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Decreased heart rate variability may predict the progression of carotid atherosclerosis in type 2 diabetes

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Abstract Heart rate variability (HRV), a measure of autonomic function, can predict survival outcomes. Cardiovascular disease is a known complication of diabetes, and we aimed to determine if autonomic dysfunction was associated with carotid artery atherosclerotic plaques in type 2 diabetic patients. We assessed frequency domain HRV from power spectral analysis of 24 h Holter ECG recordings, expiration/inspiration (E/I) ratio during deep breathing, acceleration index (AI) of R–R interval in response to head-up tilt, and the degree of carotid artery atherosclerosis in 61 type-2 diabetic patients (39 males, 45–69 years). Studies were carried out 5–6 years after diagnosis (baseline) and repeated 8 years after diagnosis (follow-up). At baseline, patients diagnosed with autonomic neuropathy, with abnormal E/I ratio and abnormal AI measurements, had decreased low frequency (LF) HRV. Baseline E/I ratio correlated with day

($r = 0.34$; $P < 0.001$) and night-time ($r = 0.44$; $P < 0.001$) LF power. Night-time HRV did not differ in patient with and without autonomic neuropathy. Reduced common carotid artery diameter and atherosclerotic intima-media thickness (IMT) both correlated with HRV at baseline. At follow-up, all HRV variables decreased significantly. Furthermore, patients with lower LF power at baseline, had a larger increase in the thickness of the carotid bulb intima-media at follow-up. Our results show that LF HRV power is associated with the extent and progression of carotid atherosclerosis in type 2 diabetes. A low LF HRV may predict the progression of atherosclerosis in these patients.

Key words Type 2 diabetes mellitus · carotid atherosclerosis · autonomic neuropathy · heart rate variability

Introduction

Autonomic neuropathy is a serious complication of diabetes [13]. It is associated with increased risk of cardiac complications [2, 6, 11, 17, 32] and stroke [47]. Previous studies have demonstrated a link be-

tween autonomic neuropathy and the metabolic syndrome in diabetes [18, 20, 23, 44, 45].

Spectral analysis of heart rate variability (HRV) is a non-invasive way to measure cardiac autonomic activity. Low frequency (LF) oscillations (0.04–0.15 Hz) reflect both sympathetic and parasympa-

thetic innervation, whereas high frequency (HF) (0.15–0.40 Hz) oscillations indicate parasympathetic activity [1, 29, 34, 37, 38]. Reduced HRV is a feature of autonomic neuropathy. In people without diabetes, augmented inflammatory activity [39], arterial hypertension [40], and the incidence of cardiac events [26, 48] are all factors associated with reduced HRV. In type 1 [9] and type 2 diabetes [27] low HRV may be related to the presence of coronary heart disease and its risk factors.

The aim of this study was first, to clarify the relationship between autonomic dysfunction, and carotid artery atherosclerosis in type 2 diabetic patients, by determining whether HRV indices were related to the progression of atherosclerosis, and second to investigate the association between HRV and conventional autonomic function tests.

Methods

■ Patients

Sixty-one patients with Type 2 diabetes were recruited from a population-based study of the diabetes incidence in Malmö, Sweden [52]. Patients were assessed 5 years after the diagnosis of diabetes (baseline examination). Their ages ranged from 45 to 69 years (median 59 years).

Among them 39 (64%) were men. One year after the initial examination, all patients underwent carotid ultrasound examination for quantification of atherosclerosis. Eight years after diagnosis, all investigations were repeated (follow-up examination). Patient characteristics have been described in detail elsewhere [19], and brief summary is given in Table 1.

Informed consent was obtained from all subjects. The study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the University of Lund.

