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Sex-based differences in the association between duration of type 2 diabetes and heart rate variability

Robert P. Nolan[1,2], Susan M. Barry-Bianchi[1], Adriana E. Mechetiuc[1], Maggie H. Chen[1]

Abstract

We examined the association between heart rate variability (HRV) and duration of type 2 diabetes among 155 female and 106 male subjects: mean±SD for duration=49.6±65.6 and 57.3±77.1 months, respectively, p=0.38. Among males, duration of diabetes was independently and inversely associated with vagal-heart rate modulation (high frequency (HF) power, 0.15–0.40 Hz, standardised ß = -0.32, p=0.001; root mean square of successive differences between R-R intervals, ß = -0.26, p=0.006) and total R-R variability (standard deviation of normal R-R intervals, ß = -0.36, p=0.001); but not among females (p≥0.80 for each HRV index). In contrast, HF was inversely associated with age of diabetes diagnosis (ß = -0.16, p=0.04) and 10-year absolute risk for coronary heart disease (ß = -0.16, p=0.04) among female subjects. Longitudinal research is needed to establish whether risk factors for early cardiac autonomic impairment differ among men and women with type 2 diabetes.

Keywords:

type 2 diabetes, duration, sex, heart rate variability, autonomic

Introduction

Impaired autonomic regulation of cardiovascular functioning is an established complication of diabetes mellitus and an independent predictor of diabetes-related mortality.[1] The prevalence of cardiac autonomic neuropathy (CAN) is approximately 25% and 34% among individuals with type 1 and type 2 diabetes, respectively, when diagnostic criteria are based upon at least two abnormalities in autonomic function tests.[2] Poor glycaemic control is central to the development of CAN. Other risk factors include age, obesity, nephropathy, distal symmetrical polyneuropathy, retinopathy, hypertension and smoking.[3-5] Symptoms of CAN may become evident within 18 months of diagnosis of type 2 diabetes.[4] Sex differences have also been found in the onset of CAN, with men presenting with autonomic impairment earlier than women.[6]

Early detection of CAN is possible using non-invasive tests that include analysis of heart rate variability (HRV), which is derived from electrocardiogram measures of variability in the continuous sampling of the R-R interval.[7,8] HRV is conventionally quantified by time domain measures that summarise total variability in the deviation of R-R intervals from a mean R-R value, or variability in the differences between adjacent R-R intervals which is indicative of vagal-heart rate modulation. Frequency domain measures are obtained by spectral analysis of recurrent cycles of R-R variation within established frequency bandwidths that correspond to sympathetic, vagal and non-neural modulation of the sinus node cycle length. Among individuals with diabetes, decreased HRV is independently associated with impaired lipid and glucose metabolism, as well as incident coronary heart disease (CHD), myocardial infarction and mortality.[2, 9, 10] With the exception of one investigation,[11] there is consistent evidence that the duration of type 1 and 2 diabetes is inversely associated with HRV measures of total R-R variability and vagal-heart rate modulation.[4, 10, 12-18] Few studies have investigated sex-based differences in factors that potentially influence HRV impairment among patients with

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diabetes in spite of the observation that (i) HRV is greater among males *vs.* females until approximately the sixth decade of life, and (ii) HRV markers of sympathetic- and vagal-heart rate modulation decline for males and females across the age span.[19,20]

Our primary aim was to evaluate sex-based differences in the association between HRV and duration of diabetes. Our secondary goal was to determine whether this association was optimally reflected in HRV markers of vagal-heart rate modulation, sympathetic-heart rate modulation or a summary index of total R-R variability.

Methods

Subjects

Participants were recruited from Ontario, Canada. All data were obtained from the baseline assessment of the Community Outreach Heart Health and Risk Reduction Trial.[21] The sample consisted of volunteers at elevated risk for CHD due to a confirmed diagnosis of diabetes (age = 35 to 74 years, n=261) with or without additional CHD risk factors that included hypertension (≥130 mmHg systolic or ≥80 mmHg diastolic) and dyslipidemia. Exclusion criteria were as follows: abnormal ECG, diagnosis of type 1 diabetes mellitus, CHD, major psychiatric illness (e.g. psychosis) or alcohol or drug dependence within the previous year.

