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Reduced Heart Rate Variability Predicts Progression of Coronary Artery Calcification in Adults with Type 1 Diabetes and Controls Without Diabetes

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Abstract

Aim: Reduced heart rate variability (HRV) is a manifestation of cardiac autonomic neuropathy, a known complication of type 1 diabetes (T1D). We evaluated whether HRV predicted coronary artery calcium (CAC) progression.

Methods: Subjects between 19 and 56 years of age with T1D or those without diabetes from the Coronary Artery Calcification in Type 1 Diabetes study underwent supine deep breathing 12-lead electrocardiograms. The SD of consecutive RR intervals was used as a measure of HRV. CAC was measured at two visits 6.0 ± 0.5 years apart. Progression of CAC was defined as an increase in square root transformed CAC volume of $\geq 2.5\,mm^3$, excluding patients who had cardiovascular events during follow-up.

Results: Reduced HRV was associated with older age, higher hemoglobin A1c, elevated albuminuria, CAC volume at baseline, and increased fibrinogen. Higher HRV at baseline was associated with lower likelihood CAC progression (odds ratio = 0.71, 95% confidence interval 0.56–0.90, $P = 0.005$), and the adjustment for known cardiovascular risk factors did not change this strong association, including adjustment for inflammatory markers.

Conclusions: Reduced HRV predicted progression of CAC in adults with and without T1D. This association further supports the participation of autonomic neuropathy in the atherosclerosis process.

Introduction

The etiology of cardiac disease in patients with type 1 diabetes (T1D) may involve many factors, including accelerated coronary atherosclerosis, diabetic cardiomyopathy, and cardiac autonomic neuropathy.[1,2] Autonomic neuropathy is a common complication of T1D associated with older age, longer duration of diabetes, worse metabolic control, and the presence of microvascular and cardiovascular disease.[3–5] Cardiac autonomic neuropathy affects the parasympathetic nervous system, leading to reduced heart rate variability (HRV).[5] In people without diabetes, decreased HRV is associated with subclinical inflammation,[6] arterial hypertension,[7] and increased incidence of cardiac events.[8] An association between cardiac autonomic neuropathy and carotid atherosclerosis, assessed by ultrasound, has been reported in patients with type 2 diabetes.[9,10]

Coronary artery calcium (CAC) is a powerful predictor of clinical coronary artery disease.[11] T1D patients demonstrate greater extent and progression of CAC than subjects without diabetes,[12] and two cross-sectional studies have reported an association between reduced HRV and the presence of CAC in these patients.[13,14]

In this article, we evaluated HRV as a predictor of CAC progression using data from the prospective Coronary Artery Calcification in Type 1 Diabetes (CACTI) Study.

Research Design and Methods

Study participants

The CACTI Study is a prospective cohort study of 1,416 participants, designed to identify causes of accelerated development and progression of coronary atherosclerosis in participants with T1D. Participants completed the baseline examination in 2000–2002 at the age of 19–56 years. The study assessed the extent of CAC in 652 men and women with T1D and 764 non-diabetes mellitus (non-DM) control subjects as reported previously.[12] All participants were asymptomatic

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for coronary artery disease and had no history of coronary artery bypass graft surgery, myocardial infarction, coronary angioplasty, or angina. Participants with T1D had diagnosis of T1D confirmed by an endocrinologist and had been treated with insulin within 1 year of diagnosis. Non-DM control participants were generally spouses, friends, and neighbors of the T1D participants. The study protocol was reviewed and approved by the Colorado Combined Institutional Review Board, and informed consent was obtained from all participants before enrollment. Complete information about CAC progression after 6.0 ± 0.5 years of follow-up, including data about HRV, was available for 915 patients, excluding those people who had a cardiovascular event ($n = 18$).

Patient evaluation

We measured the current height and weight and calculated body mass index (BMI) as kg/m^2. Resting systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured three times while the subjects were seated, and the second and the third measurements were averaged. Hypertension was defined as SBP $\geq$140 mm Hg, DBP $\geq$90 mm Hg, or current antihypertensive treatment. Participants completed standardized questionnaires including medical history, medication inventory, Rose angina, current and past smoking status, physical activity, food frequency, daily insulin dose, family history of diabetes, coronary artery disease, and hypertension.

