Heart Rate Variability and Sensorimotor Polyneuropathy in Type 1 Diabetes

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OBJECTIVE—Reduced heart rate variability (HRV) is classically viewed as an early phenomenon in diabetic sensorimotor polyneuropathy (DSP). We aimed to determine the characteristics of HRV across the spectrum of clinical DSP in type 1 diabetes.

RESEARCH DESIGN AND METHODS—Eighty-nine diabetic subjects and 60 healthy volunteers underwent assessment of RR interval variation (RR_{var}) during deep breathing and clinical and electrophysiological examination. We examined the distribution of age-standardized RR_{var} across the spectrum of clinical DSP, identified variables associated with RR_{var} in multivariate regression, and compared RR_{var} with validated measures of neuropathy.

RESULTS—Age-standardized RR_{var} had a significant, step-wise, inverse relationship with ordinal categories of increasing DSP severity ($\beta = -5.4$, $P < 0.0001$) among subjects with diabetes. Case subjects with DSP had substantially lower age-standardized RR_{var} compared with diabetic control subjects without DSP ($\beta = -5.2$, $P < 0.01$), although there was substantial overlap of RR_{var} between diabetic case subjects and control subjects and the healthy volunteer cohort. In multivariate analysis, advanced age was independently associated with lower RR_{var} in both healthy volunteers and diabetic subjects, whereas higher glycated hemoglobin A_{1c} and systolic blood pressure were independently associated with lower RR_{var} in diabetic subjects. RR_{var} had a significant association with validated measures of large and small fiber neuropathy.

CONCLUSIONS—HRV may be a biomarker for clinical DSP and is associated cross-sectionally with both early and late measures of neuropathy. The low HRV observed in some control subjects without DSP and in most case subjects with severe DSP may signify that HRV has different prognostic implications in these groups, requiring further longitudinal study.

Diabetes Care 35:809–816, 2012

Decreased heart rate variability (HRV)—the instantaneous beat-to-beat variation in heart rate during respiration—has long been identified as an early marker of autonomic neuropathy in diabetes, often preceding any other manifestation of neuropathy (1). Since these first observations, the classic dogma has been that decreased HRV is an early, asymptomatic finding in diabetic polyneuropathy (2). In normal individuals, heart rate varies significantly throughout the respiratory cycle—increasing with inspiration and decreasing with expiration—in a phenomenon known as sinus arrhythmia (3). These physiologic changes are mediated by sympathetic and parasympathetic nerves in the autonomic nervous system. Pathophysiologically, decreased HRV is thought to reflect impaired parasympathetic innervation of the vagus nerve, the longest autonomic nerve, in a length-dependent fashion akin to injury of peripheral somatic nerves in diabetic sensorimotor polyneuropathy (DSP) (4).

HRV during deep breathing is one of several cardiovascular autonomic reflex tests (CARTs) that are part of the gold standard diagnostic criteria for cardiac autonomic neuropathy (CAN) (5), a clinical condition characterized by dysfunction of the autonomic innervation of the cardiovascular system (6). Although the isolated finding of decreased HRV is considered sufficient criterion for possible or early CAN, a second abnormal CART is required for a definite or confirmed diagnosis (5). HRV can be assessed by a variety of metrics, categorized into "time" and "frequency" domains, but HRV with deep breathing in the time domain remains the most commonly used CART and has the greatest specificity for CAN (4).

Recently, several large prospective studies have demonstrated that CAN, defined by HRV criteria, is an independent predictor of cardiovascular disease (CVD) (7,8) and mortality in diabetes (9–12). Intervention trials have shown that intensive glycemic control and a strategy aimed at lifestyle change with pharmacological correction of hyperglycemia, hypertension, dyslipidemia, and microalbuminuria decrease the incidence and progression of CAN (13–15).

To date, several studies have explored the relationship between HRV and microvascular complications of diabetes (16,17). Decreased HRV has been associated with new onset renal impairment (16) as well as cardiovascular mortality in patients with diabetic nephropathy (17). However, the nature of the relationship between HRV and DSP has not been well established (18).

In light of this question and the early diagnostic value of HRV in autonomic neuropathy, we aimed to cross-sectionally evaluate HRV among type 1 diabetic participants in the Toronto Diabetic Early Neuropathy Cohort (19) to determine whether it can serve as a biomarker for DSP based on the gold-standard definition (20). To further elucidate the temporal and pathophysiological relationship underlying decreased HRV, we compared it with validated measures of peripheral neuropathy including functional large fiber sensory and motor measures, as well as functional and morphological small fiber measures.

