% (16–25%).

All three CARTs and the late heart-to-mediastinum ratio were higher in the control subjects compared with the normoalbuminuric participants with diabetes ($P \leq 0.02$). The CARTs and the late heart-to-mediastinum ratio were comparable between participants with diabetes and normoalbuminuria or macroalbuminuria ($P \geq 0.05$). The HRV indices were comparable between groups ($P \geq 0.05$), except total power, which was higher in the control subjects compared with the normoalbuminuric participants ($P = 0.03$). More persons with macroalbuminuria had bilateral sudomotor dysfunction in hands and feet compared with persons with normoalbuminuria ($P = 0.006$ for both).

Prevalence of Impaired Cardiac Autonomic Function

Defined based on the CARTs, 14 participants (18%) had definite CAN and 63 had no CAN (82%). In our population, the mean ± SD late heart-to-mediastinum ratio was 2.9 ± 0.39 in the healthy control subjects compared with 2.5 ± 0.45 in the participants with diabetes. The lower 5% percentile for the late heart-to-mediastinum ratio in the healthy control subjects was 2.4. With this applied as a cutoff to the participants with diabetes, a total of 9 participants (30%) with normoalbuminuria and 15 participants (50%) with macroalbuminuria had impaired cardiac autonomic function.

Correlations Between the Measures of Cardiac Autonomic Function and MFR

Correlations between the measures of cardiac autonomic function and MFR are presented in Table 2. All measures, except LF-to-HF ratio and the Valsalva test ratio, were positively correlated to MFR in unadjusted analysis (model 1, $P \leq 0.004$) and after adjustment for age (model 2, $P \leq 0.01$). After further adjustment for heart rate, the late heart-to-mediastinum ratio was positively associated with MFR ($P = 0.003$), but all the HRV indices lost significance ($P \geq 0.07$). After additional adjustment for other risk factors (model 4), the late heart-to-mediastinum ratio (β per 1 SD increase, 0.24; $P = 0.03$) and the 30-to-15 ratio (β per 1 SD increase, 0.25; $P = 0.04$) remained positively associated with MFR. In the final model, model 5, the late heart-to-mediastinum ratio (β per 1 SD increase, 0.28; $P = 0.01$) and the 30-to-15 ratio (β per 1 SD increase, 0.30; $P = 0.01$) remained positively associated with MFR.

Figure 1 illustrates the unadjusted correlation of MFR to the late heart-to-mediastinum ratio (Fig. 1*A*) and the 30-to-15 ratio (Fig. 1*B*). The levels of MFR according to CAN status based on the CARTs are illustrated in Fig. 2*A*.

Agreement Between the Measures of Cardiac Autonomic Function

Pearson correlation coefficients between the measures of cardiac autonomic function are presented in Supplementary Table 1. Of the HRV indices and CARTs, only the 30-to-15 ratio correlated positively with the late heart-to-mediastinum ratio (unadjusted, $P = 0.002$). This association persisted after adjustment for age ($P = 0.003$), but lost significance after adjustment for additional risk factors ($P = 0.72$). The HRV indices and CARTs were, with few exceptions, strongly correlated.

Exclusion of Participants With Known Coronary Artery Disease or Ischemia Identified by Cardiac PET

To avoid the potential confounding effect of ischemic heart disease, we performed all analyses excluding the participants with known coronary artery disease or reversible and/or irreversible ischemia identified by cardiac PET ($n = 10$).

For the comparisons of late heart-to-mediastinum ratio, CARTs, and HRV indices among the three groups, results were confirmatory.

When evaluating the prevalence of impaired cardiac autonomic function using CARTs, 10 participants had definite CAN (14%) and 59 had no CAN (86%). Applying a cutoff for the late heart-to-mediastinum ratio of 2.4 (as described previously) showed that 9 participants with normoalbuminuria (30%) and 9 participants with macroalbuminuria (42%) had impaired cardiac autonomic function.

