Predictors of Abnormal Cardiovascular Autonomic Function Measured by Frequency Domain Analysis of Heart Rate Variability and Conventional Tests in Patients With Type 1 Diabetes

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OBJECTIVE — Frequency domain analysis of heart rate variability (HRV) is used to assess cardiovascular autonomic function. There are no prospective data on the sensitivity of its various components to glycemia or other diabetes-related risk factors compared with conventional tests and with other complications of diabetes.

RESEARCH DESIGN AND METHODS — In 1985, possible risk factors of future complications were determined in 115 children with type 1 diabetes. In 1996, the presence of complications (HRV analysis, conventional tests of autonomic function, urinary albumin excretion rate [UAER], and retinopathy) were assessed in 83 of these patients (age 32 ± 1 years, duration of diabetes 22 ± 1 years).

RESULTS — Poor glycemic control (measured as lifetime glycemic exposure or HbA_{1c} in 1985) was the most important independent predictor of decreases in all measures of absolute power of HRV (total power [TP] and very low frequency, low frequency [LF], and high frequency [HF] power) and square root of the mean square of R-R interval differences but not of changes of normalized measures or ratios (normalized HF and LF, LF/HF). Other significant independent predictors of autonomic dysfunction were late age of onset of diabetes, female sex, and high BMI. To examine the sensitivity of the various tests to glycemia, the patients were divided into tertiles based on lifetime glycemic exposure (A_{1c} months). Glycemic exposure in the tertiles averaged 194 ± 25 A_{1c} months (20 years of HbA_{1c} 0.8% above normal), 556 ± 19 A_{1c} months(20 years of HbA_{1c} 2.3% above normal), and 963 ± 30 A_{1c} months (20 years of HbA_{1c} 4% above normal). Tests of complications that were significantly abnormal in patients already in the lowest tertile and were correlated with glycemia were TP and severity of retinopathy. Of conventional tests, only the ratio of length of R-R intervals during expiration to inspiration (E/I ratio) was significantly related to glycemic exposure, but it required high glycemic exposure (20 years of HbA_{1c} 4% above normal) to be abnormal. UAER was significantly increased only in the highest tertile of glycemic exposure.

CONCLUSIONS — TP and retinopathy score were much more sensitive to antecedent glycemia than conventional tests of autonomic function or UAER and were significantly abnormal in patients exposed to ~20 years' duration of an HbA_{1c} 0.8% above normal.

Diabetes Care 23:1686–1693, 2000

Diabetic autonomic neuropathy is associated with increased mortality (1,2), a prolonged QT interval (1), and cardiorespiratory arrest (1,2). The Diabetes Control and Complications Trial (DCCT) demonstrated conclusively that improved glycemic control reduces the development and progression of neurological complications in type 1 diabetes (3). In the DCCT, autonomic dysfunction was assessed by measuring R-R variability, the Valsalva ratio, and measurement of blood pressure in the supine and standing positions (4).

Clinically, most tests used to assess cardiovascular autonomic dysfunction, such as the Valsalva ratio and the handgrip and orthostatic tests, are time-consuming and require local standardization. Heart rate variability (HRV) analysis is a rapid noninvasive tool to assess cardiovascular autonomic dysfunction. It allows quantitation of total power (TP) of HRV and identification of very low frequency (VLF), low frequency (LF), and high frequency (HF) components (5,6). Sympathetic activation and tachycardia reduce TP of R-R interval variability, whereas the reverse is true for vagal activation, which is a major contributor to the HF component (6). Interpretation of the LF component is less straightforward and has been suggested to reflect the integrity of sympathetic and vagal nerves controlling HRV in the standing position and mainly vagal modulation in the supine position (6,7). In diabetic patients with predominantly vagal dysfunction, the HF component is reduced, whereas the presence of sympathetic dysfunction decreases VLF and LF components (5). There are at present no prospective data on predictors of abnormalities in the various components of HRV in type 1 diabetes.

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Received for publication 26 January 2000 and accepted in revised form 18 July 2000.

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Abbreviations: DCCT, Diabetes Control and Complications Trial; E/I ratio, ratio of length of R-R intervals during expiration to inspiration; ETDRS, Early Treatment of Diabetic Retinopathy Study; HF, high frequency; HFnorm, normalized high frequency component; HRV, heart rate variability; LF, low frequency; LFnorm, normalized low frequency component; RMSSD, square root of the mean square of R-R interval differences; TP, total power; UAER, urinary albumin excretion rate; VLF, very low frequency.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

In the present study, we hypothesized that poor glycemic control would predict abnormalities in one or several of the components of HRV. We wished to identify such component(s) and determine whether HRV components are more sensitive to glycemia than conventional tests of autonomic nervous function, urinary albumin excretion rate (UAER), or retinopathy.