RESEARCH DESIGN AND METHODS

Subject selection and evaluation

Eighty-nine subjects with type 1 diabetes were accrued from the Endocrinology and Neurology Clinics at the Toronto General Hospital/University Health Network. An additional 60 healthy volunteers were recruited by community advertisement and sampling among family and friends of diabetic subjects. Volunteers were selected to ensure that the distribution of age (by

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Received 26 August 2011 and accepted 6 January 2012.
DOI: 10.2337/dc11-1652

decade of life) and sex among healthy volunteers was the same as type 1 diabetic subjects. Together, this cohort continues to be longitudinally followed in a study funded by the Juvenile Diabetes Research Foundation (operating grant no.17-2008-715) examining the concurrent and predictive validity of various measures of neuropathy. Predictive validity, the ability of a baseline test to predict the future onset of neuropathy in subjects without neuropathy at baseline, is actively being pursued in the longitudinal follow-up of this cohort.

A stratified accrual strategy according to the Toronto Clinical Neuropathy Score (TCNS) (21) was used to create a cohort of type 1 diabetic subjects that represented the spectrum of nerve injury, from lack of detectable nerve injury to severe DSP. The TCNS is a 19-point validated clinical neuropathy score in which cumulative scores of 0–5, 6–8, 9–12, and 13–19 represent absent, mild, moderate, and severe neuropathy, respectively (21). Subjects were selected to ensure representation from each of the four TCNS stages at the time of study accrual. All subjects had a diagnosis of type 1 diabetes, were at least 18 years of age, provided informed consent, and did not have neuropathy attributable to causes other than diabetes on the basis of detailed medical history, family history, history of toxin exposure, renal failure, or presence of abnormal serum or urine protein electrophoresis. A comprehensive medical and neurologic evaluation of each participant involved assessment of neuropathy-related symptoms, signs (blood pressure and heart rate), lifestyle factors and comorbidities (including smoking), and biochemical tests (glycated hemoglobin A_{1c} [HbA_{1c}], serum lipids, and urinary albumin excretion).

The current report evaluates the baseline cross-sectional data from examinations conducted between November 2008 and May 2010. Approval of the protocol and consent procedures was obtained from the Research Ethics Board of the University Health Network.

Assessment of HRV

All participants underwent assessment of HRV using the Dantec Keypoint Workstation (Natus Medical, San Carlos, CA). Participants avoided caffeinated beverages, smoking, alcohol, and large meals for at least 2 h before the assessment. The absence of hypoglycemia or marked hyperglycemia was confirmed with capillary blood glucose measurements. A minimum of 30 min in the supine position preceded all electrocardiogram recordings. Two surface electrodes were placed on the chest to obtain an electrocardiogram tracing. A baseline 1-min recording was obtained during normal breathing in a quiet room. The subsequent recording was obtained over 1 min during deep breathing at a frequency of 6 breaths per min (5 s in and 5 s out), with time cues provided by the technician. The built-in software algorithm (22) generated a plot of RR intervals versus time. Positive and negative peaks (maximum and minimum RR intervals) during each respiratory cycle were identified using the three-point search algorithm (22). Peaks with amplitudes greater than 75% above or below the median RR interval were excluded to minimize the effect of ectopic beats and irregular breathing. HRV was expressed as the RR interval variation (RR_{var}) , the difference between the shortest and the longest RR intervals during 1 min given as a percentage of the mean of all peaks [$(RR_{max} - RR_{min})/RR_{mean} \times 100\%$]. This mathematical algorithm for RR_{var} was selected among several other formulas because of its small scatter, small number of outliers, and superior correlation with existing nerve conduction parameters during repeated testing in healthy volunteers and those with diabetes (22). The interobserver variability on repeat testing of five subjects was 30%, corresponding to an estimated coefficient of variation of 12% in RR_{var} with deep breathing units (22).

Classification of DSP case and control subjects

The case definition for DSP was consistent with the clinical and electrophysiological criteria set forth by the American Association of Neurology, the American Academy of Electrodiagnostic Medicine, and the American Academy of Physical Medicine and Rehabilitation (20). Clinical criteria required the presence of more than one symptom (numbness, tingling, weakness, foot pain, or ataxia) or sign (abnormal knee or ankle reflexes, temperature, light touch, monofilament, or vibration sensation), in keeping with a distal symmetrical neuropathic pattern of onset and progression. Electrophysiological abnormality was defined by at least one abnormal nerve conduction study (NCS) parameter in both dominant-side sural and peroneal nerve distributions assessed using the Counterpoint instrument (Natus Medical) with age- and height-adjusted thresholds for abnormality (23). Two sural nerve parameters (action potential amplitude and conduction velocity) and three peroneal nerve parameters (distal compound muscle action potential amplitude, F-wave latency from ankle stimulation, conduction velocity from fibular head stimulation) were tested. Type 1 diabetic control subjects without DSP were divided into two groups based on the presence or absence of sural NCS abnormalities. Mild, moderate, and severe DSP were defined by the first, second, and third tertile of TCNS score, respectively.

Assessment of small fiber morphology and function

As described previously (19), corneal nerve fiber length (CNFL) was assessed using corneal confocal microscopy (CCM) with the Rostock Cornea Module of the Heidelberg Tomograph II (Heidelberg Engineering, Smithfield, RI) (24). In brief, the mean CNFL from both eyes was calculated from high contrast images of the subbasal nerve plexus in Bowman's layer of the cornea using analytical software (CCM Image Analysis tool v0.6 provided by Drs. R. Malik and M. Dabbah; University of Manchester) and expressed in millimeters per squared millimeters of Bowman's layer.