In analysis of the correlations between the measures of cardiac autonomic function and MFR, the late heart-to-mediastinum ratio and the 30-to-15 ratio were positively

Table 1—Clinical characteristics and measures of cardiac autonomic function

	Control subjects (n = 30)	Normoalbuminuria (n = 30)	Macroalbuminuria (n = 30)	P (control subject vs. normoalbuminuria)	P (normoalbuminuria vs. macroalbuminuria)
Female	12 (40)	12 (40)	13 (43)	1.0	0.79
Age (years)	59.8 ± 9.9	59.8 ± 9.1	58.2 ± 9.9	0.99	0.52
Known diabetes duration (years)		32.6 ± 12.7	41.4 ± 13.3		0.01
BMI (kg/m^2)	24.7 ± 3.4	25.6 ± 4.1	27.2 ± 4.2	0.38	0.15
24-h systolic blood pressure (mmHg)	127 ± 13	135 ± 9	138 ± 11	0.009	0.26
24-h diastolic blood pressure (mmHg)	79 ± 8	78 ± 6	77 ± 5	0.88	0.48
Heart rate (bpm)	61 ± 8	69 ± 12	72 ± 12	0.003	0.41
HbA_{1c} (mmol/mol)	35.8 ± 1.9	61.3 ± 8.3	66.3 ± 11.7	<0.0001	0.70
HbA_{1c} (%)	5.4 ± 0.17	7.8 ± 0.76	8.2 ± 1.07	<0.0001	0.70
LDL cholesterol (mmol/L)	3.4 ± 0.7	2.3 ± 0.7	2.1 ± 0.8	<0.0001	0.32
eGFR (mL min^{-1} 1.73 m^{-2})	82.8 ± 13.1	89.1 ± 10.4	62.5 ± 23.1	0.043	<0.0001
UACR (mg/g)*	6 [5–10.5]	3 (3–5)	121 [53–283]	0.0002	<0.0001
Smokers	4 (13)	4 (14)	4 (14)	0.96	1.0
Alcohol (beverages/week)	8.5 (4–14)	7 (4–18)	6.5 (1–14)	0.87	0.91
Physical activity (hours/week)	5.0 ± 4.2	4.9 ± 5.3	6.1 ± 11.4	0.93	0.61
Treatment					
Antihypertensive	3 (10)	17 (57)	30 (100)	0.0001	<0.0001
RAAS inhibition	3 (10)	14 (47)	29 (97)	0.002	<0.0001
β-Blocker	0 (0)	1 (3)	8 (27)	1.0	0.03
Aspirin	1 (3)	11 (37)	19 (63)	0.0012	0.04
Lipid-lowering	0 (0)	21 (70)	24 (80)	<0.0001	0.37
Amlodipine	0 (0)	4 (13)	10 (33)	0.11	0.07
Known coronary artery disease	0 (0)	0 (0)	7 (23)	—	0.01
MFR	3.0 ± 0.79	3.1 ± 0.79	2.1 ± 0.92	0.74	<0.0001
HRV measures					
Time and frequency domains					
SDNN intervals (ms)	39.4 [28.6–53.0]	30.0 [20.5–49.6]	18.5 [13.3–34.8]	0.057	0.057
RMSSD (ms)	25 [20.4–39.45]	19.25 [10.1–30.9]	13.0 [6.5–20.1]	0.065	0.21
LF power (ms^2)	196.7 [78.3–308.7]	73.1 [24.7–238.3]	21.1 [14.6–92.5]	0.045	0.099
HF power (ms^2)	70.9 [45.9–131.5]	43.2 [12.0–87.1]]	22.3 [5.3–66.0]	0.077	0.20
LF/HF (ratio)	2.05 [1.38–4.25]	2.07 [1.34–3.52]	2.06 [0.89–3.46]	0.64	0.44
Total power (ms^2)	606.1 [253.6–1,106.2]	291.7 [118.6–644.5]	101.9 [54.6–447.4]	0.03	0.08
CARTs					
30-to-15 ratio (response to standing)	1.24 ± 0.17	1.12 ± 0.11	1.03 ± 0.25	0.0002	0.12
E-to-I ratio (deep breathing)	1.24 ± 0.15	1.15 ± 0.12	1.12 ± 0.0	0.02	0.28
Valsalva test ratio	1.77 ± 0.41	1.48 ± 0.19	1.39 ± 0.34	0.0045	0.33
CAN					
No CAN	28 (100)	22 (85)	13 (43)	0.047	0.03
CAN	0 (0)	4 (15)	10 (57)		