Assessment protocol

Participants were recruited through random-digit dialling, media advertisements, information sessions and physician referrals. Participants were instructed to refrain from drinking caffeinated beverages, smoking and strenuous exercise at least 4 hours prior to assessment. Medications were not tapered or discontinued. HRV measurements were obtained between 8:00 am and 12:00 pm. ECG was recorded over a 3-minute interval with subjects seated in a semi-reclined position. Following adaptation to the laboratory setting, baseline measurements for height, weight, BMI, waist circumference and two separate blood pressure measurements (30 minutes apart) were taken by trained research assistants. Anthropometric data was obtained by psychometric assessment.

Duration of diabetes was based on the self-reported date of diagnosis. Blood assays for fasting glucose, total lipoprotein cholesterol (total-C), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and triglycerides were provided by each subject's family physician. The Framingham index of 10-year absolute CHD risk was calculated for each subject on the basis of sex, age, total-C, HDL-C, systolic blood pressure, diabetes and smoking.[22]

This investigation conformed to the principles outlined in the Declaration of Helsinki and ethical approval was obtained from the research ethics boards of all participating institutions. Participants signed an informed consent prior to all assessment procedures.

HRV analysis

ECG data were recorded using GE Marquette Holter Monitors with analysis conducted on the MARS 8000 platform (Milwaukee, WI, USA). Each ECG tracing was screened for artefacts to ensure that QRS complexes were properly labelled. Rhythms other than normal sinus rhythm were excluded. HRV data were analysed offline. The following HRV frequency domain measures were calculated using spectral analysis of R-R intervals with fast-Fourier transformation: low frequency power (LF, 0.04–0.15 Hz) which reflects sympathetic and vagal modulation of the R-R interval, and high frequency power (HF, 0.15–0.40 Hz) which reflects vagal modulation of the R-R interval within the frequency range of respiration. The LF/HF ratio was calculated as an index of sympathetic modulation of the R-R interval. Time domain indices of HRV included SDNN and RMSSD. It is noteworthy that R-R interval is inversely associated with estimated heart rate, e.g. an R-R interval of 600 ms equals a heart rate of 100 beats/minute, while an R-R interval of 1000 ms equals a heart rate of 60 beats/minute.

Statistical analysis

Complete data were available for all participants. Clinical characteristics and medications were assessed for female *vs.* male subjects by analysis of variance (continuous variables) and Pearson χ^2 analyses (categorical variables). The independent association between HRV (HF, LF, LF/HF ratio, RMSSD and SDNN) and sex, duration of diabetes (measured in months) and the sex-by-diabetes duration interaction was evaluated using multivariable linear regression. The regression model for each HRV index was adjusted for potential confounding variables: age at diagnosis of diabetes, the severity of CHD risk factors (Framingham index of 10-year absolute CHD risk[22]), R-R interval, obesity (BMI ≥ 30), antihypertensive medications (ß-blockers, calcium channel blockers, angiotensin converting enzyme inhibitors, diuretics or angiotensin II receptor blockers), glucose control medications (oral or insulin) and exercise (20 minutes/day, 3–5 days/week).[9, 16, 23, 24] Follow-up multivariable linear regression analyses were conducted for HRV measures that were significantly associated with sex or the interaction between sex and duration of diabetes. The linear regression model for each of these HRV variables evaluated the independent association between HRV and duration of diabetes in separate analyses for male and female subjects, while adjusting for the previously noted covariates: age at diagnosis of diabetes, 10-year absolute CHD risk,[22] R-R interval, obesity, antihypertensive medications, glucose control medications and exercise.

Table 1. Clinical characteristics and medications of the study participants.

