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Time- and Frequency-domain Measures of Heart Rate Variability Predict Cardiovascular Outcome in Patients with Type 2 Diabetes

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Abstract

Aims: To evaluate the association between impaired heart rate variability (HRV) and cardiovascular disease (CVD) in patients with type 2 diabetes (T2DM).

Methods: A total of 655 patients with T2DM who underwent cardiovascular autonomic function testing were consecutively recruited and followed up prospectively. Time- and frequency-domain HRV were assessed for 5 minutes by beat-to-beat heart rate recording. We estimated the development of CVD events during a follow-up period.

Results: During a median follow-up of 7.8 years, 9.6% (n=49) of patients developed CVD (10.6 per 1,000 patient-years). The mean age and diabetes duration were 54.9 ± 8.6 years and 9.4 ± 7.3 years, respectively. Patients who had cardiovascular autonomic neuropathy (CAN) had decreased HRV compared with those with normal autonomic function. Multivariable cox hazard regression analysis revealed the lowest 10th percentile of the SD of the normal-to-normal interval (HR 2.62; 95% CI 1.30–5.31), total power (HR 2.81; 95% CI 1.37–5.79), low-frequency power (HR 2.68; 95% CI 1.28–5.59), and high-frequency power (HR 2.24; 95% CI 1.09–4.59) were significant predictors for developing CVD in patients with T2DM.

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Authors' contributions

S.A.C analyzed data and wrote manuscript. Y.M.P contributed to statistical analysis. J.S.Y and S.H.L acquired the researched data and reviewed and edited the manuscript. S.R.K reviewed and edited the manuscript. Y.B.A contributed to the study concept and design, researched data and reviewed the manuscript. S.H.K designed the study and the guarantor of this study. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that there are no relevant conflicts of interest

Conclusions: Time- and frequency-domain measures of HRV independently predicted cardiovascular outcome in patients with T2DM.

Keywords

Cardiac autonomic neuropathy; heart rate variability; type 2 diabetes; cardiovascular outcome

1. Introduction

Cardiovascular disease (CVD) is major cause of morbidity and mortality in individuals with type 2 diabetes (T2DM) and is the largest contributor to the costs of managing complications of T2DM [1–3]. T2DM is associated with a 2- to 3-fold increased risk of CVD in men and 3- to 4-fold increased risk in women compared to normal populations [4]. Although there has been a significant improvement on risk management of coronary heart disease (CHD) over the past decade, many patients with T2DM have not achieved overall target of multifactorial risk factor for preventing CVD [5]. In Korea, 56.5% of patients with T2DM had glycated hemoglobin (HbA_{1c}) $\geq 7\%$, and 50.2% of patients with T2DM did not reach the low-density lipoprotein (LDL)-cholesterol <100 mg/dL, putting at risk many patients with T2DM to CVD development despite hypoglycaemic, antihypertensive, and lipid-lowering treatment [6]. Thus, modifying multiple cardiovascular (CV) risk factors and early detection of a high-risk population in patients with T2DM is needed.

Cardiovascular autonomic neuropathy (CAN) is a common but mostly underestimated complication in patients with T2DM. CAN is the impairment of autonomic control of the CV system [7]. CAN's prevalence has been reported from 2.5% to 50% in patients with T2DM according to diagnosis criteria, the test method, and age and diabetic duration of study participants [8]. Clinically, CAN might manifest as resting tachycardia, exercise intolerance, orthostatic hypotension, and silent myocardial ischemia [9]. Risk factors influencing CAN include poorly controlled glycaemic status, microvascular complications, hypertension, obesity, smoking, and dyslipidemia [8, 10].

Several studies have reported the association between CAN and adverse CV events and silent ischemia [11, 12]. In addition, a meta-analysis showed that patients with CAN had a 1.96-fold higher frequency of silent myocardial ischemia than patients without CAN [9]. Although the pathophysiological mechanisms that explain the relationship between CAN and increased CV morbidity or mortality have not been clearly identified, CAN implies CV mortality and may have prognostic importance in CVD [9, 11, 12]. Current clinical guidelines by the American Diabetes Association emphasizes patients with T2DM should be assessed for symptoms and signs of CAN during the history and physical examination [1]. However, CAN might be asymptomatic and could be overlooked in early stages of CAN in patients with T2DM [1]. Accordingly, screening for early-stage CAN is very important for preventing its complications for reducing CV morbidity and mortality.