RESEARCH DESIGN AND METHODS

Subjects

The patients were diagnosed with type 1 diabetes between 1968 and 1978. At diagnosis, the patients were <15 years old and living in Helsinki. In 1985–1986, glycemic control, signs (numbness, Achilles and patellar tendon reflexes, perception of pain, vibration, and temperature) and symptoms of diabetic neuropathy, sensory and motor nerve conduction velocities, UAER, and severity of diabetic retinopathy were assessed.

In 1996, the measurements performed 10 years earlier were repeated by the same investigator (A.S.), with the exception of the nerve conduction studies. In 1996, cardiovascular autonomic function tests were performed, and current and historical physical activity was recorded (8). Between 1986 and 1996, five male patients died: two because of cardiovascular causes (at ages of 22 and 31 years), and three because of unknown causes (at ages 29, 31, and 32 years). Three patients were excluded because of other illnesses or pregnancy. Another 5 patients had moved, and 19 patients could not be located. No patient had evidence of coronary heart disease (Minnesota codes A3.1.2.1, A3.1.2.4, A3.1.2.4, A3.1.2.5, and A3.1.2.7) in 1996. One patient in 1985 and 23 patients in 1996 were using one or several antihypertensive drugs (20 ACE inhibitors, 7 diuretics, 6 Ca^{2+} channel blockers, and 4 β-blockers). A total of 83 (75% of those alive from the original cohort) patients completed the follow-up study. There were 29 normal subjects (age 32 ± 1 years, blood pressure 120 ± 2/77 ± 2 mmHg, BMI 23.7 ± 0.6 kg/m^2, and HbA_{1c} 5.0 ± 0.2%) studied as a nondiabetic control group for the autonomic function tests. Informed written consent was obtained after the purpose, nature, and potential risks of the study were explained to the participants. The protocol was approved by the ethical committees of Helsinki City and Helsinki University Central Hospitals.

Glycemic control

Glycosylated hemoglobin (HbA_1) was measured until 1987 using microcolumn chromatography with Quick-Step columns (Isolab, Acron, OH). In 1987, the HbA_1 assay was replaced by measurement of HbA_{1c} using high-pressure liquid chromatography (Bio-Rad, Richmond, CA). In 1987, both HbA_1 and HbA_{1c} were measured, and these data were used to convert HbA_1 to HbA_{1c} values: $HbA_{1c} = -0.219 + 0.886 \times HbA_1$ ($r = 0.96$, $n = 110$, $P < 0.0001$). The intra-individual coefficient of variation of measured and calculated HbA_{1c} values was 7.0 ± 0.4%. Lifetime glycemic exposure was calculated by multiplying the number of HbA_{1c} units above normal by the number of months between the preceding and succeeding intervals (A_{1c} months) (9). At least one yearly HbA_{1c} measurement was available for 35% of the patients during the first 6 years of disease (lack of data was mostly due to diagnosis before the availability of HbA_1 measurements) and in 82% during years 7–18 of the disease.

Assessment of diabetic complications

Cardiovascular autonomic function tests (1996). The patients refrained from vigorous exercise for 24 h, from smoking and alcohol for 12 h, and from eating and drinking caffeine for 2 h before the examination. The examination was postponed in case of hypoglycemia 24 h before the test. Tests were performed with patients in the supine position, except for the orthostatic test, which was performed in the standing position, and the controlled breathing test, which was performed in supine and standing positions. The tests were performed, as previously described in detail (10), in the following order: controlled and deep breathing test, Valsalva test, isometric handgrip test, and orthostatic test (11). The HRV analysis (12) was done using the CAFTS-system (Medicro Oy, Kuopio, Finland). The signal powers were calculated as integrals under the respective part of the power spectral density function and were expressed in absolute units (square milliseconds) and as a ratio (LF/HF) thought to measure "sympathovagal balance" (13). To obtain components independent of total variability, LF and HF components were normalized (LFnorm and HFnorm) by dividing them by TP−VLF (6). In RESULTS, only data on parameters recorded in the supine position are presented because glycemic exposure predicted abnormalities in these parameters slightly better than in those recorded in the standing position (data not shown). R-R and QT intervals were measured from resting electrocardiogram tracings (lead V_5) (14,15).

Peripheral neuropathy (1985). Motor and sensory nerve conduction velocities were measured using a Neuromatic 2000 DISA electromyography unit (Dantec Electronics, Bristol, U.K.). Sensory nerve action potentials were recorded using surface electrodes (Dantec 13 K 60 and L 37), and antidromic sensory conduction velocities of sural, ulnar, median, and radial nerves were measured (16). The peroneal, median, and ulnar compound motor action potentials and conduction velocities were measured using conventional methods (17,18).