	Females (n=155) M±SE (%)	Males (n=106) M±SE (%)	p-value
Age (years)	57.2±8.6	55.1±8.7	0.06
Duration of type 2 diabetes (months)	49.6±65.6	57.3±77.1	0.38
Age at diagnosis of diabetes (years)	53.1±8.8	50.3±9.1	0.02
BMI (kg/m^2)	32.9±7.0	30.2±6.4	0.002
Obesity: BMI ≥ 30	98 (63.2%)	46 (43.4%)	0.002
Waist circumference:			
females > 88 cm or males > 102 cm	134 (86.5%)	61 (57.5%)	<0.001
Hypertension	112 (72.3%)	64 (60.4%)	0.04
Dyslipidemia	99 (63.9%)	67 (63.2%)	0.91
Total-/high-density cholesterol ratio (mmol/L)	4.3±1.5	4.5±1.4	0.34
Triglycerides (mmol/L)	2.5±3.5	2.4±1.8	0.72
Smokers	21 (13.5%)	11 (10.4%)	0.44
Framingham 10-year absolute CHD risk[22]	14.3±7.0	23.8±11.8	<0.001
Fasting glucose (mmol/L)	8.0±2.6	8.8±3.9	0.07
Self-reported exercise (20 minutes 3–5 times per week)	65 (41.9%)	53 (50.0%)	0.20
Diabetic medications			
Oral medications	89 (57.4)	60 (56.6)	0.87
Insulin	6 (3.9)	6 (5.7)	0.50
Oral medications and insulin	2 (1.3)	1 (0.9)	0.80
Antihypertensive medications	106 (68.4)	72 (67.9)	0.94
Beta-blocker	106 (68.4)	72 (67.9)	0.94
Angiotensin converting enzyme inhibitor	50 (32.3)	43 (40.6)	0.17
Calcium channel blocker	30 (19.4)	17 (16.0)	0.49
Angiotensin II receptor blocker	28 (18.1)	16 (15.1)	0.53
Diuretic	39 (25.2)	23 (21.7)	0.52
Lipid lowering agents	73 (47.1)	57 (53.8)	0.29

BMI = body mass index; CHD = coronary heart disease

Natural logarithm transformations were made to duration of diabetes as well as HRV measures of HF, LF and LF/HF ratio in order to correct for skewness. Statistical significance for all analyses was determined by two-tailed tests with $p<0.05$. Data were analysed using SPSS version 17.0 for the Mac OS-X (SPSS Inc., Chicago, IL, USA). All authors had full access to the data. The primary author (RPN) takes full responsibility for the integrity of the data.

Results

The sample consisted of 155 female participants (age range = 37.1 to 74.1 years) and 106 male participants (age range = 36.9 to 72.7 years). Clinical characteristics and medications for subjects are presented in table 1. In comparison to female subjects, males reported a younger age at which diabetes was diagnosed. Mean BMI was above 30 for both males and females. Nevertheless, obesity was more prevalent among female subjects as defined by BMI ≥ 30 and by sex-adjusted criteria for waist circumference. Hypertension was also more common among female subjects. The Framingham index of 10-year absolute CHD

risk[22] was significantly greater among males. There were no significant sex-based differences in diabetic medications (oral or insulin), antihypertensive medications or lipid lowering agents.

Association between HRV and duration of diabetes among females and males

Table 2 shows that vagal-heart rate modulation was significantly greater among female *vs.* male subjects when estimated by HF power, but not by RMSSD. Conversely, sympathetic-heart rate modulation as measured by LF/HF ratio was significantly lower among female *vs.* male participants. A sex-based difference was not observed for mean LF power and SDNN.

Table 3 indicates that HRV markers of vagal-heart rate modulation (HF power and RMSSD) and total R-R variability (SDNN) were significantly and inversely associated with the interaction between sex and duration of diabetes. This indicated that with increased duration of diabetes there was a significantly greater decline in each of these HRV indices among male *vs.* female subjects. LF power and the LF/HF

Table 2. Unadjusted sex-based differences in heart rate variability.

	Females n=155 M±SE	Males n=106 M±SE	p-value
lnHF power (Hz/ms^2)	4.60±0.10	4.28±0.12	0.04
lnLF power (Hz/ms^2)	5.20±0.09	5.22±0.11	0.91
lnLF/HF ratio power (Hz/ms^2)	0.60±0.06	0.94±0.08	0.001
RMSSD (ms)	22.29±1.09	19.63±1.11	0.10
SDNN (ms)	45.32±1.88	49.18±2.78	0.23
R-R interval (ms)	797.26±10.00	804.62±12.56	0.65

HF = high-frequency spectral bandwidth for R-R interval, 0.15–0.40 Hz; LF = low-frequency spectral bandwidth, 0.04–0.15 Hz; M±SE = mean ± standard error; RMSSD = root mean square of differences between successive R-R intervals; SDNN=standard deviation of normal-to-normal R-R intervals.