During the 1970s, Ewing et al. devised simple bedside tests of short-term R-R differences to detect CAN in normal subjects and patients with diabetes [13]. In 1981, Akselrod et al. introduced frequency-domain measures (power spectral analysis) of the heart rate variability (HRV) [14]. Frequency-domain measures have been reported to provide clinical assessment

of parasympathetic and sympathetic activities and balances and quantify the rhythmic oscillations in HRV [15]. In early stages, CAN could be detected by decreased HRV with deep breathing, which implies parasympathetic dysfunction in patients with T2DM [1]. Takase et al. reported that high-frequency (HF) power, as a direct measure of vagal nerve integrity, was reduced in early CAN in patients with T2DM [16]. Thus, frequency-domain measures of HRV might be a diagnostic method to detect early CAN in patients with T2DM [16].

Previous studies reported the association between impaired HRV and CAN in patients with type 1 diabetes mellitus (T1DM) and T2DM [17, 18]. However, few studies evaluated the various HRV variables and CV outcomes in patients with T2DM.

Therefore, we analyzed time- and frequency-domain measures of HRV compared with standard CV autonomic function test (AFT) and evaluated the association between HRV variables and CV outcome in patients with T2DM.

2. Materials and Methods

2.1. Subjects

We consecutively recruited 1,043 patients aged 25–69 years and performed cardiac AFT at the university-affiliated diabetes center of St. Vincent's Hospital in South Korea between March 1, 2009, and February 28, 2010, and followed up until June 30, 2017.

Patients with T1DM, gestational diabetes, severe illness (including liver cirrhosis, end-stage renal disease, malignancy, and sepsis), a history of CVD or arrhythmia (including atrial fibrillation and bundle branch block), and who were receiving drugs that could influence AFT (including antiarrhythmic drugs, tricyclic, and tetracyclic antidepressants) were excluded. All participants provided written consent, and the Catholic Medical Center Ethics Committee had previously approved this study protocol.

At enrollment, all participants were interviewed about medical history and had an anthropometry investigation, which was detailed in previous publications [19]. In brief, the definition of hypertension was systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, or use of antihypertensive medication. Subjects were classified as current smokers, past smokers, or nonsmokers based on the questionnaire. Alcohol consumption was defined as drinking one to two drinks per day for 6 months or longer [20].

Laboratory variables, including a fasting plasma glucose (FPG), postprandial 2-hour glucose (pp2hr), and the lipid parameters were measured using an automated enzymatic method (736–40; Hitachi, Tokyo, Japan) after 8-hour fasting, and HbA_{1c} was measured using high-performance liquid chromatography (Bio-Rad, Montreal, QC, Canada) at baseline and at least every 6 months during the follow-up period. Estimated glomerular filtration rate was calculated using the four-component Modification of Diet in Renal Disease equation [21].

Diabetic retinopathy was investigated through retinal photographs, which an ophthalmologist used for diagnostic purposes. Urinary albumin excretion rates were evaluated from spot urine specimens using immunoturbidimetry (Eiken, Tokyo, Japan).

Diabetic nephropathy was defined as microalbuminuria, which means a urine albumin-to-creatinine ratio equal or above 30 mg/g of creatinine in spot urine specimens, which was reconfirmed at least three times in 6 months [21]. Participants have received outpatient follow-up care every 3 or 4 months from enrollment to June 30, 2017.

2.2. Cardiovascular AFT

All participants were instructed to fast and avoid alcohol, caffeine, and nicotine for 12 hours before the HRV measurements. After 10 minutes of rest, subjects underwent HRV measurement between 8:00 am and 12:00 pm to avoid diurnal variation by using the DiCAN (Medicore, Seoul, Korea). Subjects were seated on a chair in a comfortable position and were equipped with electrodes on their wrists and left foot to derive HRV for 5 minutes.

An examiner performed CV AFT using the standard battery of CV AFTs according to Ewing's method [13, 19]. The E/I difference was calculated as the mean expiration to inspiration difference in R-R intervals over six consecutive breaths during inspiration at a rate of six breaths per minute. The 30:15 ratio was the ratio of the longest R-R interval during beat 20–40 after standing, to the shortest R-R interval during beat 5–25 after standing. The Valsalva maneuver was the ratio of the longest R-R interval to the shortest R-R interval during forced exhalation into the mouthpiece of a manometer to 40 mmHg for 15 seconds [9].

An E/I difference ≤ 10 , a Valsalva ratio ≤ 1.10 , and a posture ratio ≤ 1.00 were considered abnormal. Each of the parameters classified as normal = 0 or abnormal = 1, for a total maximum score of 3. The staging of CAN was defined as follows: a score of 0 was defined as normal autonomic function; a score of 1 was defined as early-stage CAN; and a score of at least 2 was defined as a definite diagnosis of CAN [7, 8]. Orthostatic hypotension was defined as a fall in blood pressure >20 mmHg or systolic blood pressure or >10 mmHg of diastolic blood pressure in response to postural change from supine to standing [9].