Cooling detection threshold was obtained using the Medoc TSA-II NeuroSensory Analyzer (Ramat-Yishai, Israel) and the method of limits, as described previously (25). In brief, a stimulator was applied to the dorsum of the right foot at a temperature of 32°C and the temperature was gradually decreased to the first level detected by the patient as being cooler than the preceding stimulus. Five test trials were performed and averaged to establish a mean cooling detection threshold in °C.

As described previously (26), axon-reflex mediated neurogenic vasodilatation in response to cutaneous heating by the laser Doppler imaging flare technique (LDI_{FLARE}) was measured using equipment from Moor Instruments (Axminster, U.K.). In brief, a skin-heating probe was applied to the dorsum of the right foot and heated to the skin to 44°C for 20 min. Laser Doppler imaging using the moorLDI2 (Moor Instruments) (26) was used to register movement of erythrocytes in dermal capillaries over a 6 cm × 6 cm area. The LDI_{FLARE} area was calculated in squared centimeter using Moor LDI software (version 3.11).

Statistical analysis

Analysis was performed using SAS version 9.0 for Windows. Differences in categorical variables between healthy volunteers and type 1 diabetic subjects with and without DSP were assessed using the χ^2 test, whereas continuous variables were assessed using ANOVA. Results were expressed as

mean ± SD. The interquartile range (25th, 75th percentile), 90% distribution (5th, 95th percentile), and 95% distribution (2.5th, 97.5th percentile) were identified for RR_{var} and age-standardized RR_{var} values during normal and deep breathing. Age-standardized RR_{var} was calculated from a linear regression model for the existing dataset that included RR_{var} as the dependent variable and age and neuropathy subgroups as independent variables. Dependent predictor variables for the univariate and multivariate linear regression models with RR_{var} were assessed by standard regression diagnostics. We estimated 99.3% power to detect a significant difference in the diabetes subgroup alone in our cohort of 60 healthy volunteers, 62 diabetic control subjects without DSP, and 21 diabetic subjects with DSP. This estimate was based on $a = 0.05$, mean RR_{var} (40%), and standard deviation (10%) derived from the report of a comparably aged cohort using the same HRV measurement method (22), and assuming an anticipated 20% difference between diabetic subjects with and without DSP.

RESULTS—The clinical characteristics of the 149 study participants are summarized in Table 1. The cohort consisted of 60 healthy volunteers and 89 subjects with type 1 diabetes (62 control subjects without DSP and 27 case subjects with DSP). Individuals with type 1 diabetes and DSP were significantly older and had longer diabetes duration and higher systolic blood pressures. Subjects with type 1 diabetes had higher BMI, systolic blood pressure, and HbA_{1c} but lower LDL cholesterol and higher HDL cholesterol levels, compared with healthy volunteers. The TCNS, a clinical indicator of nerve injury severity, was higher among type 1 diabetic subjects than healthy volunteers. Generally, NCS parameters were most abnormal among type 1 diabetic case subjects with DSP compared with control subjects without DSP and healthy volunteers. Both RR_{var} during normal and deep breathing were generally lower in those with diabetes compared with healthy volunteers, but diabetic control subjects without DSP had the highest mean values. This was accounted for by the younger age distribution in this subgroup. To adjust for this effect we present age-standardized values. The age-standardized RR_{var} was significantly lower in type 1 diabetic case subjects with DSP, compared with both type 1 diabetic control subjects without DSP and healthy volunteers during both normal ($P < 0.0001$) and deep ($P < 0.0001$) breathing. The difference in age-standardized RR_{var} between healthy volunteers and type 1 diabetic control subjects without DSP was significant during normal (t-statistic = 2.00, $P < 0.05$), but not during deep (t-statistic = 1.43, $P = 0.16$), breathing.

To explore the relationship of age-standardized RR_{var} during deep breathing among healthy volunteers and subjects with type 1 diabetes, we examined its distribution across a broad spectrum of nerve injury (Fig. 1). Subjects with type 1 diabetes had a significantly lower age-standardized RR_{var} during deep breathing than healthy volunteers (t-statistic = 4.85, $P < 0.0001$). As shown in Fig. 1, second to sixth

Table 1—*Clinical characteristics of the 60 healthy volunteers and the 89 type 1 diabetic participants according to DSP status*