ratio were not significantly associated with sex, duration of diabetes or the sex-by-diabetes duration interaction. The multivariable linear regression models also indicated that HF power was inversely associated with the age at which diabetes was diagnosed and with 10-year absolute CHD risk, while it was positively associated with R-R interval. A statistical trend (p=0.07) was observed for an inverse association between HF power and antihypertensive medications. This suggested that antihypertensive medications may have been a proxy variable for the presence of pathophysiology of the microvasculature.[25] The multivariable linear regression model for RMSSD was consistent with the above-noted model for HF power with the exception that RMSSD was not associated with age of diabetes diagnosis. Finally, the regression model for SDNN indicated an inverse association with obesity and a positive association with R-R interval. A

statistical trend (p=0.06) was also found for a positive association between SDNN and exercise.

Table 4 presents a sex-based analysis of the association between duration of diabetes and select HRV measures using multivariable linear regression models. Each select HRV index was previously shown in table 3 to co-vary significantly with the interaction between sex and duration of diabetes. Duration of diabetes was inversely and significantly associated with markers of vagal-heart rate modulation (HF power, RMSSD) and total R-R variability (SDNN) among male subjects only. In addition, HF power and SDNN were inversely associated with obesity among males. Among female subjects, HF power was inversely associated with age at which diabetes was diagnosed and with 10-year absolute CHD risk. Finally, R-R interval was positively associated with HF power, RMSSD and SDNN for both male and female subjects.

Discussion

Heart rate variability is well-known to decline more rapidly with increasing age among individuals with *vs.* without diabetes.[16] In the present study, mean HRV values were comparable to those reported in previous investigations of men and women with type 2 diabetes where HRV was measured using a short-term assessment protocol.[26, 27] Moreover, HRV markers of vagal-heart rate modulation (HF power and RMSSD) and total R-R variability (SDNN) derived from our sample were within a range that was similar to patients post-myocardial infarction.[28, 29]

The major finding in the present study is that within the context of impaired HRV there are significant sex-based differences in the association between duration of diabetes and vagal-heart rate modulation and total R-R variability.

Table 3. Multivariable linear models of heart rate variability and duration of diabetes.

	LnHF (Hz/ms^2)		LnLF (Hz/ms^2)		LnLF/HF (Hz/ms^2)		RMSSD (ms)		SDNN (ms)	
	Std. ß	p-value	Std. ß	p-value	Std. ß	p-value	Std. ß	p-value	Std. ß	p-value
Sex	0.14	0.19	0.21	0.09	0.07	0.59	0.15	0.16	0.34	0.004
Duration of diabetes (months)	−0.02	0.74	−0.04	0.58	−0.02	0.77	0.02	0.82	−0.01	0.93
Sex * duration of diabetes	−0.30	0.01	−0.19	0.15	0.18	0.21	−0.24	0.05	−0.39	0.003
Age at diagnosis of diabetes	−0.16	0.006	−0.10	0.12	0.10	0.18	−0.03	0.61	−0.07	0.28
10-year absolute CHD risk[22]	−0.16	0.01	−0.19	0.009	−0.01	0.90	−0.16	0.01	−0.01	0.94
R-R interval	0.52	<0.001	0.40	<0.001	−0.23	<0.001	0.59	<0.001	0.39	<0.001
Obesity: BMI ≥ 30	−0.05	0.36	−0.06	0.31	−0.01	0.87	0.06	0.26	−0.13	0.02
Antihypertensive medications	−0.09	0.07	−0.05	0.41	0.07	0.25	−0.07	0.16	−0.02	0.71
Glucose control medications: oral or insulin	−0.04	0.49	−0.07	0.31	−0.03	0.68	−0.02	0.75	−0.01	0.91
Exercise	0.04	0.39	0.04	0.50	−0.01	0.84	0.02	0.66	0.10	0.06
	Adj. R^2 = 0.38 p<0.001		Adj. R^2 = 0.23 p<0.001		Adj. R^2 = 0.07 p=0.001		Adj. R^2 = 0.40 p<0.001		Adj. R^2 = 0.26 p<0.001	

BMI = body mass index; CHD = coronary heart disease

Table 4. HRV markers of vagal-heart rate modulation and total R-R variability among male and female participants.