The following HRV variables were measured during 5 minutes by time- and frequency-domain analysis, which allows a precise description of the different fluctuations: standard deviation of the normal-to-normal interval (SDNN), the root mean square of differences between adjacent R-R intervals (RMSSD), total power (TP), low frequency (LF) (0.04–0.15 Hz) power, and HF (0.15–0.40 Hz) power. TP is the variance of the normal-to-normal interval over a temporal segment.

2.3. Cardiovascular disease (CVD)

The primary endpoint of this study was development of CVD, which was defined as CHD, stroke [22]. CHD included myocardial infarction (MI), non-MI acute coronary syndrome, heart failure related to CHD, or death attributable to CHD. MI was defined as one of the following criteria: detection of a rise and/or fall of cardiac biomarkers with at least one marker of clinical cardiac ischemia and the absence of noncardiac causes of biomarker elevation and cardiac symptoms or signs or electrocardiography using World Health Organization criteria [23, 24]. Stroke was defined as a neurological deficit due to cerebrovascular causes that persisted beyond 24 hours or led to death within 24 hours [23, 25]. Physicians reviewed all patients' medical records and asked the patients whether they

visited other hospitals for evaluation and management of CVD. If a subject experienced more than one event, the time of the first event was considered in the analysis.

2.4. Statistical analysis

All HRV variables were expressed as the mean $\pm$ standard deviation, median (interquartile range), or frequency. Normal distribution was tested by the Kolmogorov-Smirnov test. Univariate analysis, using independent *t*-test was used for continuous symmetrical variables, Mann-Whitney test was used for continuous asymmetrical variables, and Chi-square test was used for categorical variables.

HRV variables were assessed in percentiles and were dichotomized at the lowest 10th percentiles as suggested abnormally for reduced values. The log-rank (Mantel-Cox) test was used to distinguish the association of the lowest 10th percentiles of HRV variables and CVD. We computed hazard ratios (HRs) and 95% confidence intervals (CIs) for the associations between HRV variables and CVD risk using Cox proportional hazards regression; the lowest 10th percentile of HRV variables was compared with all other percentiles of HRV variables. The proportional hazards assumption was assessed using an interaction term with survival time in the regression model and with log-log survival plots. There was no significant departure from proportionality in hazards over time. Potential confounders were identified *a priori* based on a literature review. The following covariates were included in multivariable adjusted models: model 1 was adjusted for age, sex, and diabetes duration; model 2 was further adjusted for HbA_{1C}; model 3 added an adjustment for the CAN stage. Statistical significance was evaluated with two-sided tests, with the level of significance at 0.05. Statistical analyses were performed using SAS version 9.3 (SAS Institute, Cary, NC, USA).

3. Results

Of 1,043 subjects screened, 321 subjects were excluded; 185 patients who were over age 70 years, 67 patients with previous CVD, 18 patients with T1DM, 4 patients with end-stage renal disease, 3 patients with liver cirrhosis, 4 patients with atrial fibrillation, 32 patients without baseline electrocardiography, 57 patients who were taking drugs influencing AFT, 18 patients with malignancy, and 655 subjects with T2DM were finally recruited for this study (Fig. 1). A total of 508 (77.6%) of the 655 subjects completed the follow-up, and 49 subjects (9.6%) have developed a CVD during a median follow-up time of 7.8 years, which corresponds to 10.6 per 1,000 person-years.

Baseline characteristics of the participants are presented in Table 1. The mean age and diabetes duration were 54.9 ± 8.6 years and 9.4 ± 7.3 years, respectively; 56.3% were women in this study. In the overall participants, patients who experienced CVD had more hypertension (p value = .036), diabetic retinopathy (p value < .001), and higher systolic blood pressure (p value = .046), FPG (p value = .049), pp2hr (p value = .035), total cholesterol (p value = .008), and triglycerides (p value = .004) than those without CVD.

Moreover, patients with CVD are more likely to have abnormal CAN, abnormal E/I ratio (p value = .012), abnormal Valsalva ratio (p value = .001), presence of orthostatic hypotension (p value = .013), and CAN stage (p value = .018). Previous hypoglycemic drugs and

antihypertensive drug use did not show significant differences between the two groups. The median value and interquartile range of TP, LF, HF, and LF/HF ratio in this study were 309.5 (153.8–345.8) (milliseconds [ms])², 46.4 (19.9–104.9) ms², 62.1 (26.2–153.4) ms², and 2.4 (0.7–2.9), respectively. The mean heart rate, median value, and interquartile range of SDNN and RMSSD in HRV were 76.1 ± 12.1 beats/min, 25.4 (18.1–33.7) ms and 14.3 (10.0–21.8) ms, respectively (Table 1). There were no significant differences in median value of TP, HF, LF, and LF/HF ratio between CVD (+) and CVD (–) (Table 1).