Laboratory analyses

After subjects fasted overnight, blood was collected and centrifuged, and separated plasma was stored at 4°C until assayed. Total plasma cholesterol and triglyceride levels were measured using standard enzymatic methods, high-density lipoprotein (HDL) cholesterol was separated using dextran sulfate, and low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula. High-performance liquid chromatography was used to measure hemoglobin A1c (HbA1c) (Variant II™ HbA1c analyzer, Bio-Rad, Hercules, CA). Plasma glucose was measured using a standard hexokinase method. Homocysteine was determined by the Abbott (Abbot Park, IL) IMx™ automated procedure. High-sensitivity C-reactive protein (hsCRP), plasminogen activator inhibitor 1, and fibrinogen were measured in the laboratory of Dr. Russell Tracy at the University of Vermont (Colchester, VT) as previously reported.[15] Albumin excretion rate (AER) measurements in overnight or morning spot urine collections were measured by radioimmunoassay and expressed in $\mu g/min$.

CAC

All participants underwent a coronary calcium scan using an ultrafast Imatron C-150XLP electron beam computed tomography scanner (GE/Imatron, San Francisco, CA) to obtain two sets of high-resolution, noncontrast, contiguous 3-mm tomographic images acquired at 100-ms exposure. Scanning started from near the lower margin of bifurcation of the main pulmonary artery with the subject holding his or her breath for 35–40 s and proceeded caudally. Calcified coronary artery lesions were identified as those with a minimum density of 130 Hounsfield units and a minimum area of three pixels (1.03 mm). A calcium score for each region was calculated by multiplying the area by the density score (1 for 130–199, 2 for 200–299, 3 for 300–399, and 4 for >399 Hounsfield units). A total CAC score in Agatston units was calculated by adding up scores for all slices separately for left main, left anterior descending, circumflex, and right coronary arteries.[16] The calcium volume score was determined by the workstation using a standard algorithm. The scanner was recalibrated every day with a phantom. Effective radiation dose for an electron beam computed tomography sequence was 0.7–1.0 mSV for men and 1.0–1.3 mSV for women. A single technician obtained and scored all electron beam computed tomography scans, and the average of two scores obtained 5 min apart was used.

CAC was measured twice at the baseline and twice at a follow-up 6.0 ± 0.5 years later and averaged at each visit.

Electrocardiogram

Study participants were asked to fast overnight for at least 12 h and to abstain from caffeine, alcohol, and smoking. Participants were examined in the morning, between the hours of 7 a.m. and 11 a.m. Study participants underwent a supine resting electrocardiogram (ECG), which was recorded using the MACPC electrocardiographic machine (Marquette Electronics, Inc., Milwaukee, WI). Examinations were conducted in a quiet room, with a stable room temperature. Participants were asked to relax but not fall asleep and to breathe normally and remain still without talking during the ECG recording. Following the resting ECG, a second supine ECG was recorded while study participants took in a deep breath for 10 s. Study participants were instructed to take in a long, slow deep breath while the technician recorded the ECG.

A standard acquisition procedure was used including the 12 SL Analysis Program of computerized evaluation of cardiac rhythm and morphology. All technicians were certified by the Epidemiological Cardiology Research Center (EPICARE) reading center prior to conducting ECGs for the study, and quality control scores were assigned to each ECG to ensure quality recordings. The ECG recordings were transmitted electronically via modem to the EPICARE Center (Dr. Ronald J. Prineas, Wake Forest University School of Medicine, Winston-Salem, NC) for analysis, interpretation, and calculation of HRV. The ECGs were coded using the Minnesota code and the NOVACODE ECG software[17] to determine ECG abnormalities. The SD of consecutive RR intervals of the entire record in Lead II was used as a measure of HRV. The R-R intervals were sampled at 5 Hz, with premature beats identified and corrected by linear interpolation with the previous and following beats. Autoregressive power spectral analysis was used to determine the spectral power in the following bands: high frequency (0.15–0.45 Hz), low frequency (0.04–0.15 Hz), and very low frequency (0.01–0.04 Hz).[18]

Statistical analysis

Data are presented as arithmetic means and SDs for continuous variables (geometric means and ranges for log-transformed variables) and percentages for categorical variables. Two-sample t test was used for continuous variables, and the χ^2 test was used for categorical variables. HRV, triglycerides, hsCRP, homocysteine, fibrinogen, and AER were log-transformed for analysis.

Unadjusted Pearson correlations were used to examine independent correlations with HRV. Stepwise multiple linear regression was used to select variables associated with HRV, stratified by diabetes status, using $P < 0.10$ as the criterion for entry and $P < 0.05$ for removal from the model.