	LnHF (Hz/ms²)				RMSSD (ms)				SDNN (ms)			
	Females		Males		Females		Males		Females		Males	
	Std. ß	p-value	Std. ß	p-value	Std. ß	p-value	Std. ß	p-value	Std. ß	p-value	Std. ß	p-value
Duration of diabetes (months)	−0.02	0.80	−0.32	0.001	0.01	0.88	−0.26	0.006	−0.01	0.95	−0.36	0.001
Age at diagnosis of diabetes	−0.16	0.04	−0.15	0.11	0.01	0.93	−0.10	0.24	0.01	0.93	−0.14	0.16
10-year absolute CHD risk[22]	−0.16	0.04	−0.13	0.15	−0.12	0.10	−0.14	0.11	−0.12	0.16	0.10	0.33
R-R interval	0.56	<0.001	0.41	<0.001	0.59	<0.001	0.56	<0.001	0.43	<0.001	0.30	0.002
Obesity: BMI ≥ 30	0.06	0.42	−0.20	0.01	0.11	0.11	−0.05	0.53	−0.08	0.33	−0.19	0.03
Antihypertensive medications	−0.08	0.25	−0.10	0.18	−0.11	0.13	−0.01	0.85	−0.05	0.55	0.01	0.87
Glucose control medication: oral or insulin	−0.06	0.43	−0.01	0.87	0.01	0.93	−0.04	0.64	0.0003	0.997	−0.02	0.85
Exercise	0.05	0.46	0.06	0.46	0.03	0.64	0.05	0.53	0.11	0.14	0.11	0.23
	Adj. R^2 = 0.35 p<0.001		Adj. R^2 = 0.42 p<0.001		Adj. R^2 = 0.35 p<0.001		Adj. R^2 = 0.48 p<0.001		Adj. R^2 = 0.18 p<0.001		Adj. R^2 = 0.32 p<0.001	

Among male participants, HF power, RMSSD and SDNN decreased significantly with greater duration of diabetes. In addition, obesity among males (BMI ≥ 30) was independently and inversely associated with HF power and SDNN. In contrast, HRV indices were not significantly associated with diabetes duration among female subjects. Rather, diminished HF power was associated with increased age at which diabetes was diagnosed and with increased severity of CHD risk factors as measured by the Framingham index of 10-year absolute CHD risk.[22] In addition, HRV markers of combined sympathetic and vagal modulation of heart rate (LF power) or sympathetic-heart rate modulation (LF/HF ratio) were not associated with duration of diabetes for male and female subjects.

Two divergent hypotheses may account for our finding of a sex-based difference in the association between HRV and duration of diabetes. First, HRV may have declined among males with greater duration of diabetes due to increased susceptibility to cardiac autonomic impairment as a result of glycaemic exposure. In comparison to women with diabetes, men have an increased prevalence of diabetic neuropathy, the age of onset of neuropathy occurs approximately 4 years earlier, and symptoms of autonomic impairment are more severe.[6] Accordingly, early signs of cardiac autonomic impairment as measured by HRV would be expected to be more pronounced among males in our sample, particularly since age did not differ significantly according to sex. This hypothesis is supported by our observation that male subjects presented with significantly lower vagal-heart rate modulation (HF power) and significantly greater sympathetic-heart rate modulation (LF/HF ratio), in comparison to female subjects.

An alternative hypothesis would assert that the absence of an association between HRV and duration of diabetes among women in our sample was attributable to a sex-specific 'floor effect' in markers of vagal-heart rate modulation and total R-R variability, due to a clustering of factors that inhibit cardiac autonomic functioning. Diabetes was diagnosed among female subjects at a mean age of 53 years which is proximate to the mean age of menopause.[30] Endogenous oestrogen in pre-menopausal women is a protective factor for cardiovascular health[31] and it may also promote autonomic heart rate modulation in women with diabetes.[32] Although we controlled for several potential confounding factors in our analysis of HRV, we did not assess the potential influence on HRV of oestrogen or oestrogen replacement therapy. Nevertheless, age of diagnosis of diabetes was independently and inversely associated with vagal-heart rate modulation among female subjects, as estimated by HF power. Risk factors for CHD may have also inhibited HRV among female subjects. Female subjects had a greater prevalence of hypertension (72.3% *vs.* 62.4%, respectively) and obesity as defined by conventional criteria for BMI and sex-adjusted waist circumference (63.2% *vs.* 43.4% and 86.5% *vs.* 57.5%, respectively), in comparison to male subjects. Further support for this alternative hypothesis is based on our observation that HF power among female subjects was inversely associated with CHD risk factor severity as measured by the Framingham index of 10-year absolute CHD risk.[22]