Clinical characteristic	Healthy volunteers (n = 60)	Type 1 diabetes (n = 89)		ANOVA P value for trend*
		Control subjects without DSP (n = 62)	Case subjects with DSP (n = 27)	
Female sex, n (%)	28 (47)	33 (53)	17 (63)	0.36
Age (years)	37.9 ± 16.9	33.5 ± 13.7	48.2 ± 13.3	0.0002
Diabetes duration (years)	—	18.1 ± 13.3	32.0 ± 12.5	<0.0001
Current/recent smoking, n (%)	13 (22)	9 (15)	5 (19)	0.59
Height (m)	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	0.43
Weight (kg)	73.1 ± 17.3	76.1 ± 14.5	83.7 ± 23.2	0.08
BMI (kg/m^2)	24.9 ± 5.1	25.3 ± 4.2	28.4 ± 5.9	0.03
Blood pressure (mmHg)				
Systolic	124 ± 14	123 ± 14	136 ± 16	0.0003
Diastolic	75 ± 10	70 ± 8	75 ± 10	0.01
Heart rate at rest (bpm)	68 ± 11	68 ± 13	72 ± 13	0.34
HbA_{1c} (%)	5.6 ± 0.4	7.5 ± 1.2	8.4 ± 1.6	<0.0001
Cholesterol (mmol/L)				
Total	4.6 ± 1.0	4.5 ± 0.8	4.4 ± 1.0	0.65
LDL	2.7 ± 0.8	2.4 ± 0.7	2.3 ± 0.7	0.03
HDL	1.4 ± 0.4	1.7 ± 0.4	1.7 ± 0.5	0.02
Triglycerides (mmol/L)	1.0 ± 0.5	0.9 ± 0.6	1.1 ± 0.8	0.28
TCNS, median [IQR]‡	0 [0, 2]	2 [1, 5]	8 [5, 12]	<0.0001
Sural nerve amplitude potential (μV)	18 ± 9	11 ± 5	3 ± 2	<0.0001
Sural nerve conduction velocity (m/s)	51 ± 5	46 ± 4	40 ± 3	<0.0001
Peroneal nerve amplitude potential (mV)	6 ± 2	6 ± 2	2 ± 1	<0.0001
Peroneal nerve conduction velocity (m/s)	48 ± 3	43 ± 3	37 ± 5	<0.0001
RR_{var} with normal breathing (%)				
Mean ± SD	23 ± 16	27 ± 16	12 ± 11	0.0003
Age-standardized mean ± SD	25 ± 5	23 ± 4	16 ± 4	<0.0001
IQR	12, 29	15, 36	6, 14	
90% Distribution	15, 69	6, 60	3, 36	
95% Distribution	13, 72	6, 66	2, 43	
RR_{var} with deep breathing (%)				
Mean ± SD	37 ± 20	44 ± 23	21 ± 16	<0.0001
Age-standardized mean ± SD	40 ± 8	38 ± 6	27 ± 6	<0.0001
IQR	20, 48	26, 52	10, 26	
90% Distribution	15, 79	12, 100	4, 45	
95% Distribution	13, 96	11, 113	3, 74	

Data are means ± SD unless otherwise indicated. [IQR] represents the interquartile range. bpm, beats per minute. *P values for categorical variables were calculated with the χ^2 test, and ANOVA was used for continuous variables. ‡Scores of 0–5 are considered to represent low likelihood of DSP, 6–8 represent likelihood of mild neuropathy, 9–12 represent likelihood of moderate neuropathy, and 13–19 represent likelihood of severe neuropathy.

