Sympathetic and Parasympathetic Neuropathy Are Frequent in Both Type 1 and Type 2 Diabetic Patients

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OBJECTIVE — The aim of this study was to evaluate the frequency of sympathetic versus parasympathetic neuropathy among type 1 and type 2 diabetic patients.

RESEARCH DESIGN AND METHODS — There were 43 patients with type 1 and 17 with type 2 diabetes who were investigated. Sympathetic nerve function was assessed by measurement of the vasoconstriction (VAC) index by laser Doppler perfusion imaging of a locally heated finger followed by indirect cooling. Parasympathetic nerve function was assessed by R-R interval variation during deep breathing as measured by the expiration/inspiration (E/I) ratio. Results were expressed as age-corrected z scores in SD; VAC index >1.64 SD and E/I ratio <−1.64 SD were considered abnormal.

RESULTS — VAC index was abnormal in 40% with type 1 and 41% with type 2 diabetes, whereas the E/I ratio was abnormal in 42% with type 1 and 65% with type 2 diabetes. There was a clear association between VAC index and E/I ratio among type 1 ($r_s = 0.525$; $P = 0.0002$) but not among type 2 ($r_s = 0.10$) diabetic patients. Among type 2 diabetic patients, the degree of dysfunction was most severe regarding parasympathetic function ($P = 0.0167$).

CONCLUSIONS — Sympathetic and parasympathetic neuropathy were frequent in both type 1 and type 2 diabetic patients. However, there was a difference between the two types of diabetes. Sympathetic and parasympathetic nerve functions correlated in type 1 but not in type 2 diabetic patients. The explanation for this discrepancy might be that parasympathetic nerve function was most severely affected among type 2 diabetic patients.

Diabetes Care 27:2936–2941, 2004

Diabetic autonomic neuropathy is associated with an increased mortality (1–3). Autonomic neuropathy includes both parasympathetic and sympathetic dysfunction. Prospective studies on patients after myocardial infarction suggest that parasympathetic neuropathy is related to increased cardiovascular mortality (4,5). Recent studies indicate, however, that sympathetic neuropathy may be important as well. Among diabetic patients, sympathetic denervation impairs myocardial coronary blood flow (6) and myocardial contractility (7). To clarify the relationship between autonomic neuropathy and cardiovascular morbidity and mortality, it is important to evaluate both parasympathetic and sympathetic nerve functions.

Reduced R-R interval variation, as measured by the expiration/inspiration (E/I) ratio during deep breathing, is a reliable sign of parasympathetic neuropathy with a coefficient of variation of 4.8% (8,9). Recently, prolonged registration of electrocardiogram up to 24 h with spectral analysis of the R-R intervals by time domain or frequency domain analysis has come into use (10). However, there is a close correlation between parasympathetic neuropathy as demonstrated by a 1-min deep breathing test and parasympathetic neuropathy as demonstrated by spectral analysis (11–13). Indeed, compared with spectral analysis, the E/I ratio is considered as most informative regarding the assessment of parasympathetic nerve function (14). Although spectral analysis may have the advantage of assessing not only parasympathetic (high frequency variation) but also sympathetic (low frequency variation) nerve function (15), the advantage of spectral analysis has recently been challenged (16). To investigate sympathetic nerve function, tests other than spectral analysis may be preferred.

To obtain a convenient and quantitative test of sympathetic nerve function, we have developed a laser Doppler perfusion imaging (LDPI) test in which alterations in finger skin blood flow upon local heating followed by indirect cooling are evaluated by assessment of the vasoconstriction (VAC) index. When the VAC index was compared before and after sympathectomy, we could clearly show that the VAC index is a test of sympathetic nerve function (17). In a pilot study, we assessed the value of the VAC test in the evaluation of the sympathetic nerve function among diabetic patients and found it promising (18). We have now conducted a new study of a group of type 1 and type 2 diabetic patients who have been followed prospectively regarding autonomic nerve function for 14 years (19,20). On the latest follow-up in 1998, the VAC test

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Received for publication 25 February 2004 and accepted in revised form 26 August 2004.

Abbreviations: E/I, expiration/inspiration; LDPI, laser Doppler perfusion imaging; VAC, vasoconstriction.