Then, we assessed the relationship between traditional CAN stage and HRV variables in patients with T2DM. As expected, there were significantly reduced trends of HRV variables according to the CAN stage, SDNN (p for trend < .001), RMSSD (p for trend < .001), TP (p for trend = .001), HF power (p for trend = .001), and LF power (p for trend < .001) (Table 2, Fig. 2A-2C). But LF/HF ratio did not show significant difference according to the CAN stage (p for trend = .087) (Fig. 2D).

Multivariable Cox proportional hazard regression analysis revealed the lowest 10th percentile of HRV variables were independent predictors for developing CVD in patients with T2DM. The lowest 10th percentile of SDNN (HR 2.62; 95% CI 1.30–5.31, p = .007), RMSSD (HR 2.40; 95% CI 1.18–4.91, p = .016), TP (HR 2.81; 95% CI 1.37–5.79, p = .005), LF power (HR 2.68; 95% CI 1.28–5.59, p = .009), and HF power (HR 2.24; 95% CI 1.09–4.59, p = .029) were independent significant predictors for developing CVD in patients with T2DM after adjusting for age, sex, diabetes duration, HbA_{1C}, and traditional CAN stage (Table 3). The cumulative HR stratified by TP at baseline in this study is shown in Fig 3 (Fig 3A; TP, 3B; LF power, 3C; HF power).

In addition, we found that HRV variables had different effects on CHD and stroke development (Table S2). However, we could not validate this finding because of a small number of patients with cardiovascular outcomes such as CHD and stroke. Further research is needed to determine the different effects of HRV variables on CHD or stroke.

We also assessed baseline characteristics of subjects with lower HRV variables. Subjects with the lowest quartile of TP were older, had longer diabetic duration, and higher FPG and HbA_{1C} (Table 4). Additionally, time- and frequency-domain HRV variables have a significantly negative correlation with glycemic control status (Table S3).

4. Discussion

We demonstrated an independent association between reduced time- and frequency-domain parameters of HRV and development of CVD in patients with T2DM. This study suggested that the lowest 10th percentile of HRV variables, including SDNN, RMSSD, TP, HF power, and LF power, were significant predictors for adverse CV outcomes after adjustment for age, diabetic duration, HbA_{1C}, and CAN stage.

Previous studies consistently reported that CAN predicts CV risk and sudden death in patients with T2DM [26, 27]. The Atherosclerosis Risk in Communities (ARIC) study showed that lowest quartiles of HRV variables have a 1.47–1.55-fold higher risk of CVD in patients with T2DM. In addition, the Detection of Ischemia in Asymptomatic Diabetes study

showed that CAN was associated with development of CHD, heart failure, or CV death in patients with asymptomatic T2DM without previous CHD [28].

HRV reflects the changes in heart rate caused by fluctuations in sympathetic and parasympathetic function under steady conditions [29]. Clinically, HRV was interpreted through time-domain analysis of normal-to-normal (NN) intervals and frequency-domain analysis or the HRV signals. Time-domain analysis of HRV is determined by the heart rate at any point in time or the intervals between successive normal complexes. SDNN is the simplest variable to calculate the standard deviation of NN intervals, the square root of variance; while RMSSD is the square root of the mean squared difference of successive NN intervals [29]. Frequency-domain analysis of HRV, so-called power spectral density analysis, provides information about how variance distributes as a function of frequency; TP, LF (frequency range: 0.04–0.15 Hz), and HF (frequency range: 0.15–0.4 Hz) are useful in separating sympathetic from parasympathetic abnormalities [14, 29].

Few studies have focused on HRV variables in Korean patients with T2DM. Park et al. reported that subjects with diabetes have lower HRV values than normal subjects (SDNN; 36.2 ± 15.5 vs. 30.1 ± 14.4 , p value = .024) [30]. HRV variables of this study were also much lower than previously observed in normal healthy Korean populations or Chinese populations (e.g., lowest 10th percentile of TP in normal Korean population is 347.0 ms^2 , higher than 76.4 ms^2 , which is comparable to values reported in previous studies) [31, 32].