Continued on p. 1282

Table 1—Continued

	Control subjects (n = 30)	Normoalbuminuria (n = 30)	Macroalbuminuria (n = 30)	P (control subject vs. normoalbuminuria)	P (normoalbuminuria vs. macroalbuminuria)
^{123}I-MIBG imaging	n = 14	n = 30	n = 30		
Late heart-to-mediastinum ratio	2.9 ± 0.39	2.6 ± 0.38	2.3 ± 0.50	0.01	0.05
Sudomotor function					
Feet (μS)		69.9 ± 14.4	55.2 ± 24.4		0.008
Sudomotor dysfunction**		11 (38)	18 (62)		0.006
Hands (μS)		58.6 ± 17.0	47.5 ± 18.7		0.02
Sudomotor dysfunction**		8 (27)	18 (62)		0.006

Data represent total number (%), mean ± SD, or median [IQR]. CAN was defined as no CAN when no pathological CART results were detected or if only one abnormal CART result was detected and as definite if two or three abnormal CART results were detected, according to the American Diabetes Association criteria. RAAS, renin-angiotensin-aldosterone system. *UAER for control subjects. **We applied age- and sex-stratified electrochemical skin conduction thresholds when evaluating sudomotor dysfunction in hands and feet.

associated with MFR unadjusted (model 1; P = 0.002) and after adjustment for age (model 2; $P \leq 0.006$). The association between MFR and the late heart-to-mediastinum ratio remained significant after adjustment for additional risk factors (model 4) (β per 1 SD increase, 0.25; P = 0.046), whereas the 30-to-15 ratio lost significance (P = 0.08).

Supplementary Fig. 1 illustrates the correlation of MFR to the late heart-to-mediastinum ratio (Supplementary Fig. 1*A*) and the 30-to-15 ratio (Supplementary Fig. 1*B*). The levels of MFR according to CAN status based on the CARTs are shown in Fig. 2*B*.

Pearson correlation coefficients between the measures of cardiac autonomic function are presented in Supplementary Table 2. Results were largely confirmatory of the results presented for all participants (Supplementary Table 1), with the exception that the late heart-to-mediastinum ratio was not correlated with the 30-to-15 ratio, and HF power was not correlated with SDNN or LF-to-HF ratio.

Additional Analyses

Analyses only including the participants with diabetes were generally confirmatory; MFR was positively correlated with late heart-to-mediastinum ratio (P = 0.01), total power ($P < 0.001$), and 30-to-15 ratio ($P < 0.001$). These associations persisted after adjustment for age (model 2, $P < 0.001$). Only the association between MFR and the

Table 2—Unadjusted and stepwise adjusted associations between measures of cardiac autonomic function and MFR

