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A 14-year prospective study of autonomic nerve function in Type 1 diabetic patients: association with nephropathy

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Abstract

Aims Prospective studies of autonomic nerve function are rare. We have followed the progression of autonomic dysfunction in relation to nephropathy over 14 years in Type 1 diabetic patients.

Methods Autonomic nerve function was assessed by heart-rate responses to deep breathing (E/I ratio) and tilting (acceleration and brake indices) and by the postural blood pressure reaction in 58 patients, 43 of whom were reassessed after 14 years. Nephropathy was evaluated by the degree of albuminuria (albuminuria > 20 μg/min or > 0.03 g/24 h) and glomerular filtration rate (^{51}Cr-EDTA plasma clearance). The acceleration index had deteriorated after 7 years ($P = 0.0155$), whereas the E/I ratio ($P = 0.0070$) and the diastolic postural blood pressure reaction ($P = 0.0054$) had deteriorated 14 years after the baseline examination (age-corrected values). All those with albuminuria at the third examination showed signs of autonomic neuropathy at baseline (10 of 10) compared with only nine of 22 without ($P = 0.0016$). Multiple regression analysis showed that the association between autonomic dysfunction and future albuminuria was due to the E/I ratio. In addition, individuals with an abnormal postural diastolic blood pressure fall ($n = 7$) at baseline showed a greater fall in glomerular filtration rate more than others 7–14 years later [29 (16.5) ml/min/1.72 m^2 vs. 11 (9) ml/min/1.72 m^2; $P = 0.0074$].

Conclusion Autonomic nerve function had deteriorated after 14 years. Autonomic neuropathy and abnormal postural diastolic blood pressure falls at baseline were associated with future renal complications.

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Keywords autonomic neuropathy, nephropathy, neuropathy, proliferative retinopathy, retinopathy, symptoms of neuropathy

Abbreviations DCCT, Diabetes Control and Complication trial; E/I ratio, expiration/inspiration ratio; GFR, glomerular filtration rate

Introduction

Autonomic neuropathy is a serious complication of diabetes [1,2] that may contribute to the development of nephropathy [3–6] although there are few data. To our knowledge, only Sampson *et al*. [7] have presented long-term observations (15 years) of autonomic dysfunction in Type 1 diabetic patients. In 1984–85, we investigated autonomic nerve function in a group of Type 1 diabetic subjects [8] later followed up 7 years after the original examination [9]. Here, we report after 13–14 years of observation. The aims of the study were to clarify whether autonomic neuropathy was progressive and whether

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Table 1 Clinical features at baseline and at the final examination 14 years later amongst Type 1 diabetic patients

	All patients		Patients followed up on four occasions	
	Baseline	14 year later	Baseline	14 years later
Number of patients	58	48	34	34
Age (years)	33 ± 10	46 ± 9	33 ± 9	47 ± 9
Duration of diabetes (years)	13 ± 9	26 ± 8	13 ± 8	27 ± 8
HbA_{1c} (%)	7.15 ± 1.48	7.41 ± 1.20	7.10 ± 1.38	7.29 ± 0.94
Supine blood pressure (mmHg)				
Systolic	130 ± 19	135 ± 22	130 ± 17	133 ± 20
Diastolic	77 ± 10	82 ± 11	76 ± 11	80 ± 10
Hypertension (= 140/90 mmHg)	14/58 (24%)	15/48 (31%)	7/34 (21%)	9/34 (26%)
Anti-hypertensive therapy	1 (2%)	14/48 (29%)	1/34 (1%)	9/34 (26%)
Albuminuria	18/36 (50%)	11/45 (24%)	7/22 (32%)	6/33 (18%)
Retinopathy	24/58 (41%)	42/46 (91%)*	13/34 (38%)	29/32 (91%)*

Results are given as mean ± SD or number (%).
*Retinopathy not assessed in two patients.

renal complications were secondary to autonomic nerve dysfunction.