Our findings indicate that there may be significant sex-based differences in the progression of early cardiac autonomic impairment in persons with type 2 diabetes. Previous cohort studies have reported that HRV markers of vagal-heart rate modulation or total R-R variability are inversely associated with duration of diabetes[4, 10, 13, 15, 18] although one study failed to observe a significant association.[11] Alternatively, a longitudinal analysis that was conducted in the Atherosclerosis Risk in Communities

study found that markers of total R-R variability (SDNN) and vagal-heart rate modulation (RMSSD) were significantly reduced among individuals with diabetes at a mean follow-up of 9 years.[16] The Diabetes Control and Complications Trial [17] also reported a longitudinal analysis which indicated that markers of vagal-heart rate modulation were significantly reduced at a 6.5-year follow-up. Interestingly, the rate of HRV decline was therapeutically improved among subjects randomised to intensive glucose control therapy; however, it was not specified whether this finding applied equally to men and women with diabetes. Additional longitudinal research is needed to specify whether risk factors for cardiac autonomic impairment differ significantly among men and women with diabetes, particularly following the age of onset for menopause among women which is characterised by an increased incidence of hypertension, dyslipidemia and cardiovascular events.[31, 33]

A major limitation of this study is that it was cross-sectional by design. Therefore, only indirect evidence is provided for sex-based differences in the association between duration of diabetes and HRV. In addition, differences in HRV that we observed between men and women with diabetes may have been attributable to intrinsic disorders that were not monitored in our study, such as subclinical diabetic cardiomyopathy.[34] As noted above, we did not assess the potential influence on HRV of oestrogen or oestrogen replacement therapy. So the degree to which our findings have been influenced by the occurrence of menopause among women or by exposure to oestrogen replacement therapy is unknown. Finally, although we found no relationship between diabetic medications and HRV, the maintenance of glycaemic control is well-known to prevent or delay the onset of CAN and to moderate its progression.[2]

In summary, HRV is commonly observed to be diminished among individuals with diabetes, which may be an early indication of CAN. Our current study found that duration of diabetes was independently and inversely associated with HRV markers of vagal-heart rate modulation (HF power and RMSSD) and total R-R variability (SDNN) among male subjects only. Nevertheless, among female subjects HRV was inversely associated with increased age of diabetes diagnosis, which was consistent with the onset of menopause, as well as increased severity of CHD risk factors and obesity. There is a need for more detailed information from longitudinal studies regarding potential sex-based differences in the development of cardiac complications associated with diabetic neuropathy.[6] The assessment of cardiovascular autonomic function is currently recommended on an annual basis for a patients diagnosed with type 1 and 2 diabetes.[35] It may be advisable to consider concomitant assessments of CHD risk factors, not only to monitor changes in ansolute CHD risk, but also to evaluate potential sources of autonomic impairment which may have particular relevance for post-menopausal

Abbreviations	
BMI	body mass index
CAN	cardiac autonomic neuropathy
CHD	coronary heart disease
ECG	electrocardiogram
HRV	heart rate variability
HF	high frequency
HDL-C	high-density lipoprotein cholesterol
LDL-C	low-density lipoprotein cholesterol
QRS	Electrocardiogram wave complex that corresponds to ventricular depolarization
RMSSD	root mean square of successive differences of normal-to-normal R-R intervals
R-R	Interval between 2 successive electrocardiogram R waves, measured in milliseconds
SDNN	standard deviation of normal-to-normal R-R intervals
total-C	total lipoprotein cholesterol

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Declaration of Conflicting Interests

The authors declare that they do not have any conflict of interest.

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