Retinopathy. Fundus photographs were taken through maximally dilated pupils in 1985 and 1996. Quantitation of the severity of retinopathy was done using the Early Treatment of Diabetic Retinopathy Study (ETDRS) (19) by the same ophthalmologist (P.Su.).

Nephropathy. In 1985, one, and in 1996, three timed overnight urine collections were performed to determine the UAER using radioimmunoassay and an antiserum against human albumin (Albumin antiserum; Orion Diagnostica, Espoo, Finland). Microalbuminuria was defined as a rate of 20–200 μg/min, and macroalbuminuria as a rate >200 μg/min in at least two consecutive urine samples (20).

Other measurements. Concentrations of total and HDL cholesterol in serum were determined by enzymatic colorimetric assays (F. Hoffman La Roche, Basel, Switzerland) using an autoanalyzer (Cobas Mira; F. Hoffman La Roche).

Statistical methods

Data between groups were analyzed using analysis of variance followed by pairwise comparison using Fisher's least significant differences test. Non-normally distributed data were analyzed using Kruskal-Wallis analysis of variance. TP, LF, HF, LF/HF, LFnorm, HFnorm, square root of the mean square of R-R interval differences (RMSSD), Valsalva ratio, and UAER were log-transformed because of their non-normal distribution before analysis. The χ^2 test was used to compare the prevalence of symptoms of peripheral neuropathy in 1985 and 1996. Simple correlations were calculated using Spearman's nonparametric rank correlation coefficient. Multivariate linear regression analysis was used to analyze the causes of

Table 1—*Characteristics of type 1 diabetic patients at the time of the first examination in 1985 and the second examination in 1996 and characteristics of the patients divided into three groups according to A_{1c} months*

	All patients		Tertile of glycemic exposure (1985)		
	1985	1996	Lowest	Middle	Highest
n	83	83	28	28	27
Sex (M/F)	43/40	43/40	14/14	16/12	13/14
Age at onset of diabetes (years)	9 ± 1	—	11 ± 1	9 ± 1	8 ± 1¶
Duration of diabetes (years)	11 ± 1	22 ± 1	10 ± 1	11 ± 1	13 ± 1#††
Age (years)	22 ± 1	32 ± 1	20 ± 1	20 ± 1	21 ± 1
Height (cm)	169 ± 1	172 ± 1*	169 ± 2	170 ± 2	169 ± 2
Weight (kg)	64 ± 1	74 ± 1*	61 ± 2	67 ± 2	64 ± 2
BMI (kg/m^2)	22.2 ± 0.3	25.0 ± 0.3*	21.4 ± 0.4	22.9 ± 0.4‡	22.3 ± 0.5
HbA_{1c} (%)	9.3 ± 0.2	8.5 ± 0.1*	8.2 ± 0.2	9.3 ± 0.3§	10.5 ± 0.3#‡‡
A_{1c} months	—	566 ± 37 (0–1,307)	194 ± 25 (0–407)	556 ± 19 (413–758)‖	963 ± 30 (784–1,307)#‡‡
Insulin dose (U/day)	51 ± 1	52 ± 2	45 ± 3	55 ± 3§	54 ± 2**
Insulin dose (U · kg^{-1} · day^{-1})	0.81 ± 0.02	0.71 ± 0.02*	0.75 ± 0.04	0.82 ± 0.03	0.86 ± 0.04¶
Serum cholesterol (mmol/l)	4.8 ± 0.1	4.9 ± 0.1	4.5 ± 0.1	4.7 ± 0.3	5.0 ± 0.3
Serum HDL cholesterol (mmol/l)	1.4 ± 0.04	1.4 ± 0.03	1.5 ± 0.07	1.4 ± 0.07	1.4 ± 0.05
Serum creatinine (μmol/l)	75 ± 1	91 ± 3*	75 ± 2	74 ± 2	75 ± 3
UAER (μmol/l)	8 (4–26)	8 (5–17)	5 (3–7)	11 (7–33)§	28 (9–67)#
Systolic blood pressure (mmHg)	125 ± 1	125 ± 2	123 ± 3	124 ± 2	127 ± 3
Diastolic blood pressure (mmHg)	83 ± 1	79 ± 1†	79 ± 2	84 ± 2	86 ± 2**
Heart rate (beats/min)	75 ± 1	72 ± 1†	72 ± 2	75 ± 2	79 ± 3
Corrected QT (ms)	351 ± 10	351 ± 7	379 ± 12	346 ± 21	328 ± 15
ETDRS score	21 ± 2	39 ± 2*	13 ± 1	19 ± 3	30 ± 3#††