Patients and methods

In 1984–85, 58 patients (22 women), all diagnosed with Type 1 diabetes between the ages of 15 and 25 years (age 17–56 years, median 33; duration of diabetes 2 months—30 years, median 12) were evaluated with regard to autonomic nerve function [8]. In 1989 (i.e. 4 years after the first examination), all were invited to a second examination and 44 of 58 accepted. In 1992 (7 years after the first examination), subjects were invited to a third examination and 41 of 58 agreed [9]. In 1998 (13–14 years after the original examination), all patients were invited to a fourth examination and 43 of 58 (16 women), median age 46 years (range 30–67) and median duration of diabetes 25 years (13–42), agreed to undergo autonomic nerve function tests. Fifteen did not participate in the fourth examination. Among them, three had died (one of probable myocardial infarction, one of aortic valvular disease, and one of multi-infarction dementia), two had moved and five completed a clinical examination only. A total of 34 individuals underwent autonomic nerve function in all four studies. Prospective data are based on these 34. Table 1 records their clinical features which were representative for the whole group. Informed consent was obtained from all subjects. The Ethics Committee, Lund University, Sweden, approved the study.

Cardiac autonomic nerve function tests

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 ECG 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 (E) divided by the mean of the shortest R-R interval during inspiration (I) [10].

The immediate heart-rate reaction to tilt

After 10 min rest, the subject was rapidly tilted (< 2 s) to the upright position (head up 90°) and remained there for 8 min. The initial heart rate reaction to tilt, an immediate acceleration followed by a transient deceleration, was evaluated by continuous ECG recording and determination of the acceleration [(A – B)/A × 100] and the brake index [(C – B)/A × 100]. These indices evaluate both sympathetic and parasympathetic nerve function [11]. In the formulae, A indicates mean RR interval before tilt, B indicates the shortest RR interval during the immediate acceleration, and C indicates the longest RR interval during the deceleration [12]. Due to cardiac arrhythmia, one patient could not be tested at baseline and, for the same reason, a second missed the final examination.

Definitions of abnormalities

The E/I ratio, the acceleration index, and the brake index, were also expressed in age-corrected values [i.e. z-scores in standard deviations (SD)] [13]. Values less than –1.64 SD (95% confidence interval, one-sided test) below the age-related reference values were considered abnormal.

The postural blood pressure reaction to tilt

Systolic and diastolic blood pressures were indirectly recorded in the right arm with a sphygmomanometer 4 min before tilt (recorded as supine blood pressure) and every minute thereafter. For systolic blood pressure, Korotkov phase 1, and for diastolic blood pressure Korotkov phase 5, were used. The lowest systolic blood pressure and the lowest diastolic blood pressure recorded after tilt were selected and also transferred to age-corrected values expressed in Z scores as previously described [14]. A Z score below –1.64 SD (95% confidence interval, one-sided test) was considered abnormal.

Albuminuria

Except at the final examination, albuminuria was assessed from urine tests obtained at the yearly extensive check up at the diabetes clinic. A urine sample was available for 36 of 58 subjects at baseline, 32 of 44 at the second examination, 27 of 41 subjects at the third examination and 45 of 48 at the final examination. In 36 of the individuals, albuminuria was assessed in at least three of the four examinations. Amongst the 45 patients tested for albuminuria at the final examination, 31 had been tested at baseline. Among the 36 evaluated at baseline, four of five not followed up showed albuminuria at baseline. Three of the four with albuminuria at baseline had died during the observation period. Urine albumin was measured by immunonephelometry on a Beckman Array Protein system instrument (Beckman Instruments, Fullerton, CA, USA). During the study period, the method for collecting urine samples was changed, i.e. from collection during 24 h (the first three examinations) to overnight collection (final examination). Microalbuminuria was therefore defined as an albumin excretion of 0.03–0.30 g/24 h in the first three examinations (24-h collection) and 20–200 µg/min (overnight collection) at the final examination; macroalbuminuria was defined as an albumin excretion above those levels [15]. In this report, patients with micro or macroalbuminuria are considered together as patients with albuminuria.