To evaluate the prospective relationship between HRV at baseline and CAC progression, we performed a stepwise multiple logistic regression using $P < 0.10$ as the criteria for entry and $P < 0.05$ for removal from the model, including as independent variables age, sex, presence of T1D, SBP, DBP, presence of CAC at baseline, and HRV. Additionally, we tested for interaction between T1D and HRV, and we did not find any interaction between them. Next, we performed sequential logistic regression models including only variables associated with CAC progression from model 1: male gender, age, presence of T1D, CAC at baseline, SBP, and HRV. Then we added HDL, LDL, and statin use (model 2), BMI (model 3), HbA1c (model 4), AER and angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) use (model 5), and inflammatory markers (fibrinogen, homocysteine, and hsCRP) as model 6.

Finally, to evaluate the association of HRV or resting heart rate with progression of CAC we performed a stepwise selection logistic regression including the important variables associated with CAC progression.

Coronary calcium interpolated volume from each scan was square root transformed to reduce the variance across coronary calcium scores,[19] and the square root transformed volumes were averaged for each visit. The difference between mean square root transformed coronary calcium volumes was calculated from the baseline visit to the follow-up visit. Progression of coronary calcium and atherosclerosis were defined as an increase in square root transformed coronary calcium volume of 2.5 or greater between the two visits.[19]

SAS version 9.2 (SAS Institute, Cary, NC) was used to these analyses, and all statistical tests were two sided, with $P < 0.05$ considered significant.

Results

Clinical and laboratory characteristics at baseline by CAC progression and diabetes status are shown in Table 1. We observed CAC progression in 324 subjects. Progressors with T1D ($n = 175$) were more frequently males and older and had higher SBP and DBP levels, higher BMI, lower HRV, higher resting heart rate, higher CAC volume at baseline, higher HbA1c, lower HDL cholesterol, higher LDL cholesterol, higher triglycerides, higher fibrinogen, higher homocysteine, and higher AER than nonprogressors with T1D. Progressors also more frequently used ACE inhibitors and statins than nonprogressors. We did not observe differences in smoking currently and hsCRP between progressors and nonprogressors with T1D. Non-DM subjects with CAC progression had characteristics similar to those of progressors with T1D, including sex and age, and also had higher blood pressure

Table 1. Clinical and Laboratory Characteristics at Baseline Between Progressors and Nonprogressors

	T1D			*Non-DM controls*		
	Progressors (n = *175*)	*Nonprogressors* (n = *235*)	P	*Progressors* (n = *149*)	*Nonprogressors* (n = *356*)	P
Age (years)	40 ± 8	34 ± 8	<0.0001	45 ± 7	39 ± 8	<0.0001
Male (%)	58.3	37	<0.0001	70.5	41.5	<0.0001
Smoking current (%)	14	8.4	0.07	9	8	0.69
Smoking ever (%)	26.3	16	0.01	24	22.5	0.27
Blood pressure (mm Hg)						
Systolic	120 ± 13	112 ± 12	0.03	120 ± 12	111 ± 11	<0.0001
Diastolic	78 ± 9	76 ± 8	0.02	83 ± 9	77 ± 8	<0.0001
BMI (kg/m^2)	27 ± 4	25 ± 4	0.002	28 ± 5	25 ± 4	<0.0001
HRV	17 (10–33)	25 (17–40)	<0.0001	24 (14–35)	33 (21–53)	<0.0001
Resting HR	68 ± 11	64 ± 10	0.0004	62 ± 10	59 ± 8	0.006
CVS at baseline	4.8 ± 7.77	0.35 ± 1.48	<0.0001	2.9 ± 5.0	0.2 ± 0.7	<0.0001
HbA1c (%)	8.0 ± 1.2	7.7 ± 1.2	0.05	5.6 ± 0.4	5.4 ± 0.4	<0.0001
Total cholesterol (mg/dL)	176 ± 33	171 ± 33	0.11	197 ± 35	191 ± 37	0.10
HDL (mg/dL)	54 ± 15	58 ± 16	0.01	45 ± 13	53 ± 14	<0.0001
LDL (mg/dL)	102 ± 28	96 ± 28	0.01	120 ± 33	114 ± 32	0.04
Triglycerides (mg/dL)	85 (63–111)	76 (56–98)	0.007	131 (89–185)	103 (73–136)	<0.0001
Fibrinogen (mg/dL)	259 (225–300)	249 (214–276)	0.05	262 (219–298)	252 (218–289)	0.06
Homocysteine (μmol/L)	8.3 (6.6–10)	7.0 (5.9–8.4)	<0.0001	8.8 (7.4–10.4)	8.0 (6.7–9.3)	<0.0001
hsCRP (μg/mL)	1.4 (0.9–2.0)	1.4 (0.8–2.1)	0.87	1.4 (0.9–2.0)	1.3 (0.8–1.8)	0.14
AER (μg/min)	15.4 (5–32)	6.0 (3.6–7.9)	<0.0001	5.0 (3.2–6.4)	1.3 (3.0–5.5)	0.006
ACE inhibitors use (%)	44	18.5	<0.0001	3.3	2.5	<0.0001
ARBs use (%)	4.5	2	0.16	2	—	0.007
Statin use (%)	24	8.5	<0.0001	12	3.5	0.0002