Table 1 Treatment and clinical features at baseline and at follow-up 3 years later

	Baseline	Follow up	P-value
Treatment (n (%))			
Antihypertensive	20 (33)	23 (38)	0.5696
Lipid lowering	2 (3)	11 (18)	0.0022
Insulin	3 (5)	7 (11)	0.1811
Sulphonylurea	34 (56)	38 (62)	0.5795
Biguanide	11 (18)	27 (44)	0.0028
<i>Clinical features</i>			
Retinopathy (n (%))	6 (10)	14 (23)	0.0179
Cr-EDTA clearance (ml/min)	106 ± 18	103 ± 20	0.0349
Acceleration index (SD)	−0.31 ± 1.03	−0.70 ± 1.07	0.0139
Expiration/inspiration ratio (SD)	−0.58 ± 1.04	−0.83 ± 1.03	0.1160
Blood pressure (mmHg)	139 ± 17/87 ± 10	147 ± 20/89 ± 9	0.1195/0.0330
BMI (kg/m ²)	29.3 ± 5.4	28.2 ± 4.6	0.0010
Fp-C-peptide (nmol/l)	1.00 ± 0.38	0.81 ± 0.35	0.0190
HbA1c %	7.68 ± 1.72	7.38 ± 1.44	0.0045
P-cholesterol (mmol/l)	5.78 ± 0.95	5.34 ± 1.04	0.0003
P-LDL (mmol/l)	3.72 ± 0.81	3.17 ± 0.76	<0.0001
P-HDL (mmol/l)	1.08 ± 0.26	1.27 ± 0.36	<0.0001
P-triglycerides (mmol/l)	2.26 ± 1.63	1.92 ± 1.10	0.0431

Comparison of the clinical characteristics of the 61 type 2 diabetes patients on the initial visit (5 years after the diagnosis of diabetes) and on follow-up (3 years later)

■ Measurements

Body mass index (BMI) was measured as weight (kg)/height(m²). Supine blood pressure was measured after 10 minutes rest, in the right upper arm using a sphygmomanometer.

Assessment of autonomic function

Heart rate variability (HRV): Long-term electrocardiogram (ECG) recordings were performed over 24 h using Marquette 8500 tape recorders (Milwaukee, WI, USA). Electrode positions similar to V1 and V5 were used. ECG recordings were analyzed with the Marquette Holter analysis system 8000, Heart Rate Variability software version 002A (Milwaukee, WI, USA). The Fast Fourier Transform technique was used for the assessment of frequency, domain analysis of HRV power spectral analysis. The sampling frequency was 128 Hz. The time series function consisted of sampling R–R intervals every 469 ms. (256 × 469 = 2 min). A spectral lot of 1 h, including the average of 30 spectra computed over 2-min periods (30 × 2 = 60 min), was employed. The power spectrum of HRV was divided into low frequency (LF) (0.04–0.15 Hz) and high frequency (HF) (0.15–0.40 Hz) bands [5]. In addition, total power (TP) (power in the band 0.01–0.40 Hz) was calculated. The integrals under respective power spectral density functions were measured, and expressed in absolute units after logarithmic transformation (ln (ms²)). The different parameters were analyzed for the whole 24 h period. In addition to the 24-h overall values, data from 1 h during the day (between 4 and 5 PM) and 1 h during the night (between 3 and 4 AM) are presented.

Deep breathing (R-R interval variation): Six maximal expirations and inspirations were performed over 1 min in the supine position. R–R interval was recorded throughout using continuous ECG. The E/I ratio, a index of parasympathetic vagal function, was measured as the mean longest R–R interval during expiration (E) divided by the mean shortest R–R interval during inspiration (I) [42].

The initial heart rate response to tilt: After 10 min of supine rest, the subject was passively tilted (within <2 s) to the upright position (at a 90° angle), and remained in this position for 8 min. The initial heart rate response to tilt, an immediate acceleration followed by a transient deceleration, was evaluated by continuous