Glomerular filtration rate (GFR)

Glomerular filtration rate (GFR) was assessed at the third and fourth examination. GFR was evaluated by the ^{51}Cr-EDTA plasma clearance method [16]. Reference values were taken from [17] and age-corrected values expressed as *Z* scores were calculated. A *Z* score value less than −1.64 SD (95% confidence interval, one-sided test) was considered abnormally low.

Statistical analysis

Repeated measures ANOVA followed by paired *t*-tests were used in within group comparisons. As the number of individuals was low ($n < 20$) in the different subgroups, non parametric Mann–Whitney *U*-test was used in comparisons between two groups. Chi-square, Fisher's test, and Mac Nemar's test were used for evaluating differences in frequencies. Spearman's test (r_s), Pearson's correlation coefficient and multiple regression were used to test associations. A *P*-value < 0.05 was considered significant. Data are presented as mean ± SD, if not otherwise stated.

Results

Autonomic nerve function

Cardiac autonomic nerve function

Figure 1(a) shows that the E/I ratio and the acceleration index deteriorated ($P < 0.0001$) during the 14 years of observation. However, there was a difference between the two tests. The acceleration index had deteriorated after 7 years, whereas the E/I ratio had decreased 14 years after baseline, as was the case

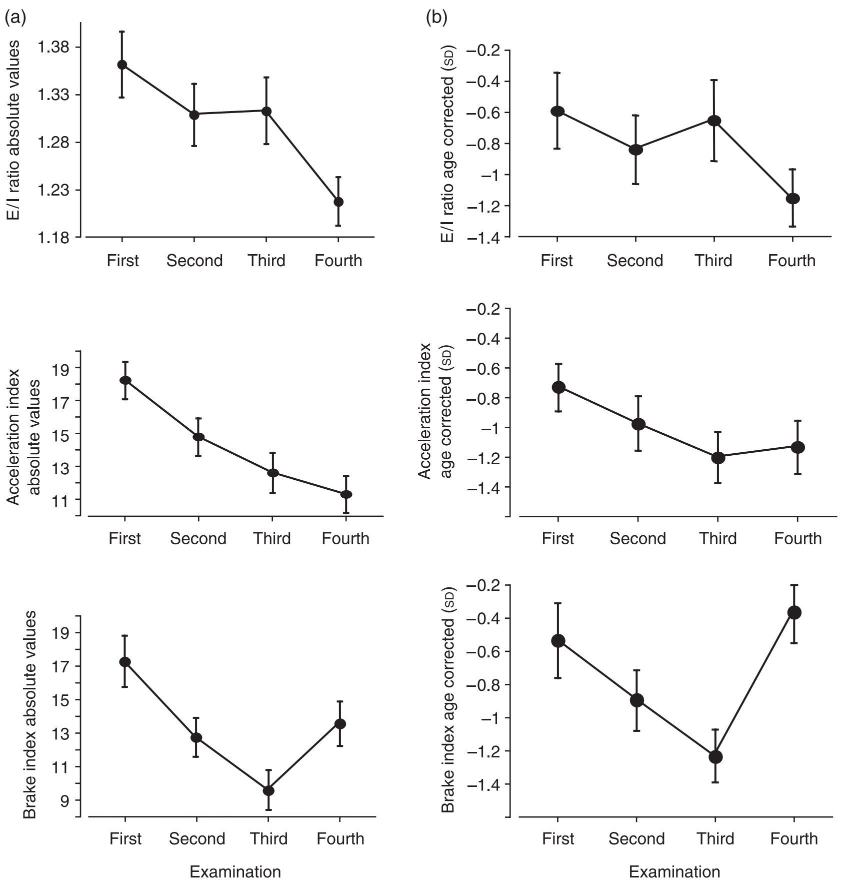

Figure 1 (a) The progression of autonomic nerve function during 14 years amongst 34 Type 1 diabetic subjects examined at all four examinations. Results expressed in absolute values and presented as mean ± SEM. The E/I ratio and the acceleration index deteriorated ($P < 0.0001$) during the 14 years of observation. (b) The progression of autonomic nerve function during 14 years amongst 34 Type 1 diabetic patients examined at all four examinations. Results expressed in age-corrected *Z*-scores and presented as mean ± SEM. The acceleration index deteriorated after 7 years ($P = 0.0155$; first vs. third examination) and the E/I ratio after 14 years ($P = 0.007$; third vs. fourth examination). The brake index decreased after 7 years ($P = 0.0066$; baseline vs. third examination) to normalize after 14 years ($P < 0.001$; third vs. fourth examination).