Data are mean ± SD values, percentages, or geometric means (interquartile range).

ACE, angiotensin-converting enzyme; AER, albumin excretion rate; ARB, angiotensin receptor; BMI, body mass index; CVS, calcium volume score; DM, diabetes mellitus; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; HR, heart rate; HRV, heart rate variability; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; T1D, type 1 diabetes.

Table 2. Determinants of Heart Rate Variability in Stepwise Linear Multiple Regression

	T1D		*Non-DM controls*	
Predictor	*Estimate*	P	*Estimate*	P
Age (per 10 years)	−0.31	<0.0001	−0.27	<0.0001
HbA1c (%)	−0.13	<0.0001	—	—
Log fibrinogen (mg/dL)	−0.36	0.02	—	—
AER (μg/min)	−0.13	<0.0001	—	—
BMI (kg/m^2)	—	—	−0.01	0.008
Homocysteine (μmol/L)	—	—	−0.23	0.03

All variables simultaneously in the model. In type 1 diabetes (T1D), gender, systolic and diastolic blood pressure, high-sensitivity C-reactive peptide, triglycerides, body mass index (BMI), and homocysteine were excluded from the model. In the non-diabetes mellitus (DM) controls, all variables were excluded except age, homocysteine, and BMI.

AER, albumin excretion rate; HbA1c, hemoglobin A1c.

levels, higher BMI, lower HRV, higher resting heart rate, higher CAC volume at baseline, higher HbA1c, adverse lipid profile, higher homocysteine, and higher AER and used more medications (ACE inhibitors, ARBs, and statins).

Of note is that HRV was significantly lower in T1D compared to non-DM subjects (geometric mean and interquartile range, 21.3 [13.9–38.2] vs. 31.5 [19.8–49.4], $P < 0.0001$), and patients with T1D had more progression of CAC than non-DM subjects (42.7% [$n = 175$] vs. 29.5% [$n = 149$], $P < 0.0001$). There was no difference in HRV between men and women with T1D (22.4 [12.7–40.8] vs. 22 [14.4–36], $P = 0.76$), while among non-DM controls, men had lower HRV than women (29.3 [19.2–44.4] vs. 33.4 [21–52.7], $P = 0.02$).

HRV correlated inversely with age ($r = -0.37$, $P < 0.0001$), diabetes' duration ($r = -0.28$, $P < 0.0001$), HbA1c ($r = -0.17$, $P < 0.0001$), log AER ($r = -0.28$, $P < 0.0001$), BMI ($r = -0.09$, $P = 0.001$), SBP ($r = -0.14$, $P < 0.0001$), DBP ($r = -0.09$, $P = 0.001$), log triglycerides ($r = -0.15$, $P < 0.0001$), log homocysteine ($r = -0.18$, $P < 0.0001$), log fibrinogen ($r = -0.16$, $P < 0.0001$), log hsCRP ($r = -0.10$, $P = 0.001$), and square root transformed baseline calcium volume score ($r = -0.22$, $P < 0.0001$). Total, HDL, and LDL cholesterol did not correlate with HRV.

The results of stepwise multiple linear regression with log HRV as dependent variable are displayed in Table 2, separately for diabetes status. Variables were selected from the correlation analysis. In T1D subjects, reduced HRV was associated with older age, higher HbA1c, elevated fibrinogen, and increased AER, but not with gender, SBP, DBP, BMI, hsCRP, log homocysteine, and log triglycerides. In non-DM controls, only age, BMI, and homocysteine were associated with HRV.