Time- and frequency-domain measures of HRV might be useful in detecting early CAN and predicting mortality after MI, heart failure, and sudden death in patients with T2DM [16, 18]. Previous studies reported that HF power reflects parasympathetic activity, which is reduced in patients with T2DM compared with healthy controls, implying HF power could be a useful method of classifying patients with early CAN and those with normal autonomic function [33]. CAN did not manifest typical symptoms of CAN in the early stage and is mostly underestimated in the clinical setting; thus, HF power could differentiate early CAN with normal autonomic function [34]. LF power was reported to be a significant predictor for mortality in patients with T2DM [35].

In addition, Anders et al. reported that reduced LF power, which reflects both sympathetic and parasympathetic functions, has an association with carotid atherosclerosis in T2DM, which has an implication in the relationship between CAN and CHD [36].

The pathogenic mechanism to explain the association of HRV and CVD remains largely unknown. In patients with CHD or heart failure, increased sympathetic activity could predispose patients to arrhythmia, including ventricular fibrillation, which has been proposed as mechanisms explaining the associations of reduced HRV with increased CV mortality [37, 38]. It is less clear whether HRV variables are associated with CVD incidence in patients with T2DM. In patients without CHD or heart failure, reduced HRV could reflect subclinical cardiac disease or imbalance of the renin-angiotensin system [27]. Previous studies also reported vagal function had an association with CHD, which reflects angiographic severity [39]. In addition, the reasons that decreased HRV was associated with CVD might originate from older age, high insulin level, reduced vagal activity, reduced

baroreflex sensitivity, atherosclerosis, reduced physical activity, smoking status, depression, and obesity accompanied by obstructive sleep apnea [40].

This study suggests that a lower HRV value could be a useful indicator to distinguish early CAN in patients with T2DM. In addition, the lowest 10th percentile value of HRV in patients with T2DM could predict development of CVD after adjusting for traditional CV risk factor and CAN stage, assessed by time- and frequency-domain measures of HRV; a sensitive, noninvasive, and quantitative method, could be implemented in an outpatient basis [14].

Additionally, LF/HF ratio was not a significant predictor for developing CVD, which is similar to the result of the ARIC study [27].

This study had several limitations. First, the sample size was small and analysis of HRV was measured in a short time. However, one examiner conducted AFT using the same method for total participants and tried to regulate the respiration of participants with the same method. The traditional method was 24 hours of HRV monitoring, but many previous studies reported 5 minutes of HRV could be used except for very LF power [14]. Second, we have no normal value for the HRV variables and traditional CAN variables, including the E/I ratio, Valsalva ratio, and posture ratio, which are specific to the Korean population. In addition, ethnic differences in AFT are not confirmed [19, 41]. Third, this study had an observational cohort design. Fourth, this study did not show the relationship between the quality of diabetes control and HRV variables but between HRV variables and baseline HbA1c. Further studies are warranted to evaluate the association between long-term glycemic control status and HRV variables. But this study has plausible strength. This study is the largest study that showed the relationship between HRV and CAN and HRV and CVD in patients with T2DM and conducted long-term follow-up. In addition, our data support the effectiveness of HRV to predict CVD in patients with T2DM.

In conclusion, we investigated the importance of HRV variables on CV outcomes in patients with T2DM. Thus, we suggest that HRV assessment might be implemented and considered a diagnostic method of CV autonomic neuropathy in accordance with a diabetic complication study in patients with T2DM.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgement

None

List of abbreviations

CVD	cardiovascular disease
T2DM	type 2 diabetes
CHD	coronary heart disease

HbA_{1C}	glycated hemoglobin
CV	cardiovascular
CAN	cardiovascular autonomic neuropathy
HRV	heart rate variability
T1DM	type 1 diabetes
AFT	autonomic function test
FPG	fasting plasma glucose
pp2hr	postprandial 2 hour glucose
eGFR	estimated glomerular filtration rate
TP	total power
LF	low frequency
HF	high frequency
SDNN	normal-to-normal interval
RMSSD	root mean square of differences between adjacent R-R intervals
MI	myocardial infarction
WHO	World Health Organization
HR	hazard ratio
CI	confidence intervals
ms	millisecond
ARIC	Atherosclerosis risk in communities
NN	normal-to-normal

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Fig 1. Study outline

T2DM type 2 diabetes, AFT, autonomic function test.

Fig 2. The results of frequency-domain parameters of heart rate variability and cardiac autonomic neuropathy stage (estimated means with standard of error means).
CAN, cardiovascular autonomic neuropathy, LF, low field, HF, high field.

Fig 3. Cumulative hazard rate of the development of cardiovascular disease according to total power in patients with type 2 diabetes A. Total power (ms^2). (log-rank $p = 0.004$) B. LF power (ms^2) (log-rank $p = 0.008$) C. HF power (ms^2) (log-rank $p = 0.031$) LF, low frequency, HF, high frequency, ms, milliseconds.