Table 2 Outcome of autonomic function tests in 61 patients with type 2 diabetes

	AI abnormal (n = 7)	AI normal (n = 54)	E/I ratio abnormal (n = 7)	E/I ratio normal (n = 54)	AI or E/I ratio abnormal (n = 12)	AI and E/I ratio normal (n = 49)
Overall LF power	5.18 ± 0.59	5.40 ± 0.95	4.25 ± 0.60 ^b	5.51 ± 0.84	4.68 ± 0.77 ^b	5.57 ± 0.87
Overall HF power	3.67 ± 0.80	4.08 ± 0.84	3.26 ± 0.64 ^b	4.14 ± 0.81	3.58 ± 0.69 ^a	4.16 ± 0.85
Daytime LF power	4.71 ± 1.08	5.22 ± 1.28	3.70 ± 0.76 ^b	5.35 ± 1.18	4.19 ± 0.94 ^b	5.42 ± 1.24
Daytime HF power	3.00 ± 1.02	3.85 ± 1.19	2.92 ± 1.12	3.88 ± 1.15	3.02 ± 0.92 ^a	3.97 ± 1.20
Night-time LF power	5.50 ± 1.07	5.70 ± 1.21	4.62 ± 1.31	5.80 ± 1.10	5.21 ± 1.25	5.84 ± 1.14
Night-time HF power	4.41 ± 1.32	4.63 ± 1.11	4.14 ± 1.13	4.68 ± 1.11	4.55 ± 1.13	4.64 ± 1.14

Results of heart rate variation (HRV) by spectral analysis in subjects with and without disturbances in the acceleration index (AI), expiration/inspiration (E/I) ratio, or both at baseline. Values less than -1.64 SD (95% confidence interval, one-sided test) below the age-related reference values were considered abnormal. All measurements are $\ln/(\text{ms}^2)$, mean (standard deviation). LF = low frequency, HF = high frequency

^a $P < 0.05$ compared with corresponding group

^b $P < 0.01$ compared with corresponding group

ECG recording, and calculation of the acceleration index (AI). The AI, a measure of the initial change in R-R interval during tilt, was calculated as $(A-B)/A \times 100$, where A was the mean R-R interval before tilt, and B was the shortest R-R interval during the immediate acceleration [43]. The immediate postural increase in heart rate reflects withdrawal of parasympathetic nerve activity, and the later transient deceleration reinstatement of parasympathetic nerve activity. The AI evaluates sympathetic and parasympathetic nerve function [12, 45]. The repeatability of AI is 24% and the coefficient of variation for E/I ratio is 4.9% [19].

Definitions of abnormalities: The E/I ratio and AI were used to assess autonomic function. Data was expressed as age-corrected values (i.e., z-scores in standard deviations (SD)) [3, 15]. Values less than -1.64 SD (95% confidence interval, one-sided test) below the age-related reference values were considered abnormal.

Ultrasound investigation: Ultrasound investigation of the right carotid artery was performed using Acuson 128/XP10 (Acuson, Mountain View, CA, USA) with a linear array 7 MHz transducer. The degree of stenosis was assessed based on blood flow velocity at the location of maximum lumen diameter reduction. When no increase in flow velocity (change in Doppler shift) could be detected, the degree of stenosis was subjectively decided from eye-balling the plaque on-line and determining to what extent the plaque protruded into the lumen (maximum 30%, otherwise an increase in flow velocity should be possible to detect). From R-triggering in an ECG-tracing, end-diastolic images were recorded for off-line analysis of intima-media thickness (IMT) in the common carotid artery and the bulb of the carotid bifurcation. This previously described [49, 50] method with acceptable reproducibility [36] has been proven valid in measurements of atherosclerosis [35, 51].

Statistics

Differences between groups were evaluated with the Mann-Whitney U -test and differences over time within groups with the Wilcoxon's signed rank test. Partial correlations were evaluated, controlled for age. Tests were two-tailed and P -values < 0.05 were considered significant. Results are presented as mean \pm SD. StatView 4.5 (SAS Institute, Cary, NC, USA) was used for the statistical calculations.