To evaluate the prospective relationship between HRV at baseline and CAC progression we first fitted a base model (model 1) through a stepwise multiple logistic regression, including as independent variables age (per 10 years), sex, presence of T1D, SBP, DBP, presence of CAC at baseline, and HRV. Higher HRV at baseline was predictive of a lower risk of CAC progression (Table 3) (standardized odds ratio [OR] = 0.71, 95% confidence interval [CI] 0.56–0.90, $P = 0.005$). There was no interaction between HRV and diabetes status. Further variables were sequentially added to model 1 to evaluate their potential confounding effect on the relationship between HRV and CAC presence. The negative association between HRV and presence of CAC was independent of lipid profile and statin status (model 2), of adjustment for BMI (model 3), HbA1c (model 4), AER and use of ACE inhibitors and ARBs (model 5), and inflammatory markers as model 6. These models are displayed in Table 4.

Next, we performed a stepwise multiple logistic regression to evaluate HRV and resting heart rate as predictors of CAC progression and age, sex, presence of T1D, blood pressure levels, HDL, LDL, AER, and presence of CAC at baseline and added to this model HbA1c, BMI, statin use, and ACE inhibitors/ARBs use as independent variables. Higher HRV at baseline was still a predictor of a lower risk of CAC progression (standardized OR = 0.73, 95% CI 0.57–0.95, $P = 0.02$), and DBP, LDL cholesterol, ACE use, BMI, HbA1c,

Table 3. Prospective Multivariate Logistic Analysis with Stepwise Selection of the Relationship Between Heart Rate Variability and Progression of Coronary Artery Calcium

	Statistics for coronary artery calcification progression		
	OR	*95% CI*	P
Presence of CAC at baseline	1.44	1.30–1.61	<0.0001
Age (per 10 years)	1.54	1.24–1.92	<0.0001
Presence of type 1 diabetes	1.76	1.22–2.55	0.002
Male vs. female	2.29	1.63–3.21	0.007
Systolic blood pressure	1.03	1.02–1.05	<0.0001
HRV	0.71	0.56–0.90	0.005

Diastolic blood pressure was not selected in this model.

CAC, coronary artery calcium; CI, confidence interval; HRV, heart rate variability; OR, odds ratio.

Table 4. Relationship Between Heart Rate Variability and Coronary Artery Calcium Progression in Sequential Models

	Statistics for coronary artery calcification progression by HRV		
	OR	*95% CI*	P
Model 1 + HDL, LDL, and statin use	0.73	0.57–0.93	0.01
Model 1 + BMI	0.73	0.58–0.95	0.01
Model 1 + HbA1c	0.74	0.58–0.95	0.01
Model 1 + AER and ACE inhibitors/ARBs use	0.74	0.57–0.96	0.03
Model 1 + fibrinogen, homocysteine, and hsCRP	0.77	0.60–0.98	0.03

Model 1 includes age, sex, type 1 diabetes, systolic blood pressure, calcium volume score at baseline, and heart rate variability (HRV).
ACE, angiotensin-converting enzyme; AER, albumin excretion rate; ARB, angiotensin receptor; BMI, body mass index; CI, confidence interval; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; OD, odds ratio.

and resting heart rate were not selected for entry into the model (Table 5).

Discussion

We report for the first time that reduced HRV prospectively predicts progression of CAC, a powerful marker of cardiovascular disease risk, in adults both with and without T1D and independently of known cardiovascular disease risk factors.

T1D patients had lower HRV than non-DM subjects, reflecting cardiac autonomic neuropathy. Previously, reduced HRV has been associated with older age, HbA1c, increased albuminuria, ischemic heart disease,[6] low-grade inflammation,[20] and insulin resistance markers.[13] In fact, we found associations between reduced HRV and older age, higher HbA1c, elevated albuminuria, higher fibrinogen, and increased CAC volume at baseline in T1D subjects.

In the Pittsburgh Epidemiology of Diabetes Complications Study, women with T1D had higher risk of cardiac autonomic neuropathy than men.[21] Differences by sex were not found in the current study in T1D subjects. The EURODIAB IDDM Complications Study also found no higher risk of cardiac autonomic neuropathy in women.[4] Thus, reduced HRV is not likely to be the basis of the more adverse effect of diabetes on coronary artery disease in women than in men.