Table 1.

Baseline characteristics according to the development of cardiovascular disease in patients with type 2 diabetes

	Total (n=508)	CVD (–) (n=459)	CVD (+) (n=49)	p value
Age (years)	54.9 ± 8.6	54.8 ± 8.8	55.6 ± 7.2	.537
Female	286 (56.3)	262 (57.1)	24 (49.0)	.277
Diabetic duration (years)	9.4 ± 7.3	9.3 ± 7.1	10.2 ± 8.4	.404
BMI (kg/m ²)	24.7 ± 3.5	24.8 ± 3.6	24.3 ± 2.9	.347
Alcohol	157 (32.6)	138 (31.8)	19 (40.4)	.231
Smoking (current)	105 (21.9)	92 (21.2)	13 (27.7)	.231
Hypertension	199 (39.2)	173 (37.7)	26 (53.1)	.036
DMR	70/458 (15.3)	54/412 (13.1)	16/46 (34.8)	< .001
SBP (mmHg)	123.4 ± 16.5	122.8 ± 16.3	128.9 ± 17.3	.046
DBP (mmHg)	81.4 ± 10.2	81.1 ± 10.1	84.1 ± 11.2	.090
Laboratory variables				
FPG (mg/dL)	158.9 ± 54.2	157.3 ± 53.9	173.6 ± 54.6	.049
pp2hr (mg/dL)	247.6 ± 82.4	244.9 ± 81.9	274.9 ± 83.1	.035
HbA1c (%)	8.4 ± 1.9	8.4 ± 1.9	8.9 ± 2.2	.106
eGFR (mL•min ^{−1} •1.73m ^{−2})	96.4 ± 26.4	97.0 ± 26.2	90.4 ± 27.1	.098
Total cholesterol (mg/dL)	187.2 ± 40.0	185.1 ± 37.7	206.8 ± 54.0	.008
Triglyceride (mg/dL)	138.8 ± 94.5	131.8 ± 80.5	203.8 ± 166.1	.004
HDL-C (mg/dL)	44.1 ± 9.9	44.3 ± 9.9	42.1 ± 9.1	.140
LDL-C (mg/dL)	109.1 ± 30.7	107.7 ± 30.3	117.9 ± 32.4	.094
C-peptide (ng/mL)	1.6 ± 1.0	1.6 ± 1.0	1.9 ± 1.0	.073
UAE (mg/g)	16.6 (9.7–45.0)	16.5 (9.6–41.8)	26.1 (11.0–111.0)	.071
Medication				
Insulin	126 (24.8)	110 (24.0)	16 (32.7)	.181
Sulfonylurea	289 (56.9)	261 (56.9)	28 (57.1)	.970
Metformin	329 (64.8)	298 (64.9)	31 (63.3)	.817
ACEi/ARB	163 (32.1)	143 (31.2)	20 (40.8)	.168
Beta blocker	15 (3.0)	13 (2.8)	2 (4.1)	.623
CCB	77 (15.2)	68 (14.8)	9 (18.4)	.510
Aspirin	75 (14.8)	63 (13.7)	12 (24.5)	.043
Statin	138 (27.2)	126 (27.5)	12 (24.5)	.658
Mean HR (beats/minute)	76.1 ± 12.1	75.8 ± 11.6	78.1 ± 16.3	.357
Time-domain parameters				
SDNN (milliseconds)	25.4 (18.1–33.7)	25.4 (18.6–33.1)	23.3 (13.7–34.8)	.362
RMSSD (milliseconds)	14.3 (10.0–21.8)	14.4 (10.3–21.8)	13.3 (7.3–23.3)	.236
Frequency-domain parameters				
TP (milliseconds ²)	309.5 (153.8–345.8)	309.9 (160.0–639.9)	291.3 (88.2–823.3)	.420
HF (milliseconds ²)	46.4 (19.9–104.9)	46.7 (20.2–107.0)	43.6 (11.3–102.2)	.379

	Total (n=508)	CVD (-) (n=459)	CVD (+) (n=49)	p value
LF (milliseconds ²)	62.1 (26.2–153.4)	62.3 (26.4–153.4)	58.4 (14.4–167.9)	.633
LF/HF Ratio	1.4 (0.7–2.9)	1.4 (0.7–2.9)	1.7 (0.6–3.4)	.873
CAN				
Abnormal E/I ratio	114 (22.4)	96 (20.9)	18 (36.7)	.012
Abnormal Posture ratio	105(20.7)	94 (20.5)	11 (22.4)	.746
Abnormal Valsalva ratio	83 (16.3)	67 (14.6)	16 (32.7)	.001
Orthostatic hypotension	48 (8.5)	37 (7.4)	11 (16.4)	.013
Stage				
Normal	281 (55.3)	262 (57.1)	19 (38.8)	.018
Early	165 (32.5)	146 (31.8)	19 (38.8)	
Definite	62 (12.2)	51 (11.1)	11 (22.4)	