Data are *n*, means ± SEM, means ± SEM (range), or medians (25–75% interquartile range). Patients who participated in both examinations are included. Glycemic exposure was calculated as A_{1c} months (see RESEARCH DESIGN AND METHODS). Reference range for HbA_{1c} is 4–6%. Reference range for serum creatinine is 50–110 μmol/l for women and 55–115 μmol/l for men. In 1985, *n* = 33 for corrected QT. An ETDRS score for grading of severity of diabetic retinopathy of ≤10 is normal (19). *$P < 0.001$, †$P < 0.05$ for changes in parameters between 1985 and 1996; ‡$P < 0.05$, §$P < 0.01$, ‖$P < 0.001$ for middle vs. lowest groups of glycemic exposure; ¶$P < 0.05$, #$P < 0.001$, **$P < 0.01$ for lowest vs. highest; and ††$P < 0.01$, ‡‡$P < 0.001$ for middle vs. highest.

variation in parameters of autonomic function and selected study variables. Normally distributed data are expressed as means ± SEM. For non-normally distributed data, median (25–75% interquartile range) values are given.

RESULTS

Demographics, glycemic control, lipids, and prevalence of nephropathy and retinopathy

First examination in 1985. Patient characteristics are shown in Table 1. Of the patients, 3 were prepubertal, 7 were pubertal, and 73 were adults. Normoalbuminuria was found in 70%, microalbuminuria in 23%, and macroalbuminuria in 7%. The retinopathy score was normal (≤10) in 53% of the patients.

Follow-up examination in 1996. Glycemic control had improved from an HbA_{1c} of 9.3 ± 0.2% in 1985 to 8.5 ± 0.1% in 1996 ($P < 0.001$). Mean of all HbA_{1c} measurements during the follow-up period averaged 9.1 ± 0.1% for the entire group: 9.3 ± 0.2% for women and 9.0 ± 0.2% for men (NS). Cumulative glycemic exposure ranged between 0 and 1,307 A_{1c} months, with a mean of 566 ± 37 A_{1c} months, and did not differ between women and men (586 ± 56 and 548 ± 50 A_{1c} months, respectively).

In 1996, 74% of the patients had normoalbuminuria, 14% microalbumin-

Table 2—*Multiple linear regression analysis of determinants of abnormalities in tests measuring autonomic nervous function in patients with type 1 diabetes*

	Significant independent variables					
Dependent variable	HbA_{1c}	Sex	Age of onset	Age	BMI	r^2 (%)
Measures of absolute power of HRV						
TP (ms^2)	0.00001	—	0.001	—	—	41
VLF (ms^2)	0.0001	—	—	—	0.04	41
LF (ms^2)	0.0001	0.05	0.005	—	—	41
HF (ms^2)	0.0001	—	0.0004	—	0.05	40
RMSSD (ms)	0.00009	—	0.0004	—	—	35
Normalized measures and ratios						
LFnorm	—	0.0004	0.005	—	—	22
HFnorm	—	0.0005	—	—	0.007	22
LF/HF	—	0.00003	0.03	—	—	25
Conventional tests						
E/I ratio	0.0004	—	—	0.02	—	19
Valsalva ratio	—	0.02	—	—	0.04	19

Data are *P* values unless otherwise indicated. Data for HbA_{1c} were measured in 1985.