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

was included in the assessment. To follow the short-term changes of the VAC index, 20 of the type 1 diabetic patients were re-examined after 2 years. The aims of this study were to clarify *1*) the prevalence of sympathetic neuropathy versus parasympathetic neuropathy in type 1 versus type 2 diabetic patients and *2*) whether there are short-time alterations in VAC with time in type 1 diabetic patients.

RESEARCH DESIGN AND METHODS

— In 1985, 58 patients (22 women), all diagnosed with type 1 diabetes between the ages of 15 and 25 years, were evaluated with regard to autonomic nerve function (19). At the time of the first examination, the type 1 diabetic patients had a median age of 33 (range 17–56) years and a median duration of diabetes of 12 years (range 2 months to 30 years). In 1998 (13–14 years after the first investigation), all patients were invited to a follow-up study, and 43 of 58 patients (16 women), median age 46 (range 30–67) years and median duration of diabetes of 26 (range 13–42) years, accepted the invitation. Among patients not participating in the follow-up examinations, 3 had died (1 in 1986 of suspect myocardial infarction, 1 in 1989 of aortic valvular disease, and 1 in 1996 of multi-infarction dementia), 2 had moved out of town, and 10 were not interested in evaluation. Among type 1 diabetic patients, all were investigated regarding sympathetic and parasympathetic nerve function. Ten (23%) of 43 were smokers, and 13 (30%) had antihypertensive medication (5 on β-blockers [1 together with a diuretic], 3 on ACE inhibitors, 3 on calcium antagonists, and 2 on diuretics).

Similarly in 1985, 51 patients (11 women) with type 2 diabetes diagnosed between the ages of 40 and 45 years were evaluated with regard to autonomic nerve function (20). At the time for the first study, the type 2 diabetic patients had a median duration of diabetes of 7 (range 0–15) years. When reinvited in 1998 for the current study, 4 patients had moved out of town, 4 had died, and 13 refused (mostly because of bad health). In addition, autonomic nerve function could not be evaluated in one type 2 diabetic patient with atrial fibrillation and in four with severe ischemic heart disease. In total, 25 of the original 51 patients (3 women), median age 63 (range 56–72) years and median duration of diabetes of 23 (10–31) years, participated in the present study. Among type 2 diabetic patients, the results of 20 examinations regarding sympathetic, 21 regarding parasympathetic nerve function, and 17 regarding both functions could be evaluated. Two (12%) of 17 were smokers, and 11 (65%) had antihypertensive medication (2 on β-blockers, 1 on a β-blocker in combination with a diuretic, 1 on a β-blocker in combination with a ACE inhibitor, 2 on diuretics, 1 on a diuretic in combination with a calcium antagonist, 1 on a diuretic in combination with a ACE inhibitor, 1 on a diuretic in combination with a calcium antagonist and an α1-receptor blocker, and 2 on diuretics in combination with ACE inhibitors and calcium antagonists).

In the current study, we re-examined 20 patients (8 women) with type 1 diabetes, median age 43 (range 30–56) years and median duration of diabetes of 22 (13–36) years, after 2 years with regard to VAC index to assess the stability. The ethics committee at Lund's University approved the study. In accordance with the Helsinki Declaration, informed consent was obtained from all participants.

Sympathetic nerve function

LDPI. Using the instrument PIM 1.0 (Perimed AB, Stockholm, Sweden), a scanner system with two step motors and mirrors directs the laser light from a He-Ne laser source with a wavelength of 633 nm onto the skin surface. A pair of remote photodiodes detects the backscattered light, and after noise compensation, the energy of the output is taken as a measure of perfusion and read in arbitrary units.

Data were processed in a computer (486 DX, 33 MHz; SPC Trading, Uppsala, Sweden) using the manufacturer's software program version 2.01. Skin temperature was measured in degrees Celsius, using a skin spot sensor (Sensor 442-PI; Perimed, Stockholm, Sweden). The supporting holder of the heating device was molded with two grooves allowing measurement from two fingers. The temperature of the holder was controlled and maintained at 40 ± 0.1°C with a Peltier element (Norton CP 1.4–71-10L; Siemens, Munich, Germany) connected to a computer identical to that of the PIM scanner. Contralateral cooling was achieved by immersing the right hand and forearm in a water bath (Julabo; Paratherm IV, Juchheim Labortechnik, Schwarzwald, Germany) with a temperature of 15 ± 0.1°C. Tests were carried out according to our departmental routines as described previously (17).