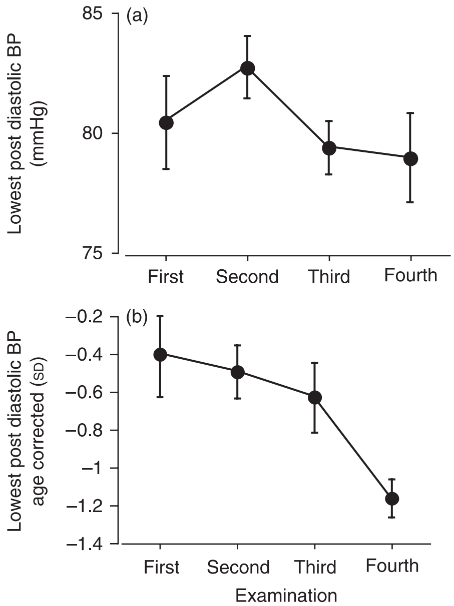

Figure 2 The postural diastolic blood pressure (lowest value) to a 10-min and 90° head-up tilt during 14 years amongst 34 Type 1 diabetic patients examined at all four examinations. Data presented as mean ± SEM. (a) Results expressed in absolute values (mmHg). There was no significant difference (ANOVA; $P = 0.0979$) in the development of postural diastolic blood pressure reaction using absolute values. (b) Results expressed in age-corrected Z scores. Using age-corrected values, the postural diastolic blood pressure reaction deteriorated (ANOVA; $P = 0.0101$) with a significant decrement in the postural diastolic blood pressure at the final examination ($P = 0.0054$ third vs. fourth examination).

for age-corrected values (Fig. 1b) ($P = 0.0155$ for the acceleration index, first vs. third examination; $P = 0.0070$ for the E/I ratio, third vs. fourth examination). The brake index also changed during the study ($P < 0.0001$), albeit differently. In agreement with the acceleration index, the brake had decreased after 7 years ($P = 0.0066$ in age-corrected values) to then, different from the acceleration index, be normalized after 14 years ($P < 0.001$ age corrected values; third vs. fourth examination). There was a significant correlation ($r_s = 0.58$; $P = 0.006$) between the age-corrected brake index at the third examination vs. the age-corrected acceleration index at the final examination.

Postural blood pressure reaction

Using absolute values, there was no significant difference (ANOVA; $P = 0.0979$) in the development of the postural diastolic blood pressure reaction (Fig. 2). However, using age-corrected values, the postural diastolic blood pressure reaction deteriorated (ANOVA; $P = 0.0101$) with a significant decrement in the postural diastolic blood pressure in the final examination ($P = 0.0054$; fourth vs. the third examination).

Individual autonomic tests results

Table 2 shows the prevalence of abnormal cardiac autonomic nerve function at baseline and after 14 years in all subjects as well as in the 34 patients examined on all four occasions. Half had signs of autonomic neuropathy (i.e. one or more abnormal test) at baseline and the prevalence was not significantly increased

Table 2 The prevalence of abnormal autonomic nerve function tests amongst Type 1 diabetic patients prospectively followed for 14 years