Data are number (percentage), means $\pm$ SD, or median (interquartile range). $P < 0.05$ was considered significant. CVD cardiovascular disease, BMI body mass index, DMR diabetic retinopathy, SBP systolic blood pressure, DBP diastolic blood pressure, FPG fasting plasma glucose, pp2hr postprandial 2hr glucose, eGFR estimated glomerular filtration rate, HDL high-density lipoprotein, LDL low-density lipoprotein, UAE, urine albumin excretion, ACEi/ARB, ACE inhibitor/angiotensin receptor blocker, CCB, calcium channel blocker, SDNN, standard deviation of normal RR intervals, RMSSD, square root of the mean squared difference of successive RR intervals, TP, total power, HF, high-frequency, LF, low-frequency CAN, cardiovascular autonomic neuropathy.

Table 2.

Mean heart rate and time- and frequency-domain parameters of heart rate variability according to the CAN stage.

	Total (n=508)	Normal (n=281)	Early (n=165)	Definite (n=62)	p for trend
Mean HR (beats/minute)	76.1 ± 12.1	76.1 ± 11.4	74.5 ± 11.9	80.0 ± 15.1	.215
Time-domain parameters					
SDNN (milliseconds)	25.4 (18.1–33.7)	27.5 (20.2–35.5)	23.7 (17.9–32.8)	19.6 (11.9–28.7)	< .001
RMSSD (milliseconds)	14.3 (10.0–21.8)	16.1 (11.4–24.1)	12.8 (10.1–19.2)	10.5 (6.5–16.5)	< .001
Frequency-domain parameters					
TP (milliseconds ²)	309.5 (153.8–345.8)	383.2 (198.4–769.6)	262.1 (138.9–539.3)	173.9 (53.5–299.8)	.001
HF (milliseconds ²)	46.4 (19.9–104.9)	60.2 (26.2–140.4)	36.1 (18.8–75.4)	22.5 (6.3–60.4)	.001
LF (milliseconds ²)	62.1 (26.2–153.4)	89.8 (40.1–197.4)	50.5 (22.0–108.8)	20.6 (6.3–66.8)	< .001
LF/HF Ratio	1.4 (0.7–2.9)	1.7 (0.8–3.2)	1.4 (0.8–2.7)	1.0 (0.3–2.5)	.087

Data are means ± SD, or median (interquartile range). $P < 0.05$ was considered significant.

CAN cardiovascular autonomic neuropathy, HR heart rate, SDNN standard deviation of normal RR intervals, RMSSD square root of the mean squared difference of successive RR intervals, TP total power, LF low-frequency, HF high-frequency, LF/HF Ratio LF [milliseconds²]/HF [milliseconds²].

Table 3.

Hazard ratios and 95% CIs for the association between the lowest 10th percentile of heart rate variability variables and risk of cardiovascular disease in patients with type 2 diabetes during an 8-year follow-up

	Model 1	Model 2	Model 3
	HR (95% CI)	HR (95% CI)	HR (95% CI)
Mean HR			
HR 100	1.00	1.00	1.00
HR > 100	3.16 (1.24–8.03) ^a	3.12 (1.23–7.92) ^a	2.86 (1.10–7.39) ^a
Time-domain parameters			
SDNN			
10 th percentile	1.00	1.00	1.00
< 10 th percentile	3.14 (1.61–6.12) ^a	3.13 (1.60–6.10) ^a	2.62 (1.30–5.31) ^a
RMSSD			
10 th percentile	1.00	1.00	1.00
< 10 th percentile	2.87 (1.45–5.68) ^a	2.79 (1.41–5.53) ^a	2.40 (1.18–4.91) ^a
Frequency-domain parameters			
TP			
10 th percentile	1.00	1.00	1.00
< 10 th percentile	3.37 (1.70–6.67) ^a	3.32 (1.68–6.55) ^a	2.81 (1.37–5.79) ^a
LF power			
10 th percentile	1.00	1.00	1.00
< 10 th percentile	3.16 (1.57–6.36) ^a	3.24 (1.61–6.50) ^a	2.68 (1.28–5.59) ^a
HF power			
10 th percentile	1.00	1.00	1.00
< 10 th percentile	2.49 (1.23–5.02) ^a	2.49 (1.23–5.02) ^a	2.24 (1.09–4.59) ^a
LF/HF ratio			
10 th percentile	1.00	1.00	1.00
< 10 th percentile	2.07 (0.92–4.66)	2.11 (0.94–4.75)	1.81 (0.79–4.14)

Multivariable Cox proportional hazard models were adjusted for the following covariates:

model 1: sex, age and diabetes duration;

model 2: model 1 + HbA1c;

model 3: model 2 + CAN stage.