Results

At baseline, patients with autonomic neuropathy, who had abnormal E/I ratio and AI, showed reduced LF

Table 3 Heart rate variability measurements at baseline and at follow-up 3 years later

	Baseline	Follow-up	P -value
Overall LF power	5.38 ± 0.92	5.22 ± 0.96	0.0044
Overall HF power	4.04 ± 0.85	3.97 ± 0.92	0.0250
Daytime LF power	5.16 ± 1.28	4.62 ± 1.17	< 0.0001
Daytime HF power	3.77 ± 1.20	3.38 ± 1.14	0.0073
Night-time LF power	5.70 ± 1.18	5.38 ± 1.15	0.0096
Night-time HF power	4.62 ± 1.12	4.30 ± 1.22	0.0018
Overall LF/HF ratio	1.36 ± 0.18	1.34 ± 0.21	0.6574
Daytime LF/HF ratio	1.44 ± 0.31	1.42 ± 0.27	0.7616
Night-time LF/HF ratio	1.26 ± 0.21	1.37 ± 0.62	0.1234

Frequency domain measurements of heart rate variability (HRV) calculated from 24 h Holter recordings, at baseline (5 years after diagnosis) and at follow up (3 years later) in 61 type 2 diabetic patients. Power measurements are $\ln/(\text{ms}^2)$. All data are mean (standard deviation). LF, low frequency; HF, high frequency

HRV (Table 2). In patients with abnormal AI alone, HRV was within normal limits. Moreover, baseline E/I ratio correlated with both day ($r = 0.34$; $P < 0.001$) and night-time ($r = 0.44$; $P < 0.001$) LF power. At night, HRV did not differ between patients with and without autonomic neuropathy, as defined by the conventional tests (Table 2).

All variables obtained during spectral analysis, including LF power during the daytime, had significantly deteriorated on the follow-up visit (LF at baseline $5.16 \pm 1.28 \ln/[\text{ms}^2]$, LF at follow-up $4.62 \pm 1.17 \ln/[\text{ms}^2]$; $P < 0.0001$). LF/HF ratios were unchanged (Table 3).

At baseline, overall LF ($r = -0.35$; $P = 0.012$) and HF ($r = -0.33$; $P = 0.013$) power, and night-time HF power ($r = -0.36$; $P = 0.007$) were inversely correlated with age. However, these correlations had disappeared at follow-up 3 years later. Several variables obtained during spectral analysis, in particular overall and daytime power correlated inversely with systolic blood pressure (Table 4).

Atherosclerosis (mean IMT, $r = -0.43$; $P = 0.001$, Fig. 1) in the common carotid artery correlated in-

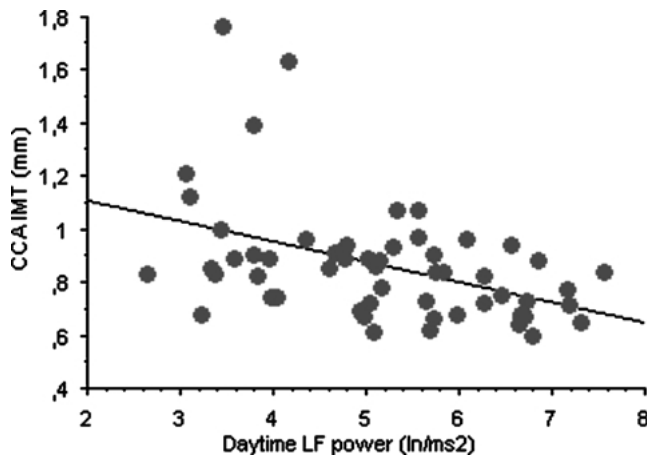


Fig. 1 The relationship between low frequency HRV and carotid artery atherosclerosis in type 2 diabetes. Daytime low frequency (LF) power of heart rate variability (HRV), calculated from 24 h Holter recordings, was inversely correlated with intima-media thickness (IMT) of the common carotid artery on initial investigation (5 years after diagnosis of type 2 diabetes). Patients with a greater carotid artery IMT had a lower daytime LF HRV ($r = -0.43$; $P = 0.001$). Data from 61 diabetes mellitus patients

versely with daytime LF power at baseline. Furthermore, a low LF power during daytime at baseline was related to a larger increase in the carotid bulb IMT during follow-up ($r = -0.39$; $P = 0.010$, Fig. 2), whereas there was no correlation at follow-up between daytime LF power and IMT.