Experimental protocol. Room temperature was 23.8 ± 0.1°C (mean ± SD). Conversation was avoided. The patients were investigated in semirecumbent posture with their left arm and hand resting at heart level. The hand was placed in a supporting holder that was molded with two grooves allowing measurement from two fingers. The tip of the middle finger was used for LDPI and was scanned over 2 × 2 cm with a scanning time of ~20 s. Temperature was registered on the tip of the index finger. After initial rest and stabilization for at least 15 min, baseline recordings were obtained for 2 min. The heating device was then switched on (40°C), and after 6 min, contralateral cooling by immersion of the right arm into water keeping 15°C for 3 min was performed. To obtain a numerical expression of the decrease in blood flow after indirect cooling, the lowest perfusion value during the first

Table 1—*Sympathetic (VAC index) and parasympathetic (E/I ratio) nerve functions in type 1 and type 2 diabetic patients*

	Type 1 diabetic patients	Type 2 diabetic patients*
n	43	17
Abnormal VAC index	17 (40)	7 (41)
Abnormal E/I ratio	18 (42)	11 (65)
Abnormal VAC index or E/I ratio	25 (58)	13 (76)
Abnormal VAC index and E/I ratio	10 (23)	5 (29)
Only abnormal VAC index	6 (14)	2 (12)
Only abnormal E/I ratio	8 (18)	6 (35)

Data are *n* (%). *Among the 21 type 2 diabetic patients tested for the E/I ratio, 17 were evaluated for the VAC index and the E/I ratio. Among the four patients tested for only the E/I ratio, two of four were abnormal. Among the three patients tested for only the VAC index one of three was abnormal.