^a p < 0.05

HR heart rate, SDNN standard deviation of normal RR intervals, RMSSD square root of the mean squared difference of successive RR intervals, TP total power, LF low-frequency, HF high-frequency, LF/HF Ratio LF [milliseconds²]/HF[milliseconds²], CAN, cardiovascular autonomic neuropathy.

Table 4.

Characteristics of study participants according to quartile of total power

	Total (n=508)	1Q (n=127)	2Q (n=126)	3Q (n=128)	4Q (n=127)	p for trend
TP (ms ²)	309.5 (153.8–345.8)	89.7 (54.9–126.3)	226.8 (194.7–260.4)	428.1 (367.5–507.5)	997.9 (801.3–1642.9)	< .001
HF (ms ²)	46.4 (19.9–104.9)	14.1 (5.7–25.9)	37.3 (19.5–58.3)	61.7 (31.4–108.7)	179.8 (78.4–304.0)	< .001
LF (ms ²)	62.1 (26.2–153.4)	16.8 (7.4–29.8)	48.6 (26.7–68.7)	99.1 (58.3–154.1)	269.8 (147.0–419.7)	< .001
LF/HF Ratio	1.4 (0.7–2.9)	1.2 (0.5–2.3)	1.4 (0.7–2.5)	1.5 (0.8–3.3)	2.0 (0.8–3.9)	.018
Age (years)	54.9 ± 8.6	56.3 ± 8.1	55.4 ± 7.8	55.2 ± 9.3	52.6 ± 8.9	.001
Duration (years)	9.4 ± 7.3	11.5 ± 7.7	9.1 ± 6.8	9.6 ± 7.3	7.4 ± 6.7	< .001
Female	286 (56.3)	78 (61.4)	71 (56.3)	70 (54.7)	67 (52.8)	.160
Hypertension	199 (39.2)	48 (37.8)	51 (40.5)	54 (42.2)	46 (36.2)	.878
Laboratory variables						
FPG (mg/dL)	158.9 ± 54.2	166.5 ± 56.9	165.0 ± 55.8	149.8 ± 49.5	153.7 ± 52.7	.015
pp2hr (mg/dL)	247.6 ± 82.4	255.7 ± 86.0	253.5 ± 81.6	242.6 ± 88.7	238.7 ± 72.8	.086
HbA1c (%)	8.4 ± 1.9	8.9 ± 2.2	8.6 ± 1.9	8.1 ± 1.8	8.2 ± 1.7	.002
TC (mg/dL)	187.2 ± 40.0	186.3 ± 41.4	184.3 ± 35.0	185.8 ± 39.5	192.3 ± 43.6	.223
TG (mg/dL)	138.8 ± 94.5	140.7 ± 99.6	134.3 ± 85.8	131.4 ± 77.1	148.9 ± 112.3	.573
HDL-C (mg/dL)	44.1 ± 9.9	44.6 ± 9.0	43.7 ± 9.1	44.9 ± 10.2	43.2 ± 11.0	.418
LDL-C (mg/dL)	109.1 ± 30.7	110.6 ± 31.7	104.7 ± 29.5	103.0 ± 31.2	118.3 ± 30.7	.219
UAE (mg/g)	16.6 (9.7–45.0)	22.9 (10.9–71.4)	16.5 (9.9–42.9)	15.4 (9.3–31.2)	15.4 (9.0–33.9)	.674
CAN stage						
Normal	281 (55.3)	51 (40.2)	65 (51.6)	79 (61.7)	86 (67.7)	< .001
Early	165 (32.5)	47 (37.0)	43 (34.1)	41 (32.0)	34 (26.8)	
Definite	62 (12.2)	29 (22.8)	18 (14.3)	8 (6.3)	7 (5.5)	

Data are number (percentage), means ± SD or median (interquartile range), or n (%). $p < 0.05$ was considered significant. Q, quartile, TP, total power, ms, milliseconds, HF, high-frequency, LF, low-frequency, FPG, fasting plasma glucose, pp2hr postprandial 2hr glucose, TC, total cholesterol, TG, triglyceride, HDL high-density lipoprotein, LDL low-density lipoprotein, UAE, urine albumin excretion, CAN, cardiovascular autonomic neuropathy.