	Model 1 unadjusted		Model 2 adjusted for age		Model 3 adjusted for age and heart rate		Model 4 adjusted for age, heart rate, and other risk factors**		Model 5 adjusted as model 4 + physical activity and medication***	
	β	P	β	P	β	P	β	P	β	P
^{123}I-MIBG imaging (n = 74)										
Late heart-to-mediastinum ratio	0.39	0.0004	0.38	0.0005	0.32	0.0027	0.24	0.03	0.28	0.01
HRV measures (n = 84)										
Time and frequency domains*										
SDNN intervals (ms)	0.34	0.0006	0.32	0.001	0.19	0.10	0.14	0.29	0.12	0.37
RMSSD (ms)	0.33	0.0009	0.30	0.002	0.16	0.20	0.14	0.30	0.12	0.39
LF power (ms^2)	0.34	0.0007	0.31	0.001	0.20	0.07	0.14	0.25	0.11	0.38
HF power (ms^2)	0.29	0.004	0.25	0.01	0.09	0.46	0.05	0.71	0.06	0.65
LF/HF (ratio)	0.10	0.32	0.13	0.18	0.15	0.10	0.09	0.30	0.06	0.55
Total power (ms^2)	0.32	0.001	0.30	0.002	0.18	0.14	0.12	0.36	0.10	0.47
CARTs										
30-to-15 ratio (response to standing, n = 83)	0.30	0.001	0.27	0.004			0.25	0.04	0.30	0.01
E-to-I ratio (deep breathing, n = 84)	0.30	0.002	0.26	0.006			0.15	0.11	0.19	0.05
Valsalva test ratio (n = 60)	0.14	0.19	0.14	0.19			−0.01	0.92	−0.002	0.99

*Log2 transformed for analyses. **Not included in adjustment for the CARTs. The β-estimates represent standardized effect. Other risk factors included sex, 24-h systolic blood pressure, BMI, HbA_{1c}, UACR (UAER in control subjects), and smoking. ***Treatment with β-blockers, amlodipine, tramadol, tricyclic antidepressants, or chlorpromazine.