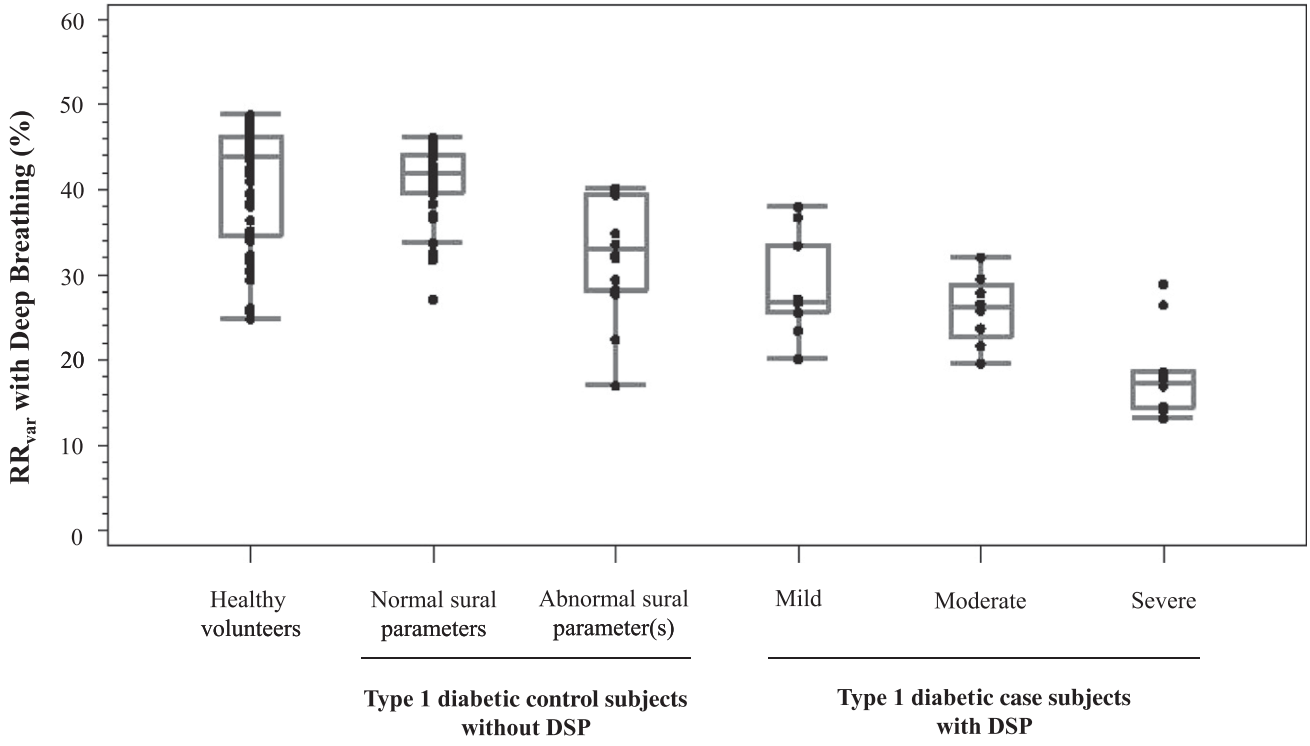

Figure 1—*Box-and-whisker plots demonstrating the distribution of age-standardized RR_{var} with deep breathing in 60 healthy volunteers and 89 type 1 diabetic subjects according to neuropathy status. Type 1 diabetic control subjects without DSP were divided into two groups based on the presence or absence of sural NCS abnormalities. Type 1 diabetic case subjects with DSP were divided into mild, moderate, and severe based on TCNS tertile.*

box-and-whisker plots, among subjects with diabetes, age-standardized RR_{var} with deep breathing had a significant, step-wise, inverse relationship with ordinal categories of increasing DSP severity, from type 1 diabetic control subjects without and with evidence of subclinical sural nerve injury to DSP case subjects divided into mild, moderate, and severe according to TCNS tertile (linear regression $\beta = -5.4$, $P < 0.0001$). In comparison with healthy volunteers, type 1 diabetic control subjects without DSP and without subclinical sural nerve injury had no significant difference in age-standardized RR_{var} with deep breathing (linear regression $\beta = 0.09$, $P = 0.08$) (Fig. 1, first and second box-and-whisker plots). Age-standardized RR_{var} with deep breathing was significantly lower among type 1 diabetic control subjects with subclinical sural nerve injury compared with control subjects without subclinical sural nerve injury (linear regression $\beta = -8.3$, $P < 0.0001$) (Fig. 1, second and third box-and-whisker plots). Case subjects with DSP had substantially lower age-standardized RR_{var} with deep breathing compared with diabetic control subjects without DSP (linear regression $\beta = -5.15$, $P < 0.01$). Among case subjects with DSP, age-standardized RR_{var} with deep breathing had an inverse relationship with increasing neuropathy severity (linear regression $\beta = -6.8$, $P = 0.05$) (Fig. 1, fourth to sixth box-and-whisker plots).

We performed a univariate and multivariate regression analysis of RR_{var} with deep breathing using the baseline variables from Table 1 and present it in Table 2. Among subjects with type 1 diabetes, RR_{var} with deep breathing was lower in univariate linear regression analysis with advanced age, higher systolic and diastolic blood pressures, higher TCNS score, and more abnormal values of large fiber NCS measures. The same association with age, TCNS score, and peroneal nerve conduction velocity was seen among healthy volunteers. In multivariate analysis, the only variable independently associated with lower RR_{var} with deep breathing in both healthy volunteers and type 1 diabetic subjects was advanced age. However, higher systolic blood pressure and higher HbA_{1c} were additionally independently associated with lower RR_{var} in type 1 diabetic subjects.

With respect to measures of small fiber neuropathy in the subjects with type 1 diabetes, univariate regression analysis demonstrated that lower RR_{var} with deep breathing was associated with shorter CNFL and lower cooling detection threshold, but no association with LDI_{FLARE} area was observed. These associations are shown graphically by way of scatterplots to compare RR_{var} with validated measures of large and small fiber neuropathy in subjects with type 1 diabetes (Fig. 2). Lower RR_{var} with deep breathing was associated with slower peroneal and sural nerve conduction velocities (linear regression $\beta = 2.22$, $P < 0.0001$; $\beta = 1.69$, $P = 0.0008$) (Fig. 2, scatterplots A and B). Lower RR_{var} with deep breathing was also associated with shorter CNFL (linear regression $\beta = 1.84$, $P = 0.0002$) and lower cooling detection threshold (linear regression $\beta = 1.51$, $P < 0.0001$), but not smaller LDI_{FLARE} area (linear regression $\beta = 3.24$, $P = 0.13$) (Fig. 2, scatterplots C–E). All associations between RR_{var} with deep breathing and measures of small and large fiber neuropathy were not independent of the relationship of age with each type of neuropathy measure (data not shown).