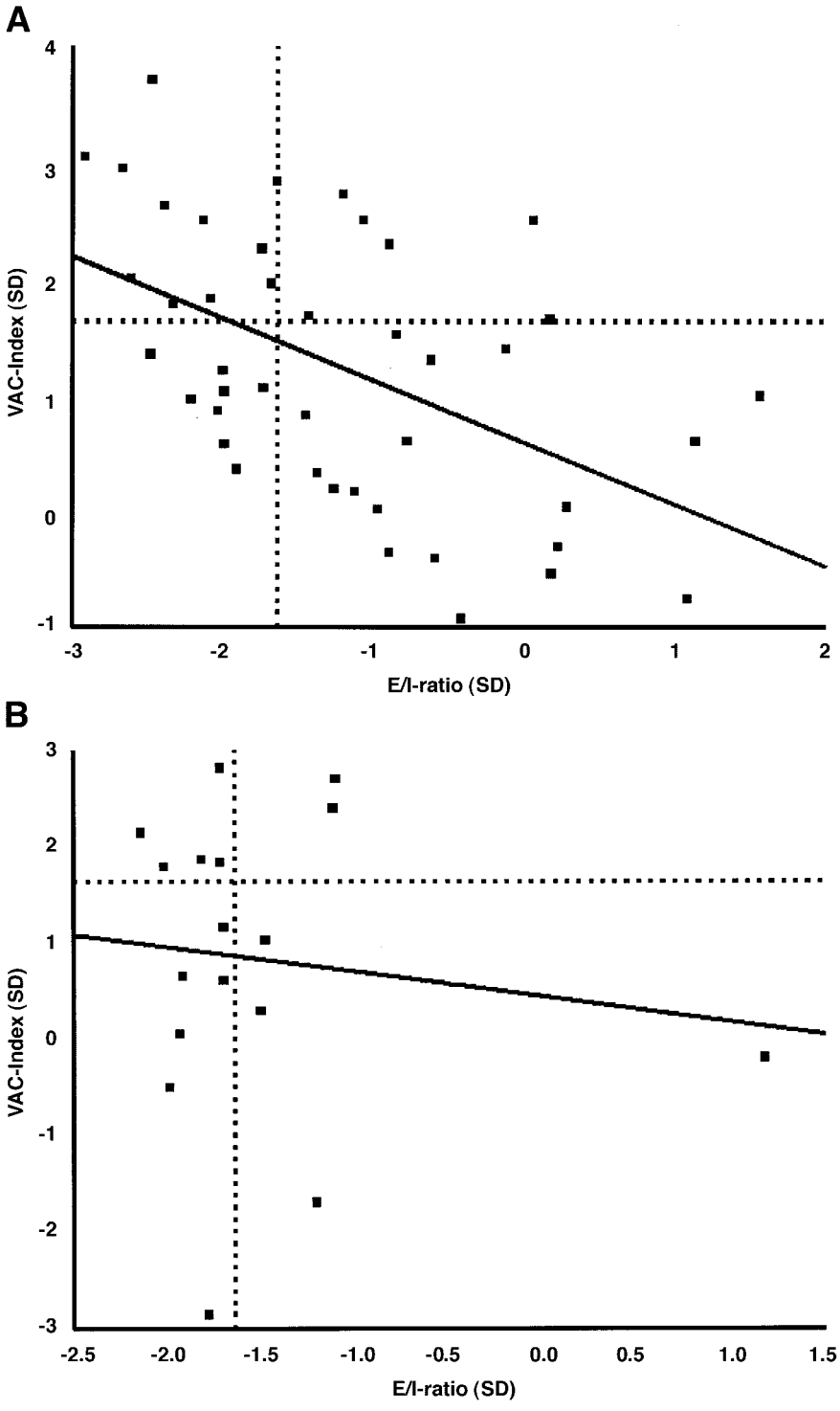

Figure 1—A: *Correlation between the VAC index and the E/I ratio among type 1 diabetic patients. The correlation was significant* (r_s *= 0.535,* P *= 0.0002). Dotted lines indicate cutoff limits for abnormal limits.* B: *Correlation between the VAC index and the E/I ratio among type 2 diabetic patients. The correlation was not significant* (r_s *= 0.10,* P *= 0.97). Dotted lines indicate cutoff limits for abnormal limits.*

minute of cooling ($LDPI_c$) was divided by the mean value of 2 min before cooling ($LDPI_h$), constructing an index for vasoconstriction ($VAC = LDPI_c/LDPI_h$). The value was converted and expressed in age-corrected *z* scores (17).

Parasympathetic nerve function

Deep breathing test (R-R interval variation). Six maximal expirations and inspirations were performed during 1 min in the supine position during the recording of a continuous electrocardiogram, and the R-R intervals were recorded. The E/I ratio, a test of parasympathetic vagal nerve function, was calculated as the mean of the longest R-R interval during expiration divided by the mean of the shortest R-R interval during inspiration (8). The E/I ratio was expressed in age-corrected values (i.e., *z* scores in SDs) (21).

Definitions of abnormalities. VAC values >1.64 SD (95% CI, one-sided test) above the age-related reference values (17) and an E/I ratio <−1.64 SD (95% CI, one-sided test) below the age-related reference values were considered abnormal (20). HbA_{1c} was measured by high-performance liquid chromatography (22). Reference values are 4.0–5.3%.

Statistical analysis

A Spearman's correlation coefficient r_s was calculated for correlation, Wilcoxon's signed rank for paired comparisons, *t* test for between-group comparisons, and Fisher's test for evaluation of differences in prevalence of abnormalities. $P < 0.05$ was considered significant. Data are presented as median (range), mean ± SE, or number (%).

RESULTS

Type 1 diabetic patients

Among type 1 diabetic patients, the VAC index was abnormal in 17 (40%) of 43, and the E/I ratio in 18 (42%) (Table 1). The VAC index and the E/I ratio were both abnormal in 10 of 43 (23%). There was a clear correlation ($r_s = 0.525$; $P = 0.0002$) between the VAC index and the E/I ratio (Fig. 1*A*). Mean HbA_{1c} and blood pressures in the whole group were 7.41 ± 0.17% and 132/76 ± 2/1 mmHg, respectively. There was only a slight correlation ($r_s = -0.308$; $P = 0.044$) between HbA_{1c} and E/I ratio, whereas there was no significant correlation between HbA_{1c} and VAC index ($r_s = 0.203$). Patients on antihypertensive treatments had a slightly higher mean systolic blood pressure (142 ± 3 mmHg vs. 127 ± 3 mmHg; $P = 0.032$) and a mean lower E/I ratio (−1.66 ± 0.21 vs. −0.99 ± 0.22; $P = 0.048$) than those without, whereas the VAC index did not