Discussion

When relating conventional tests of autonomic function (E/I and AI) to HF and LF power spectral analysis of HRV, we found a large individual overlap for HF

and LF power in comparison with conventional test variables. No definite cut-off values for spectral analysis variables could therefore be proposed to identify patients with autonomic neuropathy, defined by classical criteria. This may well explain why spectral analysis has been found not useful, in comparison with conventional tests, for the assessment of autonomic neuropathy [30, 46]. On the other hand, spectral analysis is useful in the identification of early autonomic neuropathy [10, 16], the individuals at risk for life-threatening arrhythmias [10], and in the prediction of mortality after myocardial infarction [25] and chronic heart failure [33].

Our study demonstrated progressive decrease in HRV between 5 and 8 years after the diagnosis of diabetes. Low HRV has been shown to be related to diabetes duration, in a cross-sectional study of type 1 diabetic patients [9]. Our study shows, HRV decreases with the duration of disease in type 2 diabetes.

However, a decrease in HRV would be expected to occur due to increasing age alone. Therefore, the lack of a healthy control group is an important limitation of our study. Total power in HRV analysis in healthy subjects decreases by 30% between the age groups 20–29 and 60–69 years [22], comparable to the decrease of 3% in 3 years in our study. The decrease in daytime LF power of 10% in 3 years in our study is, therefore, greater than expected due to age alone.

A further limitation of our study was that 33% of our patients were on antihypertensive therapy. As concomitant hypertension is common in type 2 diabetes, it is hard to avoid the possible effect of antihypertensive medication when investigating autonomic function in type 2 diabetes. Nevertheless, we believe that the correlation between systolic blood pressure and HRV, combined with the fact that the correlation between age and HRV at baseline had

Table 4 Spearman's correlation coefficients (r); P -values) for correlations between results of spectral analysis of heart rate variability and metabolic variables in 61 type II diabetic patients

	HbA1c	F C-peptide	BMI	Systolic BP	Diastolic BP
<i>Baseline examination</i>					
Overall LF power	-0.081; 0.545	-0.153; 0.253	-0.229; 0.086	-0.399; 0.003	-0.152; 0.255
Overall HF power	-0.083; 0.534	-0.144; 0.280	-0.173; 0.195	-0.324; 0.015	-0.245; 0.067
Daytime LF power	-0.216; 0.106	-0.220; 0.100	-0.384; 0.004	-0.373; 0.005	-0.137; 0.306
Daytime HF power	-0.265; 0.047	-0.205; 0.124	-0.319; 0.017	-0.263; 0.049	-0.131; 0.328
Night-time LF power	0.012; 0.929	0.045; 0.739	0.043; 0.750	-0.189; 0.157	-0.550; 0.682
Night-time HF power	0.043; 0.746	0.088; 0.509	0.046; 0.731	-0.235; 0.079	-0.162; 0.225
<i>Follow-up examination</i>					
Overall LF power	0.053; 0.721	-0.168; 0.265	-0.895; 0.371	-0.384; 0.010	-0.088; 0.557
Overall HF power	0.015; 0.921	-0.161; 0.287	-0.792; 0.428	-0.267; 0.074	-0.049; 0.741
Daytime LF power	0.012; 0.934	-0.273; 0.072	-0.149; 0.316	-0.398; 0.008	-0.172; 0.249
Daytime HF power	-0.129; 0.385	-0.267; 0.076	-0.088; 0.556	-0.312; 0.037	-0.097; 0.514
Night-time LF power	0.063; 0.671	0.089; 0.555	0.099; 0.509	-0.165; 0.268	0.049; 0.743
Night-time HF power	0.035; 0.817	0.062; 0.683	0.122; 0.415	-0.142; 0.340	0.069; 0.644