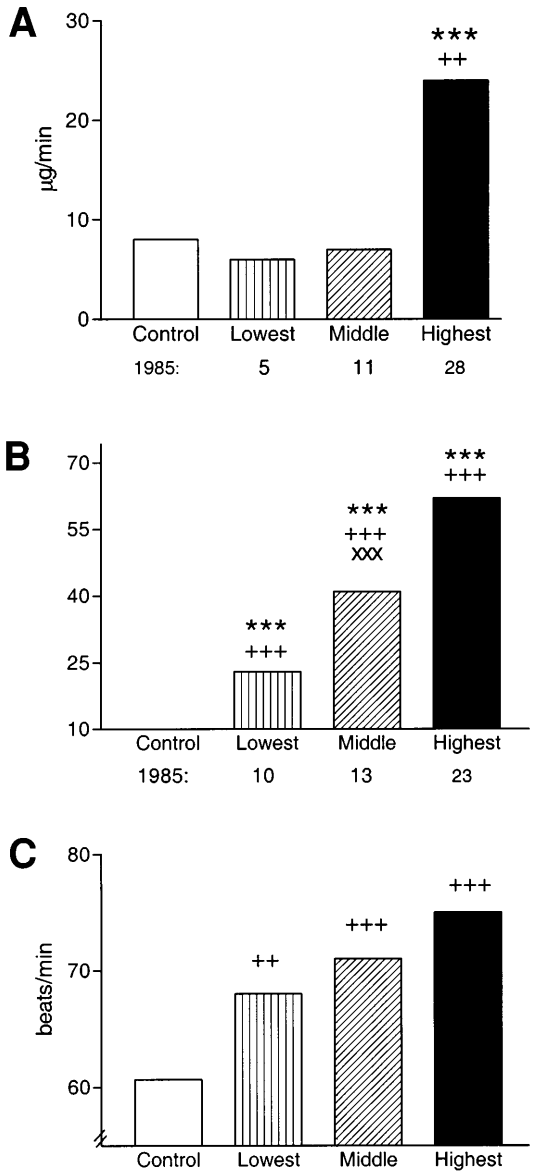

Figure 1—*Median values of UAER (A), diabetic retinopathy severity score (ETDRS) (B), and heart rate (C) in 1996 in normal subjects (Control) and patients with type 1 diabetes divided into tertiles according to glycemic exposure (A_{1c} months). The median values for each measures in patients with type 1 diabetes in 1985 are given under the x-axes.* $^{***}P < 0.001$ *for patients with highest vs. middle and lowest glycemic exposure;* $^{++}P < 0.01$, $^{+++}P < 0.001$ *for normal subjects vs. patients with type 1 diabetes;* $^{xxx}P < 0.001$ *for patients with middle vs. lowest glycemic exposure.*

uria, and 12% macroalbuminuria (NS vs. 1985). Serum creatinine had increased significantly by 21% during the 10-year period. An increase in muscle mass might have contributed to this increase, since both body height and weight increased significantly during the follow-up period (Table 1). The prevalence of retinopathy had increased significantly: in 1996, the ETDRS score was abnormal in 91% of the patients (Table 1).

Neuropathy

Frequency of abnormalities by different criteria and tests. Significant progression was observed in the symptom score (0 = no symptoms, 5 = each of the following: neurogenic impotence, gastroparesis, bladder atony, excessive sweating, and dysphagia) given in 1996 (0.5 ± 0.1) compared with 1985 (0.3 ± 0.1, $P < 0.01$). The percentage of patients with absent Achilles tendon reflexes had also increased significantly (44 vs. 63%, $P < 0.02$) from 1985 to 1996. Using the classification scheme of Ewing et al. (21), 3 of 83 patients (4%) had an abnormal Valsalva ratio (<1.20), 18 of 83 (22%) had a blunted (<10 mmHg) increase in diastolic blood pressure during sustained handgrip, and 5 of 83 (6%) had an excessive postural fall in systolic blood pressure (greater than –30 mmHg) in 1996.

Spectral power components of HRV during controlled breathing and measured in the supine position for female and male patients, respectively, with type 1 diabetes were as follows [median (25–75% interquartile range)]: VLF, 239 (107–434) and 398 (189–715) ms^2; LF, 120 (46–321) and 314 (178–630) ms^2; HF, 212 (45–735) and 289 (103–865) ms^2; TP, 782 (330–1535) and 1319 (644–2241) ms^2; and RMSSD, 23 (10–38) and 27 (15–43) ms.

Predictors of autonomic dysfunction

In simple regression analysis, the strongest predictor of most abnormalities in the tests of autonomic nervous function was HbA_{1c} measured in 1985 (VLF, HF, RMSSD: $r = -0.39$; LF, TP: $r = -0.45$; $P < 0.001$ for all). Other significant predictors were BMI, age of onset of diabetes, sex, age, and serum cholesterol (data not shown). In multiple linear regression analysis (Table 2), the 1985 HbA_{1c} value independently predicted all measures of absolute power of HRV but not LFnorm, HFnorm, or LF/HF. Of the conventional tests, the 1985 HbA_{1c} value only predicted an abnormal ratio of length of R-R intervals during expiration to inspiration (E/I ratio). The 1985 HbA_{1c} value also independently predicted abnormal TP within patients using antihypertensive drugs (data not shown). Current or historical physical activity did not correlate with measures of autonomic function (data not shown).

Glycemic exposure required to develop complications

Figures 1–3 show the prevalence of various complications in 1996 as a function of tertiles of glycemic exposure (Table 1). Both retinopathy and autonomic dysfunction, quantitated by the ETDRS and heart rate, increased linearly as a function of worsening glycemia, whereas UAER was only increased in the highest tertile (Fig. 1). All measures of absolute spectral power decreased as glycemia worsened (Figs. 2 and 3). When normalized to TP − VLF, HFnorm was lower, and the LFnorm and LF/HF were significantly higher in the patients than in the control subjects. These latter changes may possibly explain the increase in heart rate.