CONCLUSIONS—In evaluating a cohort of type 1 diabetic subjects representing the spectrum of neuropathy, we found a strong incremental association between lower HRV, as measured by RR_{var} with deep breathing, and increasing degrees of DSP severity. Despite this association with

Table 2—*Association of baseline demographics with RR_{var} with deep breathing*

Baseline clinical characteristics	Healthy volunteers (n = 60) β	*P*	Type 1 diabetes (n = 89) β	*P*
Univariate analysis				
Female sex	−1.68	0.75	−0.009	0.99
Age (years)	−0.39	0.01	−0.59	0.0002
Diabetes duration (years)	—	—	−0.50	0.16
Current/recent smoking	3.88	0.55	−0.90	0.90
Height (m)	5.60	0.86	19.19	0.50
Weight (kg)	0.11	0.51	−0.12	0.47
BMI (kg/m^2)	0.40	0.48	−0.87	0.15
Blood pressure (mmHg)				
Systolic	−0.36	0.06	−0.62	<0.0001
Diastolic	−0.23	0.38	−0.92	0.0008
Heart rate at rest (bpm)	−0.39	0.12	−0.32	0.09
HbA_{1c} (%)	−8.88	0.24	−3.80	0.06
Cholesterol (mmol/L)				
Total	−2.63	0.38	−2.60	0.48
LDL	−2.85	0.46	1.47	0.75
HDL	−2.26	0.76	−5.12	0.48
Triglycerides (mmol/L)	−4.83	0.41	−7.86	0.09
Large fiber neuropathy measures				
TCNS	−3.55	0.005	−1.87	0.0008
Sural nerve amplitude potential (μV)	0.30	0.32	1.30	0.002
Sural nerve conduction velocity (m/s)	1.08	0.06	1.69	0.0008
Peroneal nerve amplitude potential (mV)	0.96	0.41	3.09	0.0009
Peroneal nerve conduction velocity (m/s)	1.91	0.009	2.22	<0.0001
Small fiber neuropathy measures				
CNFL (mm/mm^2)	0.79	0.20	1.84	0.0002
LDI_{FLARE} area (cm^2)†	1.67	0.27	3.24	0.13
Cooling detection threshold (°C)	0.55	0.40	1.51	<0.0001
Multivariate analysis‡				
Age (years)	−0.34	0.04	−0.51	0.003
Systolic blood pressure (mmHg)	−0.25	0.22	−0.36	0.03
HbA_{1c} (%)–forced	−1.22	0.89	−4.70	0.02

Data represent linear regression slope (β) and *P* value for the relationship between RR_{var} (the dependent variable) and the independent variables listed in the first column. bpm, beats per minute. †Axon-reflex mediated neurogenic vasodilatation by the laser Doppler imaging flare method. ‡The multivariate model included the variables that were significant in univariate analysis in either the healthy volunteer or type 1 diabetes cohorts. Direct measures of neuropathy, which included the TCNS, nerve conduction studies, and small fiber measures, were not considered in the multivariate analysis.

late-stage clinical DSP, we observed a wide range of HRV, including low HRV, among our healthy volunteer cohort who had the same sex and age distribution as type 1 diabetic subjects. This observation prompted us to search for independent predictors of HRV that may preexist in healthy individuals. Our regression analysis highlights the independent association between lower HRV and advanced age in both healthy volunteers and subjects with diabetes, as well as the inverse association of HRV with blood pressure and glycemic exposure in diabetic subjects. HRV was found to have a significant positive association with validated measures of small and large fiber nerve function, suggesting that it may perform as a marker of DSP in both early and late stages of neuropathy.

Decreased HRV has classically been identified as the earliest manifestation of autonomic neuropathy (1), which may mark the first committed step toward neuropathic diabetes outcomes such as clinical DSP (2). However, it has been well-established that HRV has prognostic utility in predicting nonneuropathic diabetes outcomes such as CVD (7,8) and mortality (9–12). There exist many putative intermediaries on the causal pathway to death, such as hypertension (27) and abnormal cardiac electrophysiological parameters (28,29). However, the mechanism by which decreased HRV, or CAN, specifically leads to CVD and death remains unresolved (30). Does damage to autonomic nerves (represented by low HRV) independently lead to CVD and mortality, or is subsequent damage to somatic nerves, as would be suggested by the prevailing framework for the natural history of DSP, a necessary intermediary in this causal pathway? With the consideration of other microvascular complications of diabetes, a recent study found that those with low HRV were at higher risk for developing end-stage renal disease (16), implying that end-stage renal disease may be on the causal pathway between low HRV and mortality. Although preliminary as a result of the cross-sectional nature of the current study, our results suggest that DSP may play a role in the causal relationship between low HRV and mortality. The current study shows a clear association between lower HRV and increasing degrees of DSP severity, which are in agreement with existing literature (18). Although previous studies have identified DSP to be an independent predictor for developing abnormal/low HRV prospectively (31), no study has examined whether the opposite relationship—low HRV independently predicting future DSP onset—is also true. The current study also revealed a subset of individuals without evidence of DSP that have HRV as low as those with severe DSP and thus raises a fundamental question about the implications of CAN as an independent predictor of nonneuropathic outcomes in diabetes. Specifically, does the low HRV that we observed in diabetic subjects with absent or mild DSP carry the same prognostic importance for CVD and mortality as the low HRV that is present in case subjects with severe DSP? Prospective follow-up of the current cohort and other validation studies will be required to establish whether HRV is an independent predictor of DSP onset and whether HRV can predict CVD and mortality independent of DSP as an intermediary.