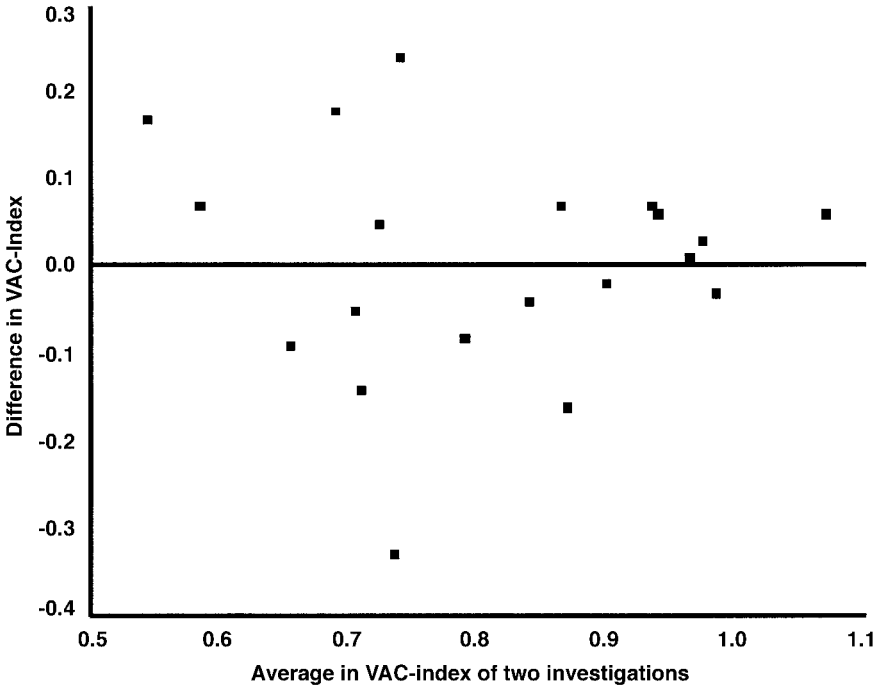

Figure 2—*VAC index in the first and second investigation among 20 type 1 diabetic patients. Difference between the tests on* y *axis and mean value on the* x *axis (Altman plot). There was no systematic difference between the two tests.*

differ. There were no differences between smokers or nonsmokers regarding the E/I ratio or the VAC index.

Type 2 diabetic patients

Among type 2 diabetic patients, the VAC index was abnormal in 7 of 17 (41%) and the E/I ratio in 11 (65%). The VAC index and the E/I ratio were both abnormal in 5 of 17 (29%). In contrast to type 1 diabetic patients, there was no significant correlation ($r_s = 0.10$, $P = 0.97$) between the VAC index and the E/I ratio in type 2 diabetic patients (Fig. 1*B*). The reason for this could be that parasympathetic nerve function was more severely affected than sympathetic nerve function among type 2 diabetic patients; 16 of 17 had an E/I ratio <-1.0 SD, whereas only 9 of 17 had a VAC index >1.0 SD ($P = 0.0167$). Mean HbA_{1c} and blood pressures in the whole group were 7.24 ± 0.33% and 139/82 ± 3/2 mmHg, respectively. There were no correlations between HbA_{1c} and the E/I ratio ($r_s = -0.101$) or the VAC index ($r_s = 0.023$). There were no significant differences for the E/I ratio (-1.64 ± 0.09 vs. -1.32 ± 0.38) or the VAC index between patients with or without antihypertensive treatment and smokers or nonsmokers, respectively. Compared with type 1 diabetic patients without antihypertensive treatment, type 2 diabetic patients had a low E/I ratio (-1.52 ± 0.15; $P = 0.051$).