LFnorm, HFnorm, and LF/HF were not related to glycemic exposure.

CONCLUSIONS — We compared the sensitivity of various components of HRV, conventional tests of autonomic function, retinopathy, and UAER to lifetime glycemia. Of all the tests studied, TP was best correlated with glycemia in simple and multiple regression analyses. In addition to TP, its components VLF, LF, and HF were also inversely related to glycemia. Glycemic exposure did not predict abnormalities in either HFnorm, LFnorm, or LF/HF, although the latter was significantly increased in the diabetic patients. Thus, TP seems to be the parameter most sensitive to glycemia in type 1 diabetes.

We confirmed previous data showing that age, sex, and obesity contribute to variation in some measures of HRV. In healthy subjects, aging decreases spectral power of all HRV components but, according to most studies, does not change LF/HF (7). In type 1 diabetic patients, age was a significant independent determinant only of the E/I ratio in multiple linear regression analysis (Table 2). A novel finding was that a young age of onset of type 1 diabetes independent of glycemic control, sex, serum cholesterol, diabetes duration, and BMI predicted more normal results of several parameters, including TP, LF, HF, RMSSD, LFnorm, and LF/HF. This remained true even when age was included in the model (Table 2). Thus, although patients with young age of onset had greater glycemic exposure, they had less severe autonomic dysfunction than those with similar glycemic exposure and late age of onset. This could be due to a generally better ability of nerves to regenerate and remodel in young compared with old subjects (22,23).

Female sex was associated with a higher HFnorm, a lower LFnorm, and a lower LF/HF in normal subjects and patients with type 1 diabetes. TP was not influenced by sex. The data are consistent with those of the Pittsburgh Epidemiology of Diabetes Complications Study (24) and with cross-sectional data from nondiabetic patients (25–27). A reduction in both parasympathetic and sympathetic control of HRV is associated with obesity (28–30) and weight gain (31) in nondiabetic subjects. In the DCCT, greater body weight was related to the presence of cardiovascular autonomic dysfunction as measured by R-R variability (32). Similarly, in the type 1 diabetic patients studied here, BMI was inversely and independently correlated with the VLF, HF, and HFnorm components (Table 2). The relationship between BMI and the LF component was of borderline significance in multiple linear regression analysis. The Pittsburgh study also found LDL cholesterol to predict abnormalities in autonomic nervous function (24). In the present study, serum cholesterol measured in 1985 was significantly but weakly correlated with several measures of autonomic nervous function. However, when included in the same model with HbA_{1c}, serum cholesterol was no longer a significant predictor of abnormal test results (Table 2), suggesting that the relationship between cholesterol and autonomic nervous function might have been mediated via glycemic control.

The present data extend previous results by analyzing which parameter of spectral power analysis of HRV is most sensitive to hyperglycemia. Both of the 1985 HbA_{1c} and glycemic exposure measures were independent predictors of TP and all of its components when expressed in absolute units of power (square milliseconds) and RMSSD. Indeed, the present data suggest that identification of the different spectral components is not necessary to detect an effect of chronic hyperglycemia on cardiovascular autonomic function. The correlations between the 1996 HbA_{1c} and components of HRV (data not shown) were weaker than those for the 1985 HbA_{1c}, indicating that past glycemic control was a more important determinant of abnormalities in spectral power analysis than was glycemia prevailing during the 3 months before testing. Glycemic control did not predict an increase in LFnorm or a decrease in HFnorm or LF/HF, most likely because the normalization procedure involved division of LF and HF by TP, which was the factor most influenced by chronic hyperglycemia. The normalization process revealed, however, that in all tertiles of glycemia, parasympathetic nervous function was on average more defective than sympathetic function, resulting in relative sympathetic overactivity. This result is consistent with a model of diabetic autonomic dysfunction in which loss of parasympathetic nervous function precedes loss of sympathetic function (33). If loss of both occurs, heart rate starts to decline and becomes fixed. This may explain the poorer correlation between past glycemia and heart rate ($r = 0.19$, NS) than any measure of HRV (in absolute units) ($r = -0.39$ to -0.45, $P < 0.001$).