Table 5. Prospective Multivariate Logistic Analysis with Stepwise Selection of the Relationship Between HRV and Progression of Coronary Artery Calcium

	Relative risk for coronary artery calcification progression		
	OR	*95% CI*	P
Presence of CAC at baseline	5.27	3.62–7.68	<0.0001
Age (per 10 years)	2.00	1.56–2.56	<0.0001
Presence of type 1 diabetes	1.89	1.23–2.90	0.003
Male vs. female	1.51	1.02–2.23	0.04
Systolic blood pressure	1.03	1.01–1.04	0.0001
HDL cholesterol	0.98	0.96–0.99	0.002
Log AER	1.33	1.12–1.58	0.0009
Statin use	2.10	1.17–3.78	0.01
HRV	0.73	0.57–0.95	0.02

Diastolic blood pressure, low-density lipoprotein, angiotensin-converting enzyme use, body mass index, hemoglobin A1c, and resting heart rate were excluded this model.
AER, albumin excretion rate; CAC, coronary artery calcium; CI, confidence interval; HDL, high-density lipoprotein; HRV, heart rate variability; OR, odds ratio.

In addition, the association between HRV and fibrinogen is of interest, as fibrinogen is frequently associated with nephropathy in T1D subjects, and hyperfibrinogenemia may be also an indicator of inflammatory vascular changes and endothelial dysfunction.[22] We also observed association between HRV and AER in T1D subjects; however, we found no interaction between albuminuria and fibrinogen (data not shown). This finding, in this case, could reveal the independent association of each of these factors with HRV.

Although the association between cardiac autonomic neuropathy and increased cardiovascular mortality in patients with diabetes is known, silent coronary artery disease in diabetes appears to be more related to accelerated atherosclerosis than to cardiac autonomic neuropathy.[2] However, sympathetic denervation may cause dedifferentiation of vascular smooth muscle cells.[23] These alterations are associated with extracellular matrix production and migration to the intima, changes that have been seen in atherosclerosis.[24] Therefore, the possibility that autonomic dysfunction is implicated in the atherosclerosis process is plausible.

Colhoun et al.[13] observed an association between CAC and lower HRV, independent of age, HbA1c, and triglycerides but not independent of SBP or BMI in T1D subjects. In contrast to these previous findings, in our study lower HRV was associated with the presence of CAC, independently of confounders tested, including blood pressure and BMI. We studied young patients with asymptomatic atherosclerosis; consequently, our data, in addition to those of Colhoun et al.,[13] suggest that reduced HRV could be associated with early atherosclerosis rather than being an effect of the ischemia that occurs in established coronary artery disease.

Inflammation has been associated with cardiac autonomic neuropathy in T1D subjects asymptomatic for coronary artery disease[20,25] as well in non-DM subjects without[6,26,27] and with[28,29] coronary artery disease. However, these studies were cross-sectional, making the exact understanding between cardiac autonomic dysfunction and inflammation and its possible cause–effect pathway difficult. In our prospective analysis, lower HRV was a predictor of CAC progression independent of inflammatory markers. Experimental findings suggest that the nervous autonomic system could significantly modulate inflammatory reaction.[30,31] Lanza et al.[25] in a small subset of T1D subjects showed that improved HRV

(through β-blocker use) was associated with a parallel reduction of hsCRP. Cardiac autonomic neuropathy leading to inflammation could represent one pathway through which traditional risk factors promote or trigger the atherosclerosis process, as evaluated here by CAC progression. Further prospective studies are needed to confirm this cause–effect pathway.

Of note is that a stronger association was observed between CAC progression and HRV than between CAC progression and resting heart rate.

Possible limitations of this study include the use of only one ECG and the absence of other autonomic function tests to support the presence of cardiac autonomic neuropathy. However, we believe that these facts do not invalidate our results. ECG has been used as an accepted method of evaluation of cardiac autonomic neuropathy in many studies[13,14,25] and has been used as the only method of evaluation of cardiac autonomic neuropathy in others.[32] Moreover, recording for many hours is costly and logistically complex and therefore would be difficult to carry out in a large epidemiologic study or in a clinical setting. In this study, data were collected according a standard protocol, and quality control measurements were obtained on 5% of all recorded ECGs, minimizing the potential for error and misclassification.

In summary, the results of this study show that reduced HRV, identified on a standard ECG with deep breathing, was associated with older age, presence of T1D, hyperglycemia, increased albuminuria, and higher levels of fibrinogen. Cardiac autonomic neuropathy, assessed by reduced HRV, was associated with progression of CAC in both patients with T1D and non-DM adults. This association further supports the participation of autonomic neuropathy in the atherosclerosis process with inflammatory involvement in adults, regardless of diabetes status.