	All patients		Patients followed up on four occasions	
	Baseline $n = 58$	14 year later $n = 43$	Baseline $n = 34$	14 year later $n = 34$
Any abnormal test				
Any	34 (53%)	27 (65%)	18 (52%)	17 (50%)
E/I	20 (34%)	18 (42%)	9 (26%)	13 (38%)
Acceleration index	12 (21%)	16 (37%)	6 (18%)	12 (35%)
Brake index	15 (31%)	3 (7%)	10 (29%)	3 (9%)
One abnormal test				
E/I	9 (16%)	8 (19%)	4 (12%)	7 (21%)
Acceleration index	4 (7%)	6 (14%)	3 (9%)	6 (18%)
Brake index	9 (16%)	3 (7%)	6 (18%)	0*
E/I or acceleration index or brake index	23 (40%)	19 (44%)	13 (38%)	11 (32%)
Two abnormal tests				
E/I and acceleration index	5 (9%)	7 (16%)	3 (9%)	3 (9%)
E/I and brake index	3 (5%)	0	0	0
Acceleration index and brake index	0	0	0	0
Total two abnormal tests	8 (14%)	7 (16%)	3 (9%)	3 (9%)
Three abnormal tests				
E/I, acceleration index and brake index	3 (5%)	3 (7%)	2 (6%)	3 (9%)
Two or three abnormal tests	11 (19%)	10 (23%)	5 (15%)	6 (18%)

Results are given as number (%).
E/I, expiration/inspiration ratio.
*$P = 0.0246$ vs. first study.

14 years later. Amongst those tested both at baseline and after 14 years, 10 of 12 with an abnormal E/I and five of six with an abnormal acceleration index at baseline had abnormalities in the corresponding test 14 years later. The prevalence of an abnormal postural diastolic blood pressure response was 11/58 (19%) at baseline and 12/42 (29%) after 14 years.

Autonomic nerve function and glycaemic control

There was no significant relationship between HbA_{1c} at baseline and autonomic nerve function. However, HbA_{1c} at baseline and at the second examination was related to the brake index at the third examination ($r_s = -0.40$; $P = 0.0215$ and $r_s = -0.433$; $P = 0.0116$, respectively). Further, HbA_{1c} at the second examination showed a significant negative correlation with the E/I ratio at the third examination ($r_s = -0.35$; $P = 0.0394$). There were also significant and negative correlations between HbA_{1c} at the third examination vs. the E/I ratio ($r_s = -0.40$; $P = 0.0186$) and the acceleration index ($r_s = -0.35$; $P = 0.0405$) at the fourth examination. Patients with an abnormal E/I ratio as well as those with an abnormal brake index at the third examination showed significantly higher HbA_{1c} values at the second examination (7.84 ± 1.39 vs. 6.84 ± 1.15%; $P = 0.0194$ and 7.86 ± 1.59 vs. 6.74 ± 0.93%; $P = 0.0447$, respectively). The number of patients with definite abnormalities (Table 2) did not increase.

Albuminuria

Overall, half [18 of 36 (50%); 5/18 macroalbuminuria] of patients had albuminuria at baseline as compared with eight of 39 (21%; 3/8 macroalbuminuria) after 4, 10 of 33 (30%; 2/10 macroalbuminuria) after 7, and 11 of 45 (24%; 4/11 macroalbuminuria) after 14 years. All patients with albuminuria at third examination had autonomic neuropathy at baseline, 10 of 10 as compared with nine of 22 without ($P = 0.0016$). Correlation analysis between autonomic nerve function tests, HbA_{1c}, supine blood pressures and albumin excretion at baseline vs. the albumin excretion 14 years later showed the age-corrected E/I ratio at baseline to have the closest association with the albumin excretion 14 years later (correlation coefficient –0.543). When albumin excretion, 14 years after the baseline examination (dependent variable) was included with the E/I ratio, HbA_{1c}, albumin excretion, and supine diastolic pressure variables at baseline (independent variables) in a multiple regression analysis, only the adjusted partial coefficient for the E/I ratio remained significant (–0.524; $P = 0.0054$). Those with albuminuria at the final examination (Fig. 3) showed significantly lower E/I ratios than those without at all examinations (baseline, $P = 0.0162$; second examination, $P = 0.0149$; third examination, $P = 0.0004$; fourth examination, $P = 0.0002$).

GFR

Only three of 39 (8%) subjects at the third and five of 41 (10%) at the fourth examination had an abnormally low GFR.