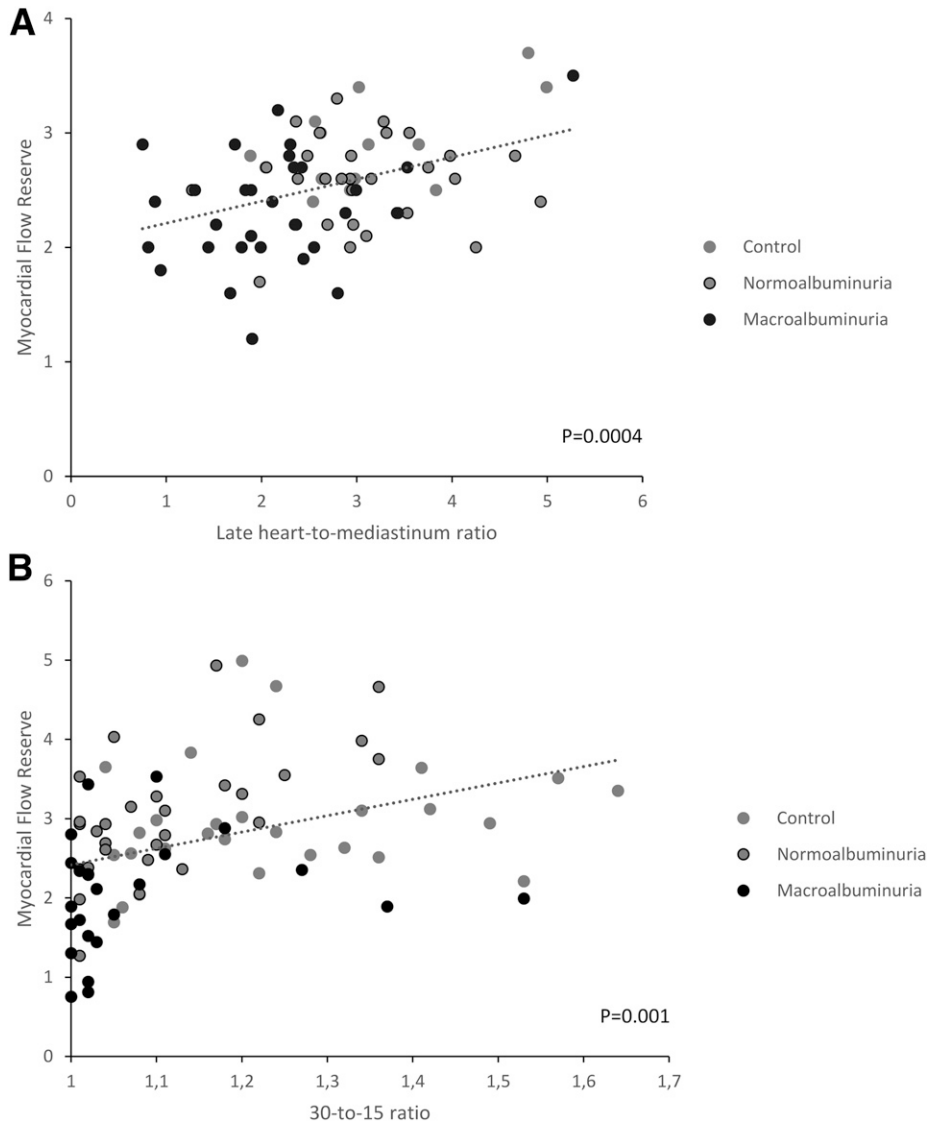

Figure 1—Correlations between MFR and measures of cardiac autonomic function: late heart-to-mediastinum ratio (*A*) and 30-to-15 ratio (*B*). All correlations were significant, $P \leq 0.001$.

30-to-15 ratio persisted after adjustment for additional risk factors (model 4, $P = 0.01$).

The myocardial washout rate of MIBG was not associated with the MFR ($P = 0.41$) or the late heart-to-mediastinum ratio ($P = 0.27$).

DISCUSSION

In this cross-sectional study in type 1 diabetes and healthy control subjects, we demonstrated a positive association between MFR and a comprehensive panel of cardiac autonomic function measures, including HRV indices, cardiac autonomic reflex tests, and the late heart-to-mediastinum ratio. We confirmed the positive association between MFR and the late heart-to-mediastinum ratio in analyses restricted to the participants free of coronary artery disease.

CAN affects both the sympathetic and parasympathetic part of the autonomic nervous system. When testing for CAN, it is therefore advised that a battery of examinations should be used to evaluate both branches of the autonomic nervous system, rather than a single test. The cardiovascular reflex tests recommended by the American Diabetes Association for the diagnosis of CAN and the HRV indices evaluate the function of both branches of the autonomic nervous system indirectly (20,21). With the introduction of radiolabeled analogs of norepinephrine, which are actively taken up by the sympathetic nerve terminals, it is now possible to directly assess the integrity of cardiac sympathetic nerve fibers (22). MIBG is an analog of norepinephrine labeled with ^{123}I to allow Gamma Camera imaging. A high heart-to-mediastinum ratio indicates an intact sympathetic system in the myocardium, whereas a low heart-to-mediastinum ratio is an indicator of impaired cardiac sympathetic integrity (23).

The aim of our study was to explore the potential link between cardiac autonomic function and impaired cardiac