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Author Disclosure Statement

No competing financial interests exist.

References

1. Retnakaran R, Zinman B: Type 1 diabetes, hyperglycaemia, and the heart. Lancet 2008;371:1790–1799.
2. Airaksinem KEJ: Silent coronary artery disease in diabetes—a feature of autonomic neuropathy or accelerated atherosclerosis? Diabetologia 2001;44:259–266.
3. Witte DR, Tesfaye S, Chaturvedi N, Eaton SE, Kempler P, Fuller JH; EURODIAB Prospective Complications Study Group: Risk factors for cardiac autonomic neuropathy in type 1 diabetes mellitus. Diabetologia 2005;48:164–171.
4. Kempler P, Tesfaye S, Chaturvedi N, Stevens LK, Webb DJ, Eaton S, Kerényi Z, Tamás G, Ward JD, Fuller JH; EURODIAB IDDM Complications Study Group: Autonomic neuropathy is associated with increased cardiovascular risk factors: the EURODIAB IDDM Complications Study. Diabet Med 2002;19:900–909.
5. Proceedings of a consensus development conference on standardized measures in diabetic neuropathy. Summary and recommendations. Diabetes Care 1992;15:1104–1107.
6. Sajadieh A, Nielsen OW, Rasmussen V, Hein HO, Abedini S, Hansen JF: Increased heart rate and reduced heart-rate variability are associated with subclinical inflammation in middle-aged and elderly subjects with no apparent disease. Eur Heart J 2004;25:363–370.
7. Schroeder EB, Liao D, Chambless LE, Prineas RJ, Evans GW, Heiss G: Hypertension, blood pressure, and heart rate variability: the Atherosclerosis Risk in Communities (ARIC) Study. Hypertension 2003;42:1106–1111.
8. Tsuji H, Larson MG, Venditti FJ Jr, Manders ES, Evans JC, Feldman CL, Levy D: Impact of reduced heart rate variability on risk for cardiac events: the Framingham Heart Study. Circulation 1996;94:2850–2855.
9. Gottsäter A, Rydén-Ahlgren A, Szelag B, Hedblad B, Persson J, Berglund G, Wroblewski M, Sundkvist G: Cardiovascular autonomic neuropathy associated with carotid atherosclerosis in type 2 diabetic patients. Diabet Med 2003;20:495–499.
10. Gottsäter A, Ahlgren AR, Taimour S, Sundkvist G: Decreased heart rate variability may predict the progression of carotid atherosclerosis in type 2 diabetes. Clin Auton Res 2006;16:228–234.
11. Raggi P, Callister TQ, Cooil B, He ZX, Lippolis NJ, Russo DJ, Zelinger A, Mahmarian JJ: Identification of patients at increased risk of unheralded acute myocardial infarction by electron-beam computed tomography. Circulation 2000;101:850–855.
12. Dabelea D, Kinney G, Snell-Bergeon JK, Hokanson JE, Eckel RH, Ehrlich J, Garg S, Hamman RF, Rewers M; The Coronary Artery Calcification in Type 1 Diabetes Study; Effect of type 1 diabetes on the gender difference in coronary artery calcification: a role for insulin resistance? The Coronary Artery Calcification in Type 1 Diabetes (CACTI) Study. Diabetes 2003;52:2833–2839.
13. Colhoun HM, Francis DP, Rubens MB, Underwood SR, Fuller JH: The association of heart-rate variability with cardiovascular risk factors and coronary artery calcification: a study in type 1 diabetic patients and the general population. Diabetes Care 2001;24:1108–1114.
14. Thilo C, Standl E, Knez A, Reiser M, Steinbeck G, Haberl R, Schnell O: Coronary calcification in long-term Type 1 diabetic patients—a study with multi slice spiral computed tomography. Exp Clin Endocrinol Diabetes 2004;112:561–565.
15. Maahs DM, Ogden LG, Kretowski A, Snell-Bergeon JK, Kinney GL, Berl T, Rewers M: Serum cystatin C predicts progression of subclinical coronary atherosclerosis in individuals with Type 1 diabetes. Diabetes 2007;56:2774–2779.
16. Agatston AS, Janowitz WR, Hildner FJ, Zusmer NR, Viamonte M Jr, Detrano R: Quantification of coronary artery calcium using ultrafast computed tomography. J Am Coll Cardiol 1990;15:827–832.