Follow-up patients

There was no significant difference ($P = 0.601$) in the VAC index between the first and second investigation (Fig. 2). Among nine patients with abnormal VAC index in the first study, eight had still abnormal VAC indexes in the second study, whereas four patients with previously normal VAC index had become abnormal.

CONCLUSIONS — The current study showed that sympathetic neuropathy (abnormal VAC index) was as frequent as parasympathetic neuropathy (abnormal E/I ratio) among type 1 and type 2 diabetic patients. Because the VAC index was a persistent finding in the patients followed up, once present, sympathetic neuropathy seemed to be stable in most cases. As previously normal VAC indexes had become abnormal in some patients at follow-up, sympathetic neuropathy might become more frequent when the duration of diabetes increases. An interesting difference between type 2 and type 1 diabetic patients was detected. Whereas the prevalence of sympathetic and parasympathetic neuropathy, respectively, was similar in type 1 versus type 2 diabetic patients, sympathetic nerve function correlated with parasympathetic neuropathy only among type 1 diabetic patients. This discrepancy might be related to the fact that the parasympathetic impairment was more severe than the sympathetic impairment in our type 2 diabetic patients. The age-corrected E/I ratio in our type 2 diabetic patients corresponded to the age-corrected E/I ratio in our type 1 diabetic patients on antihypertensive treatment but was lower than in our type 1 diabetic patients without antihypertensive treatment. We infer from this that parasympathetic nerve dysfunction in general may be more impaired in type 2 diabetic patients compared with type 1 diabetic patients. Studies of large groups of type 1 and type 2 diabetic patients are needed to clarify this issue.

Using the blood pressure reaction to handgrip as a test of sympathetic neuropathy and the heart rate reaction to deep breathing as a test of parasympathetic neuropathy, Ewing et al. (23) report that parasympathetic neuropathy preceded sympathetic neuropathy in diabetic patients. However, this concept has been challenged. Signs of sympathetic neuropathy have been described in diabetic patients without parasympathetic neuropathy (24). Indeed, Hoeldtke et al. (25) report that sympathetic neuropathy as found from abnormal sudomotor response might be an early complication developing soon after the diagnosis of type 1 diabetes. Our study confirms that sympathetic neuropathy is frequent in type 1 diabetic patients. A novel finding is that sympathetic neuropathy is frequent also in type 2 diabetic patients. Most likely, tests based on laser Doppler techniques are more efficient in the detection of sympathetic dysfunction than previous tests based on the blood pressure reaction to handgrip. However, the natural history of autonomic neuropathy may differ between type 1 and type 2 diabetic patients. The lack of correlation between the VAC index and the E/I ratio in our type 2 diabetic patients suggests that there is a difference between the two major types of diabetes in this context. As parasympathetic nerve damage seemed to be more advanced than the sympathetic damage, it might be that parasympathetic neuropathy precedes sympathetic neuropathy in

type 2 diabetes in line with Ewing et al.'s (23) original concept. Another option might be that disturbed heart function or silent coronary artery disease affects the heart rate variation. It has to be remembered that our type 2 diabetic patients had a long duration of diabetes, indicating that we may have examined a group of survivors. Accordingly, we cannot exclude that severe sympathetic nerve damage could have occurred among the patients who had developed or died in cardiac complications before the current study.

To further clarify the found discrepancy between type 1 and type 2 diabetic patients, intrinsic differences between nerve fibers have to be considered. Parasympathetic nerves comprise large myelinated nerve fibers. In contrast, sympathetic nerves are thin and nonmyelinated. The discovery that subjects with impaired glucose tolerance may develop both parasympathetic (26) and peripheral (27) neuropathy suggests that large myelinated nerve fibers are affected early in type 2 diabetes. In agreement with the loss of epidermal nerve fibers in impaired glucose tolerance (28), sympathetic nerves may be affected early in type 2 diabetes. Prospective studies of the E/I ratio and the VAC index are needed to establish the relationship between glycemic control and the duration of diabetes versus the development of parasympathetic and sympathetic neuropathy in diabetic patients, including potential differences between type 1 and type 2 diabetes.