Several studies have examined the clinical correlates and predictors of HRV, typically when abnormally low levels of HRV itself were used as the diagnostic criteria for CAN (32–36). Similar to our findings, a progressive reduction in HRV is seen with increasing age among normal subjects (33) and in patients with diabetes (34–36). These relationships have led to the recommendation of age-specific normal threshold values for a variety of CARTs, including HRV with deep breathing (5). Our results also agree with the independent association of glycemic exposure and lower HRV as assessed by HbA_{1c} (34,35). The inverse

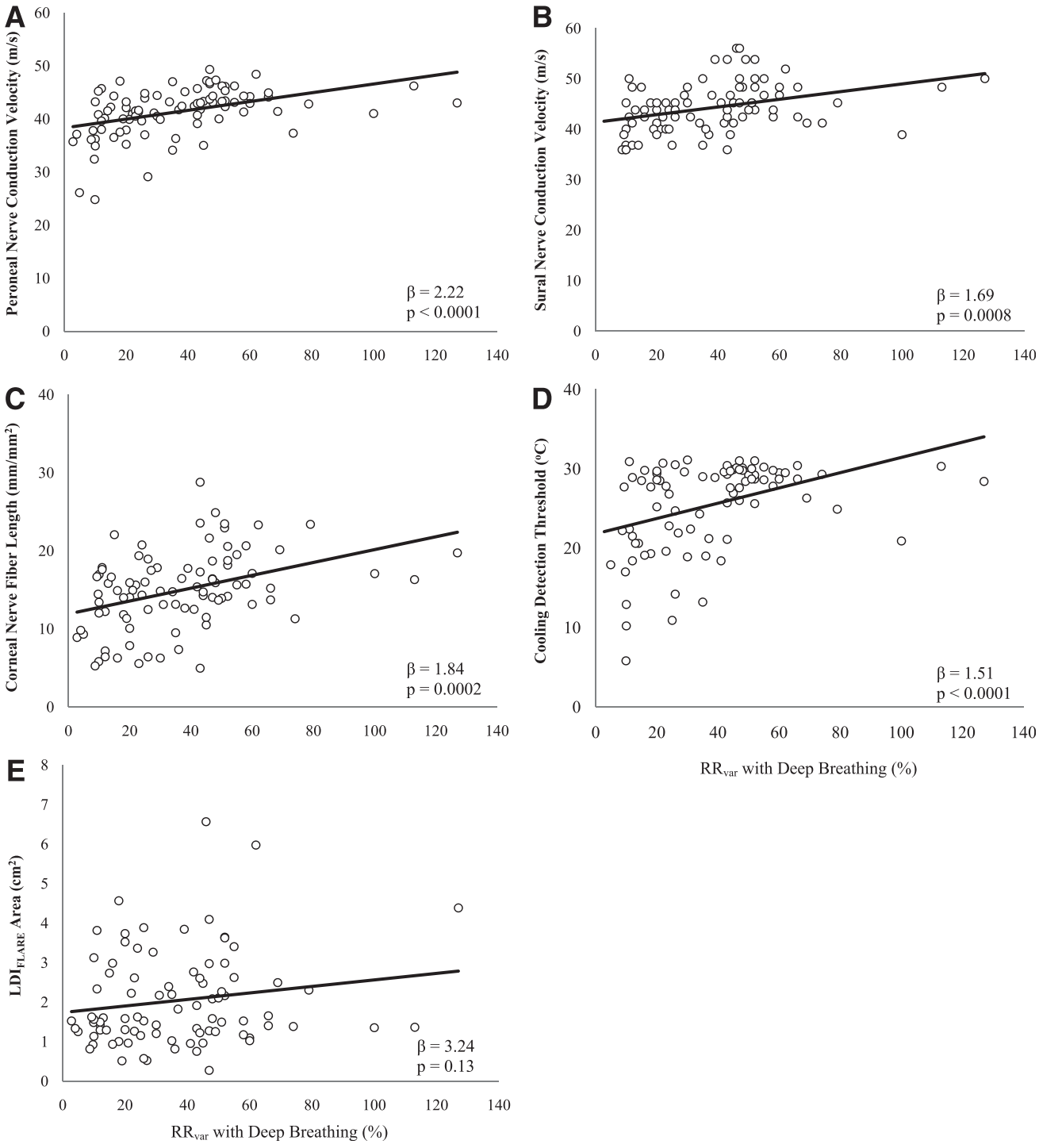

Figure 2—*Association of RR_{var} with deep breathing and validated measures of large fiber* (A *and* B) *and small fiber* (C–E) *neuropathy. Scatterplots of RR_{var} with deep breathing and measures of large fiber motor function (peroneal nerve conduction velocity,* A*); large fiber sensory function (sural nerve conduction velocity,* B*); small fiber morphology (corneal nerve fiber length,* C*); and small fiber function (cooling detection threshold,* D*; LDI_{FLARE} area,* E*), among subjects with type 1 diabetes are shown.*