17. Rautaharju PM, Park LP, Chaitman BR, Rautaharju F, Zhang ZM: The Novacode criteria for classification of ECG abnormalities and their clinically significant progression and regression. J Electrocardiol 1998;31:157–187.
18. Pitzalis MV, Mastropasqua F, Massari F, Forteo C, Di Maggio M, Passantino A, Colombo R, Di Biase M, Rizzon P: Shortand long-term reproducibility of time and frequency domain heart rate variability measurements in normal subjects. Cardiovasc Res 1996;32:226–233.
19. Hokanson JE, MacKenzie T, Kinney G, Snell-Bergeon JK, Dabelea D, Ehrlich J, Eckel RH, Rewers M: Evaluating changes in coronary artery calcium: an analytic method that accounts for interscan variability. AJR Am J Roentgenol 2004;182:1327–1332.
20. González-Clemente JM, Vilardell C, Broch M, Megia A, Caixàs A, Giménez-Palop O, Richart C, Simón I, Martínez-Riquelme A, Arroyo J, Mauricio D, Vendrell J: Lower heart rate variability is associated with higher plasma concentrations of IL-6 in type 1 diabetes. Eur J Endocrinol 2007;157:31–38.
21. Maser RE, Pfeifer MA, Dorman JS, Kuller LH, Becker DJ, Orchard TJ: Diabetic autonomic neuropathy and cardiovascular risk. Arch Intern Med 1990;150:1218–1222.
22. Mendall MA, Patel P, Ballam L, Strachan D, Northfield TC: C reactive protein and its relation to cardiovascular risk factors: a population based cross sectional study. BMJ 1996;312:1061–1065.
23. Kacem K, Bonvento G, Seylaz J: Effect of sympathectomy on the phenotype of smooth muscle cells of middle cerebral and ear arteries of hyperlipidaemic rabbits. Histochem J 1997; 29:279–286.
24. Sarmento A, Soares-da-Silva P, Teixeira AA, Azevedo I: Effects of denervation induced by 6-hydroxydopamine on cell nucleus activity of arterial and cardiac cells of the dog. J Auton Pharmacol 1987;17:119–126.
25. Lanza GA, Pitocco D, Navarese EP, Sestito A, Sgueglia GA, Manto A, Infusino F, Musella T, Ghirlanda G, Crea F: Association between cardiac autonomic dysfunction and inflammation in type 1 diabetic patients: effect of beta-blockade. Eur Heart J 2007;28:814–820.
26. Jensen-Urstad M, Jensen-Urstad K, Ericson M, Johansson J: Heart rate variability is related to leucocyte count in men and to blood lipoproteins in women in a healthy population of 35-year-old subjects. J Intern Med 1998;243:33–40.
27. Lampert R, Bremner JD, Su S, Miller A, Lee F, Cheema F, Goldberg J, Vaccarino V: Decreased heart rate variability is associated with higher levels of inflammation in middle-aged men. Am Heart J 2008;156:759.e1–e7.
28. Janszky I, Ericson M, Lekander M, Blom M, Buhlin K, Georgiades A, Ahnve S: Inflammatory markers and heart rate variability in women with coronary heart disease. J Intern Med 2004;256:421–428.
29. Lanza GA, Sgueglia GA, Cianflone D, Rebuzzi AG, Angeloni G, Sestito A, Infusino F, Crea F, Maseri A; SPAI (Stratificazione Prognostica dell'Angina Instabile) Investigators: Relation of heart rate variability to serum levels of C-reactive protein in patients with unstable angina pectoris. Am J Cardiol 2006;97:1702–1706.
30. Borovikova LV, Ivanova S, Zhang M, Yang H, Botchkina GI, Watkins LR, Wang H, Abumrad N, Eaton JW, Tracey KJ: Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature 2000;405:458–462.
31. Wang H, Yu M, Ochani M, Amella CA, Tanovic M, Susarla S, Li JH, Wang H, Yang H, Ulloa L, Al-Abed Y, Czura CJ, Tracey KJ: Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. Nature 2003;421: 384–388.
32. Soedamah-Muthu SS, Chaturvedi N, Witte DR, Stevens LK, Porta M, Fuller JH; EURODIAB Prospective Complications Study Group: Relationship between risk factors and mortality in type 1 diabetic patients in Europe. The EURODIAB Prospective Complications Study (PCS). Diabetes Care 2008; 31:1360–1366.

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