In conclusion, sympathetic and parasympathetic neuropathy are both frequent in type 1 and type 2 diabetic patients. However, disturbed sympathetic nerve function was only clearly associated with disturbed parasympathetic nerve function in type 1 diabetic patients. The VAC index and the E/I ratio, respectively, are valuable tests of sympathetic and parasympathetic nerve function, respectively. Both tests are recommended for the assessment of autonomic neuropathy in diabetic patients.

Acknowledgments— This work is part of the European Program on Standards, Measurements, and Testing: Standardized Calibration Methods and Europrobes for Laser Doppler Monitoring and Imaging of Blood Perfusion in Tissue, which is funded and administered by the Commission of the European Communities. Grants from the Albert Påhlsson Foundation, the Research Funds Malmö University Hospital, the Swedish Heart Lung Foundation, the Thorsten and Elsa Segerfalk Foundation, the Lundström Foundation, the Swedish Diabetes Association, and the Swedish Medical Research Council supported this study.

We thank Annmarie Johansson and Ann Radelius for excellent technical assistance.

References

1. O'Brien IA, McFadden JP, Corrall RJM: The influence of autonomic neuropathy on mortality in insulin-dependent diabetes. *Q J Med* 79:495–502, 1991
2. Chen HS, Hwu CM, Kuo BI, Chiang SC, Kwok CF, Lee SH, Lee YS, Weih MJ, Hsiao LC, Lin SH, Ho LY: Abnormal cardiovascular reflex tests are predictors of mortality in type 2 diabetes mellitus. *Diabet Med* 18:268–273, 2001
3. Gerritsen J, Dekker JM, TenVoorde BJ, Kostense PJ, Heine RJ, Bouter LM, Heethaar RM, Stehouwer CD: Impaired autonomic function is associated with increased mortality, especially in subjects with diabetes, hypertension, or a history of cardiovascular disease: the Hoorn Study. *Diabetes Care* 24:1793–1798, 2001
4. Kleiger RE, Miller JP, Bigger JTJ, Moss AJ: Decreased heart rate variability and its association with increased mortality after myocardial infarction. *Am J Cardiol* 59: 256–262, 1987
5. La Rovere MT, Bigger JT Jr, Marcus FI, Mortara A, Schwartz PJ: Baroreflex sensitivity and heart-rate variability in prediction of total cardiac mortality after myocardial infarction: ATRAMI (Autonomic Tone and Reflexes After Myocardial Infarction) investigators. *Lancet* 351: 478–484, 1998
6. Stevens MJ, Dayanikli F, Raffel DM, Allman KC, Sandford T, Feldman EL, Wieland DM, Corbett J, Schwaiger M: Scintigraphic assessment of regionalized defects in myocardial sympathetic innervation and blood flow regulation in diabetic patients with autonomic neuropathy. *J Am Coll Cardiol* 31:1575–1584, 1998
7. Scognamiglio R, Avogaro A, Casara D, Crepaldi C, Marin M, Palisi M, Mingardi R, Erle G, Fasoli G, Dalla Volta S: Myocardial dysfunction and adrenergic cardiac innervation in patients with insulin-dependent diabetes mellitus. *J Am Coll Cardiol* 31:404–412, 1998
8. Sundkvist G, Almér L-O, Lilja B: Respiratory influence on heart rate in diabetes mellitus. *Br Med J* 1:924–925, 1979
9. Armstrong FM, Bradbury JE, Ellis SH, Owens DR, Rosén I, Sönksen P, Sundkvist G: A study of peripheral diabetic neuropathy: the application of age-related reference values. *Diabet Med* 8 :S94–S99, 1991
10. Agelink MW, Malessa R, Baumann B, Majewski T, Akila F, Zeit T, Ziegler D: Standardized tests of heart rate variability: normal ranges obtained from 309 healthy humans, and effects of age, gender, and heart rate. *Clin Auton Res* 11:99–108, 2001
11. Nilsson H, Bergström B, Lilja B, Juul-Möller S, Carlsson J, Sundkvist G: Prospective study of autonomic nerve dysfunction in type 1 and type 2 diabetic patients: 24 hour heart rate variation and plasma motilin levels disturbed in parasympathetic neuropathy. *Diabet Med* 12: 1015–1021, 1995
12. Burger AJ, Charlamb M, Weinrauch LA, D'Elia JA: Short- and long-term reproducibility of heart rate variability in patients with long-standing type 1 diabetes mellitus. *Am J Cardiol* 80:1198–1202, 1997
13. Ducher M, Cerutti C, Gustin MP, Abou-Amara S, Thivolet C, Laville M, Paultre CZ, Fauvel JP: Noninvasive exploration of cardiac autonomic neuropathy: four reliable methods for diabetes? *Diabetes Care* 22:388–393, 1999
14. May O, Arildsen H: Assessing cardiovascular autonomic neuropathy in diabetes mellitus: how many tests to use? *J Diabetes Complications* 14:7–12, 2000
15. Laitinen T, Vauhkonen IK, Niskanen LK, Hartikainen JE, Lansimies EA, Uusitupa MI, Laakso M: Power spectral analysis of heart rate variability during hyperinsulinemia in nondiabetic offspring of type 2 diabetic patients: evidence for possible early autonomic dysfunction in insulin-resistant subjects. *Diabetes* 48:1295–1299, 1999
16. Huikuri HV, Makikallio T, Airaksinen KE, Mitrani R, Castellanos A, Myerburg RJ: Measurement of heart rate variability: a clinical tool or a research toy? *J Am Coll Cardiol* 34:1878–1883, 1999
17. Bornmyr S, Svensson H, Söderström T-B, Sundkvist G, Wollmer P: Finger skin blood flow in response to indirect cooling in normal subjects and in patients before and after sympathectomy. *Clin Physiol* 18: 103–107, 1998
18. Bornmyr S, Castenfors J, Svensson H, Wroblewski M, Sundkvist G, Wollmer P: Detection of autonomic sympathetic dysfunction in diabetic patients: a study using laser Doppler imaging. *Diabetes Care* 22:593–597, 1999
19. Bergström B, Lilja B, Österlin S, Sundkvist G: Autonomic neuropathy in type 1 diabetes: influence of duration and other diabetic complications. *Acta Med Scand* 222: 147–154, 1987
20. Bergström B, Lilja B, Österlin S, Sundkvist G: Autonomic neuropathy in non-insulin dependent (type 2) diabetes mellitus: possible influence of obesity. *J Intern Med* 227:57–63, 1990
21. Bergström B, Lilja B, Rosberg K, Sund-