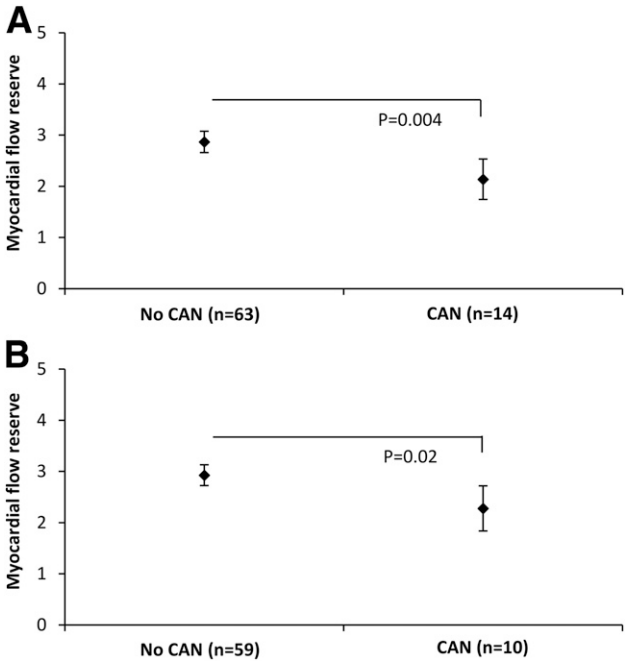

Figure 2—MFR according to CAN status: all participants (*A*) and participants with known coronary artery disease or reversible and/or irreversible ischemia identified by cardiac PET (*B*) (n = 10) were excluded. Data are presented as the mean with 95% CI. CAN diagnosis was based on CARTs using the American Diabetes Association criteria; we defined CAN as *1*) no CAN when no pathological CART results were detected or if only one abnormal CART result was detected and *2*) definite CAN if two or three abnormal CART results were detected. Differences between groups were analyzed with independent-samples t tests.

vascular function, as this could contribute to a better understanding of the pathophysiology linking CAN to risk of cardiovascular disease. The gold standard for assessing the myocardial blood flow is PET imaging (24). We used cardiac ^{82}Rb PET/CT for the assessment of MFR. MFR is calculated as the ratio between resting and maximal induced myocardial blood flow. It reflects to what extent the myocardial blood flow can increase during pharmacologically induced stress. MFR mirrors the function of the large epicardial arteries and the microcirculation of the myocardium and is a strong predictor of cardiovascular disease and death, even in the absence of known coronary artery disease (5,25). Moreover, we used the results from the ^{82}Rb PET/CT to accurately identify participants with myocardial perfusion defects, enabling us to address the potential confounding effect of coronary artery disease on the association between cardiac autonomic function and impaired cardiac vascular function.

We demonstrated associations between a wide range of different measures of cardiac autonomic function and MFR. The associations between the late heart-to-mediastinum ratio and MFR and between the 30-to-15 ratio and MFR were strongest, as they persisted even after adjustment for appropriate risk factors. A major challenge in studies investigating the possible association between cardiac autonomic function and myocardial blood flow is to address the potential confounding by the presence of ischemic heart disease, which might affect both the cardiac autonomic function and MFR. To further disentangle whether the association between cardiac autonomic function and MFR simply reflected existing clinically diagnosed coronary artery disease, we performed a separate set of analyses excluding the 10 participants with known coronary artery disease and/or ischemia revealed by cardiac PET/CT. The association between the late heart-to-mediastinum ratio and MFR remained significant in these analyses even after comprehensive adjustment. These findings implicate that cardiac autonomic dysfunction may be associated with impaired stimulated blood flow in type 1 diabetes even after eliminating the influence of coronary artery disease.

A study in persons undergoing cardiac transplantation was the first to demonstrate a role of cardiac adrenergic signals in the regulation of myocardial blood flow (26). The findings provoked speculations on how impaired MFR could aggravate existing endothelial dysfunction or atherosclerotic lesions and, in periods of increased oxygen demand, could lead to myocardial ischemia and left ventricular dysfunction, even in the absence of atherosclerotic lesions (27). Very few data on cardiac autonomic function and myocardial blood flow in type 1 diabetes have been published until now. A study in 28 persons with either type 1 or type 2 diabetes (27) reported impaired vasodilator response of coronary resistance vessels in the presence of sympathetic nerve dysfunction. Likewise, a study in 14 persons with type 1 diabetes (28) demonstrated that the myocardial blood flow differed regionally in relation to islets of persistent cardiac sympathetic integrity. Later on, the same authors could not confirm the regional differences in vasodilator reserve, but showed a relation between sympathetic dysfunction (assessed by ^{11}C hydroxyephedrine PET) and a wide range of abnormalities in myocardial blood flow (assessed by ^{13}N ammonia PET) in 28 persons with type 1 diabetes (29). Myocardial injury is an advanced condition involving several mechanisms that may or may not be linked with sympathetic integrity. More studies are needed to confirm our observed association between cardiac autonomic function and impaired cardiac vascular function in type 1 diabetes using a methodology that directly addresses the confounding effect of ischemia.