BMI = Body mass index, BP = blood pressure

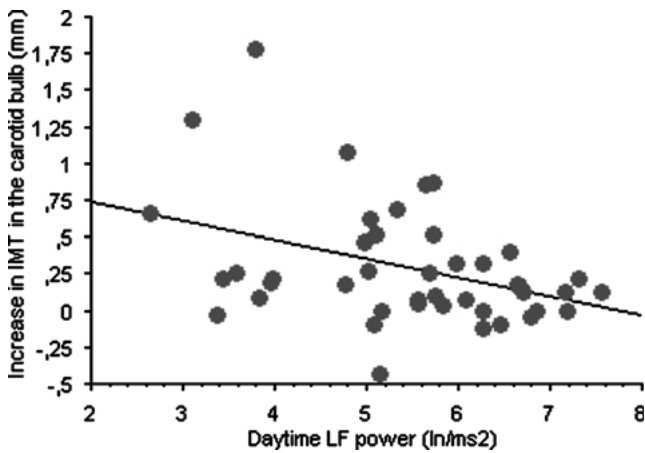


Fig. 2 The relationship between low frequency HRV and the progression of carotid artery atherosclerosis in type 2 diabetes. Daytime low frequency (LF) power of heart rate variability (HRV) was inversely correlated with the increase in intima-media thickness (IMT) of the carotid bulb (Δ -value). LF HRV was calculated from 24 h Holter monitoring 5 years after diagnosis of type 2 diabetes. Change in carotid IMT was determined from ultrasound measurements carried out 5 years after diagnosis (initial) and 3 years later (follow up). Patients with lower LF HRV had a greater increase in carotid artery intima-media thickness ($r = -0.39$; $P = 0.010$). Data from 61 patients with diabetes mellitus

disappeared at follow-up, may be explained by the progressive diabetic process disrupting the relationship between age and HRV.

Most importantly, we found relationships between the decreasing HRV and increasing carotid artery atherosclerosis among patients with type 2 diabetes. Indeed, there was a correlation not only between HRV and IMT at baseline, but also between HRV and the progression of IMT. These data are in agreement with the results of Liao and co-workers, who reported significant associations between lower HRV and coronary heart disease in both non-diabetic [26], and diabetic [27] subjects. Other investigators, however, have failed to demonstrate relationships between HRV and lower extremity vascular disease and mortality in diabetes [31].

We have shown in both this [44] and another [18] cohort that type 2 diabetic patients with autonomic neuropathy, as defined by an abnormal E/I ratio an established sign of parasympathetic neuropathy [4], display stigmata of the metabolic syndrome. This suggests that the metabolic syndrome may be the

underlying mechanism that affects the vasculature, causing autonomic neuropathy in type 2 diabetes. Spectral analysis of HRV gave further support for this concept.

LF power, a reflection of both sympathetic and parasympathetic activity [7], and baroreceptor buffering [53], during the daytime was correlated with common carotid IMT. This association may be explained by arterial wall stiffness [21]. The pathogenesis of carotid atherosclerosis may play a role in autonomic dysfunction in type 2 diabetes.

We have previously suggested that parasympathetic neuropathy favors several factors promoting atherogenesis; obesity, insulin resistance, and an increased glucose production from the liver [44]. Carotid artery atherosclerosis and increased IMT often occur together with atherosclerosis in coronary [28] and peripheral [8, 41] arteries, and increased carotid IMT predicts myocardial infarction and stroke [14]. The present study of relationships between autonomic neuropathy and carotid IMT was performed 5–8 years after the clinical diagnosis of diabetes, a disease in which macrovascular complications are known to develop steadily over time [24]. Follow-up of this cohort will reveal whether the higher carotid artery IMT is associated with autonomic neuropathy and the metabolic syndrome [18, 23, 44, 45]. Moreover, this will address if these associations increase the risk for clinically relevant atherosclerotic events as the diabetes progresses. This would be in accordance with Töyry's observation, that autonomic neuropathy predicts stroke in type 2 diabetic patients [47].

In conclusion, autonomic function evaluated using spectral analysis of HRV, was related to the extent and progression of carotid atherosclerosis 5–8 years after diagnosis in type 2 diabetic patients. Decreased LF HRV may predict the progression of atherosclerosis in these patients.

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