kvist G: Autonomic nerve function tests: reference values in healthy subjects. *Clin Physiol* 6:523–528, 1986
22. Jeppsson JO, Jerntorp P, Sundkvist G, Englund H, Nylund V: Measurement of hemoglobin A1c by a new liquid-chromatographic assay: methodology, clinical utility, and relation to glucose tolerance evaluated. *Clin Chem* 32:1867–1872, 1986
23. Ewing DJ, Campbell IW, Clarke BF: Heart rate changes in diabetes mellitus. *Lancet* 1:183–185, 1981
24. Bergström B, Manhem P, Bramnert M, Lilja B, Sundkvist G: Impaired responses of plasma catecholamines to exercise in diabetic patients with abnormal heart rate reactions to tilt. *Clin Physiol* 9:259–267, 1989
25. Hoeldtke RD, Bryner KD, Horvath GG, Phares RW, Broy LF, Hobbs GR: Redistribution of sudomotor responses is an early sign of sympathetic dysfunction in type 1 diabetes. *Diabetes* 50:436–443, 2001
26. Eriksson KF, Nilsson H, Lindgärde F, Österlin S, Dahlin L-B, Lilja B, Rosén I, Sundkvist G: Diabetes mellitus but not impaired glucose tolerance is associated with dysfunction in peripheral nerves. *Diabet Med* 11:279–285, 1994
27. Thrainsdottir S, Malik R, Dahlin LB, Wiksell P, Eriksson KF, Rosén I, Petersson J, Greene D, Sundkvist G: Endoneurial capillary abnormalities presage deterioration of glucose tolerance and accompany peripheral neuropathy in man. *Diabetes* 52: 2615–2622, 2003
28. Smith AG, Ramachandran P, Tripp S, Singleton JR: Epidermal nerve innervation in impaired glucose tolerance and diabetes-associated neuropathy. *Neurology* 5 7: 1701–1704, 2001