Another measure obtained from the MIBG scintigraphy is the myocardial washout rate, calculated as the difference in myocardial counts between the early and the late image, normalized to the mediastinum counts. The washout rate is considered to reflect adrenergic activity with a high washout rate, indicating high adrenergic activity. The clinical relevance of the washout rate is not clear in diabetes. The washout rate in our study population was not related to MFR or to late heart-to-mediastinum ratio.

We demonstrated a general impaired function of the cardiac autonomic system in persons with type 1 diabetes and normoalbuminuria compared with healthy control subjects, which is in line with previous findings (30). Furthermore, we demonstrated CAN to be more frequent

in persons with type 1 diabetes and macroalbuminuria compared with normoalbuminuria. Autonomic imbalance has been linked to chronic kidney disease and its progression in type 1 diabetes (31), and our findings add to the literature, with previous studies in type 1 diabetes (32,33) demonstrating a lower cardiac autonomic function in persons with macroalbuminuria compared with normoalbuminuria.

In relation to peripheral autonomic dysfunction, we demonstrated significantly higher prevalence of bilateral sudomotor dysfunction in the feet or hands in the participants with macroalbuminuria compared with normoalbuminuria.

For cardiac autonomic integrity assessed as the late heart-to-mediastinum ratio, our findings in type 1 diabetes are in concordance with our previous observations in type 2 diabetes, where we demonstrated the late heart-to-mediastinum ratio to be comparable in persons with type 2 diabetes and normoalbuminuria or albuminuria, but lower in healthy control subjects compared with the persons with diabetes and normoalbuminuria (4).

We found agreement between the HRV indices and the CARTs, and between the individual measures of cardiac autonomic function within these two groups. The late heart-to-mediastinum ratio was positively correlated with the 30-to-15 ratio, but not with the other CARTs or HRV indices. This correlation lost significance following adjustment for cardiovascular risk factors.

Strengths and Limitations

The strengths of this study include the robust measures of cardiac autonomic function and MFR assessed by PET imaging. Although the study is large compared with previous studies on this topic, the relatively small sample size increases the risk of type II errors. The sample size was anticipated, based on results from a previous study in type 2 diabetes with the limitation that inclusion and exclusion criteria were different in the two studies. Another limitation is the cross-sectional nature of the study, preventing us from coming to a conclusion about causal relationships. Inclusion of participants with a history of microalbuminuria, covering the full continuum of albuminuria, would have enhanced the statistical evaluation of the associations between the measures of cardiac autonomic function and albuminuria, and would have allowed us to investigate whether change in autonomic function is an early or late phenomenon in diabetic nephropathy.

Conclusion

In conclusion, we found distinct associations between measures of cardiac autonomic function and MFR. Most notably, after adjustment for relevant cardiovascular risk factors and in the participants free of coronary artery disease, the late heart-to-mediastinum ratio directly measuring the integrity of cardiac sympathetic nerve fibers was positively associated with MFR. This indicates that cardiac autonomic dysfunction, including the loss of sympathetic integrity, may be associated with impaired myocardial blood flow regulation in type 1 diabetes, even after eliminating the influence of coronary artery disease.

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Author Contributions. E.H.Z. conceived and designed the research, acquired the data, performed the statistical analysis, and drafted the manuscript. P.H. acquired the data, contributed to interpretation of the results, and reviewed/edited the manuscript. S.A.W. acquired the data, contributed to interpretation of the results, and reviewed/edited the manuscript. C.S.H, J.F., B.J.v.S., L.H., and A.K. contributed to interpretation of the results and reviewed/edited the manuscript. P.R. conceived and designed the research, contributed to interpretation of the results, and reviewed/edited the manuscript. T.W.H. conceived and designed the research, acquired the data, performed the statistical analysis, contributed to interpretation of the results, drafted the manuscript, and reviewed/edited the manuscript. E.H.Z. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Data Availability. The data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request. No applicable resources were generated or analyzed during the current study.

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