relationship between blood pressure and HRV in subjects with diabetes has also been reported in the literature (32,34–36), although, contrary with the results from our cohort, others have observed this relationship in healthy volunteers (33). Our findings are in close agreement with a study by Valensi et al. (36), who found that age, diabetes duration, and blood pressure were independent predictors of HRV with deep breathing among subjects with type 1 diabetes. Although we could not confirm a relationship between higher BMI and lower HRV, others have demonstrated such association in type 2 diabetes (36) and in unselected populations (37). Unlike previous reports (34), our study found no significant correlation between HRV and fasting

triglyceride levels or cigarette smoking. Taken together, our observations highlight the notion that decreased HRV is an age-dependent phenomenon that may often predate the onset of diabetes, although poor glycemic control is independently associated with lower values among those with diabetes. The significant inverse relationship between blood pressure and HRV may be the result of increased sympathetic tone after the parasympathetic denervation putatively associated with CAN, although the temporal relationship between these variables will require prospective study. In view of the independent association of HRV with age, present even in healthy volunteers at low risk for the development of polyneuropathy, any protocol that considers the use of HRV to identify the presence of DSP or to predict its future onset will need to incorporate age-stratified diagnostic thresholds.

HRV is primarily mediated by efferent parasympathetic B and C small nerve fiber function (38). Other measures of small fiber function include quantitative sensory tests (i.e., cooling detection threshold) (25), which assess afferent C and Aδ fibers, as well as axon reflex-mediated neurogenic vasodilatation in response to nociceptive stimuli (i.e., LDI_{FLARE}) (26), which assess afferent C fiber function. Small fibers can also be assessed morphologically using CCM to measure CNFL (19,24). In contrast, abnormalities on NCS that define the presence of DSP reflect loss of large nerve fiber function comprising myelinated Aα and Aβ fibers. Although confounded by age, the current study found a significant positive association between HRV and measures of large fiber motor and sensory function, as well as small fiber sensory function (cooling detection threshold). We also report the novel finding that lower HRV is associated with shorter CNFL. Similar to our findings, a previous study among 43 subjects with DSP found that those with CAN, defined by HRV criteria, were more likely to have abnormalities in thermal perception thresholds, sympathetic skin response, and quantitative sudomotor axon reflex tests (39). The results of the current study suggest that HRV, as a measure of small fiber neuropathy, is associated with measures of both small and large fiber neuropathy and hence is present in both early and late phenotypes of diabetic neuropathy.

Although unique as a study to evaluate the relationship of HRV and DSP, there are several limitations to the results of this study. First, we acknowledge that the measurement of HRV using RR_{var} with deep breathing as developed by Stålberg and Nogués (22) is not the only, nor most commonly operationalized, method for assessing HRV. Despite its benefits of being automated and easily integrated with concurrent NCS and algorithmic attempts to minimize the effect of ectopic beats, alternative methods such as calculation of the mean circular resultant using vector analysis (40) have been shown to further attenuate the effect of trends in heart rate over time and the effect of basal heart rate (4). Second, the relevance of this data to type 2 diabetes requires further study. Third, we acknowledge that the most important clinical outcome for establishing HRV as a biomarker will be the future onset of DSP through longitudinal follow-up. The current cross-sectional study is the first step toward this objective, although large prospective study will be required to fully elucidate the predictive validity of HRV for DSP. Fourth, although we identify the major variables that are independently associated with HRV, we likely have insufficient power to detect variables with minor but reportedly significant associations such as smoking or serum triglyceride levels. Finally, we were unable to confidently identify abnormal threshold values for RR_{var} with deep breathing, as calculated by Stålberg and Nogués (22), that could determine risk for concurrent DSP and thus elected to study its relationship as a continuous variable.

In summary, HRV may be a biomarker for clinical DSP and is associated cross-sectionally with both early and late measures of neuropathy. The current study establishes the need to prospectively examine the role of HRV in predicting DSP onset and suggests that DSP may play a role in the causal relationship between HRV and CVD and mortality.

Acknowledgments—This research was supported by the Juvenile Diabetes Research Foundation Grant 17-2008-715. B.A.P. was a Canadian Diabetes Association Scholar. S.O. was supported by a Residency Research Elective from the University of Toronto Internal Medicine Program.

No potential conflicts of interest relevant to this article were reported.

S.O. and B.A.P. researched the data and performed the statistical analysis of the data. S.O. prepared the first draft of the manuscript. V.B., A.O., and B.A.P. reviewed the manuscript for scholarly content and accuracy. V.B. and B.A.P. created the study hypothesis and objective and designed the study. B.A.P. 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.

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