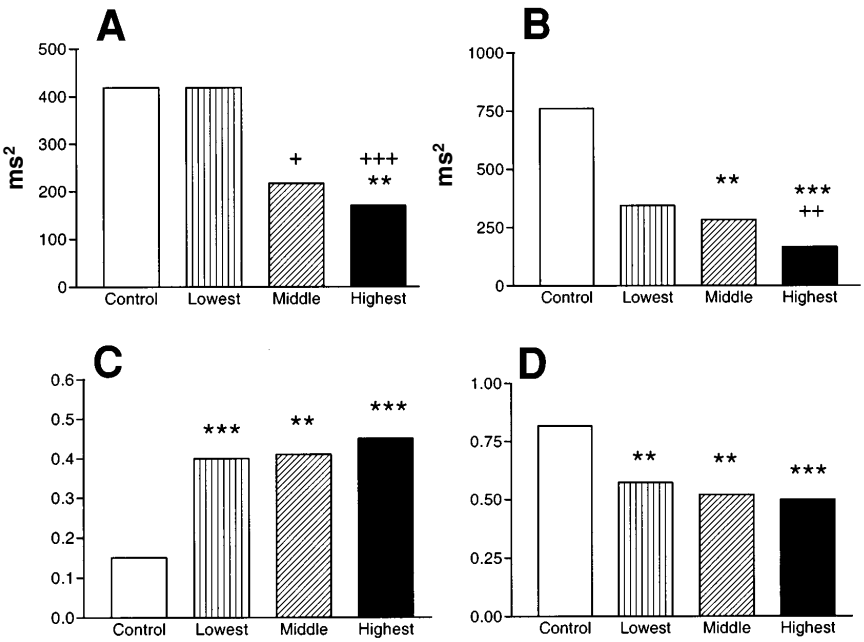

Figure 2—*Median values of LF (A) and HF (B) components in spectral analysis and their normalized (by adjusting for TP−VLF) forms, LFnorm (C) and HFnorm (D) in normal subjects (Control) and patients with type 1 diabetes divided into tertiles according to glycemic exposure (A_{1c} months). $^{+}P < 0.05$ for patients with middle vs. lowest glycemic exposure; $^{+++}P < 0.001$; $^{++}P < 0.01$ for highest vs. lowest glycemic exposure; $^{**}P < 0.01$, $^{***}P < 0.001$ for normal subjects vs. patients with type 1 diabetes.*

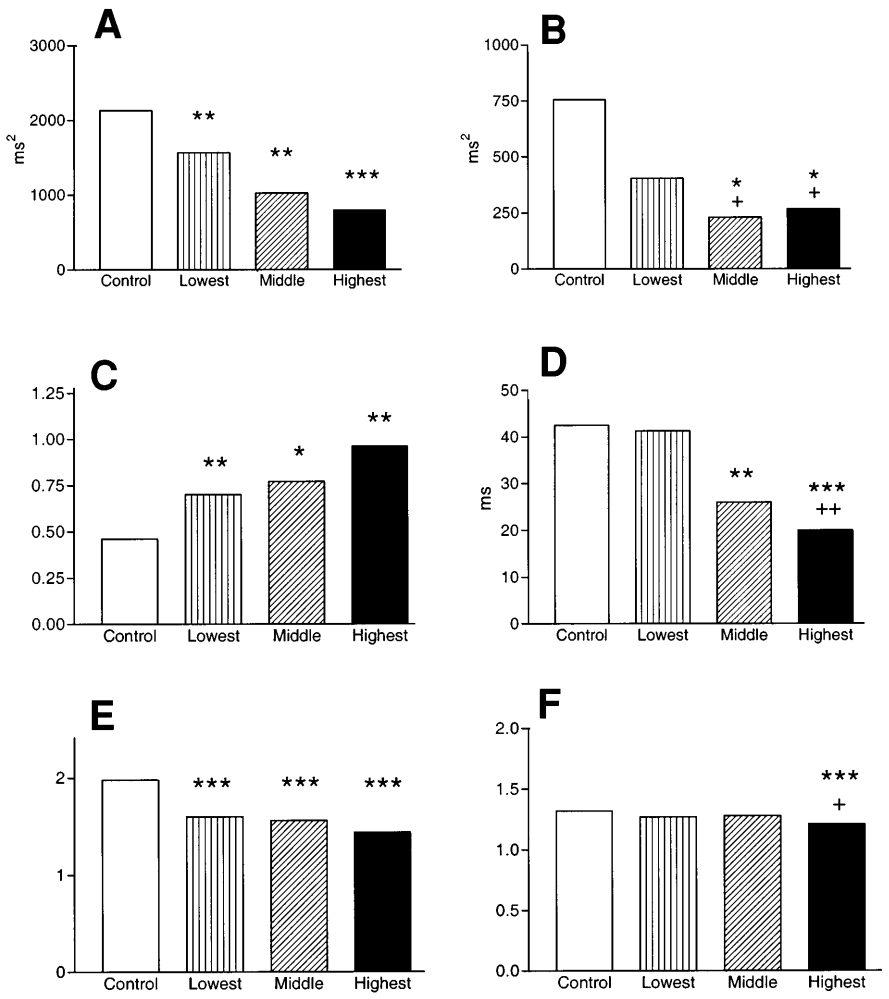

Figure 3—*Median values of TP (A), VLF (B), and LF/HF (C) of HRV; RMSSD (D); values of the Valsalva ratio (E); and E/I ratio (F) in normal subjects (Control) and patients with type 1 diabetes divided into tertiles according to glycemic exposure (A_{1c} months). $^{**}P < 0.01$, $^{***}P < 0.001$ for normal subjects vs. patients with type 1 diabetes; $^{+}P < 0.05$, $^{++}P < 0.01$ for patients with lowest vs. highest or middle glycemic exposure.*

Loss of Achilles tendon reflexes and slowing of sensory and motor nerve conduction velocities measured in 1985 were correlated with decreases in spectral power of HRV in 1996. Also, in 1996, loss of Achilles tendon reflexes and vibration perception and symptoms of autonomic dysfunction correlated with measures of absolute spectral power. These data are consistent with other data showing that peripheral somatic neuropathy correlates with cardiovascular autonomic dysfunction in patients with type 1 diabetes (34–39).

Autonomic dysfunction was related to the severity of retinopathy and nephropathy, as in several previous cross-sectional studies (32,40–46). Regarding the prospective data, we calculated glycemic exposure as suggested by Orchard et al. (9). In their study, retinopathy, nephropathy, and neuropathy, defined as the odds ratio of proliferative retinopathy, microalbuminuria, overt nephropathy, and distal symmetric polyneuropathy, were plotted against quintiles of glycemic exposure. The relationship between glycemic exposure and proliferative retinopathy was linear, and the threshold for proliferative retinopathy was ~600 A_1 months (9). In the present study, the ETDRS score was used to quantify all signs of diabetic retinopathy. As shown in Fig. 1, in the lowest tertile and with a mean of 194 A_{1c} months, the ETDRS score was already significantly increased, consistent with this score being a more sensitive measure of retinopathy than the occurrence of proliferative retinopathy. As in the Pittsburgh study (9), the relationship between glycemic exposure and retinopathy was linear. Our data are also remarkably similar with respect to the occurrence of microalbuminuria. In the Pittsburgh study, a significant increase in microalbuminuria was not observed except in the highest quintile, with >1,000 A_1 months (9). In the present study, only the highest tertile, with an average of 963 A_{1c} months, had an increased UAER (Fig. 1). Whether this difference between retinopathy and nephropathy is because the UAER is insensitive to glycemia and regulated by nonglycemic factors (47–49) cannot be determined. The 1,000–A_1 month threshold implies that it takes 21 years of duration at 4% above normal HbA_{1c} for even a modest increase (median within the microalbuminuric range, Fig. 1) in UAER to occur (9). These data question whether it is cost-effective to determine UAER yearly in patients with type 1 diabetes. Regarding neuropathy, there was a continuous linear relationship between glycemia and all measures of absolute spectral power of HRV (TP, HF, LF, and VLF). Thus, a significant decrease in TP was observed in the lowest tertile, with a mean of 194 A_{1c} months or 20 years' duration at 0.8% above normal HbA_{1c}. This result differs from data in the Pittsburgh study, in which only the highest quintile with >1,000 A_1 months had a significant increase in neuropathy, defined as the presence of distal symmetrical polyneuropathy. This suggests that spectral power of HRV (Figs. 2 and 3) decreases before the development of classic signs of autonomic neuropathy. The Valsalva ratio was not independently correlated with glycemia (Table 2), in keeping with DCCT data, in which changes in glycemia failed to alter the Valsalva ratio (50). The E/I ratio was influenced by glycemia (Table 2), but a significant decrease in the ratio was only observed in the highest glycemic tertile (Fig. 3).

In conclusion, we found TP and the ETDRS score to be the most sensitive measures of diabetes complications. More than four times less glycemic exposure (20 years' duration with HbA_{1c} 0.8 vs. 4% above the upper limit of normal) was required to detect significant abnormalities in these parameters than in other measures, such as the conventional E/I ratio or UAER. Measurement of TP seems to be a useful method that can be performed by any operator in 5 min. However, normal values for TP still need to be established, and HRV analysis requires special equipment.

Acknowledgments — This study was supported by grants from the Academy of Finland, the Sigrid Juselius Foundation (H.Y.-J.), the Finnish Cultural Foundation (A.S.), and the Diabetes Research Society (A.S.).

We thank Jorma Mäenpää, MD, Niilo-Pekka Huttunen, MD, Mikko Syvänne, MD, and the volunteers for their help. Marja Haikola and Kati Tuomola are acknowledged for excellent technical assistance.

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