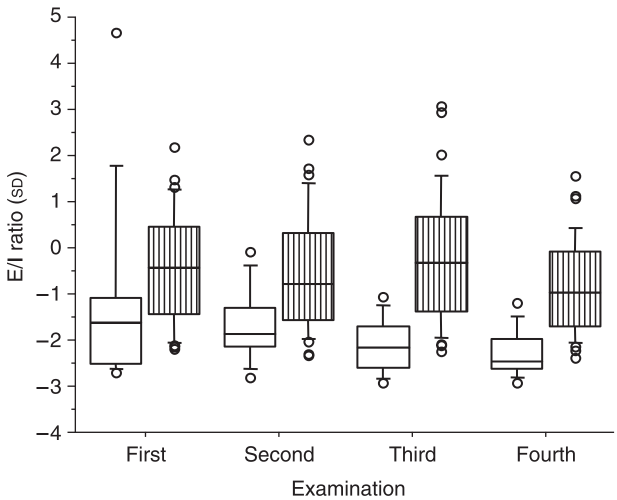

Figure 3 The E/I ratio at the four examinations in those with (□) and without (▥) albuminuria 14 years after baseline. Data presented as box and whisker plots showing the 10th, 25th, 50th (median), 75th and 90th percentiles, Values above the 90th and below the 10th percentile plotted as points. The E/I ratio was significantly lower at all four examinations in patients with albuminuria at the fourth examination vs. those without (First, $P = 0.0162$; Second, $P = 0.0149$; Third, $P = 0.0004$; Fourth, $P = 0.0002$, respectively).

There was a significant negative correlation between the brake index at baseline vs. the decrease in GFR (age-corrected) between the third and fourth examination ($r_s = -0.442$; $P = 0.0124$). The decrease in GFR between the third and fourth examination was also significantly higher in those with an abnormal postural diastolic blood pressure fall at baseline ($n = 7$) vs. those without ($n = 32$) (Fig. 4; 29 (16.5) ml/min/1.72 m^2 vs. 11 (9) ml/min/1.72 m^2; $P = 0.0074$; age corrected 2.09 (0.80) vs. 0.60 (0.74); $P = 0.0080$).

Discussion

This prospective study showed that autonomic nerve function deteriorated during the 14 years of observation. Initially, acceleration index decreased and this was later followed by deteriorations in the E/I ratio and the postural diastolic blood pressure. Low E/I ratios at baseline, not supine blood pressures, HbA_{1c} or albumin excretion, were associated with increased albuminuria 14 years later. Moreover, those with abnormal postural diastolic blood pressure falls or low brake indices at baseline deteriorated in GFR more than expected 7–14 years after the baseline examination.

Signs of autonomic neuropathy

In our prospective study, three different indices of autonomic neuropathy were used. The deep breathing test evaluates the heart-rate variation during 1 min during deep breathing and the results are expressed as the E/I ratio, a marker of parasympathetic nerve function [10]. This test is reproducible [18] and has recently

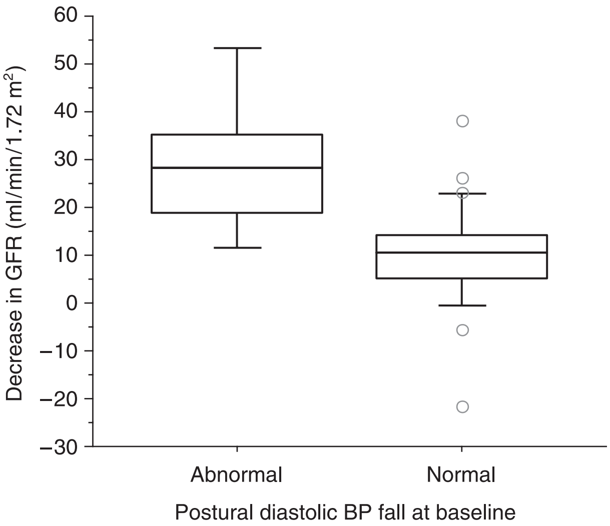

Figure 4 The decrease in GFR between the third and fourth examination amongst patients with an abnormal postural diastolic blood pressure reactions at baseline (n = 7) vs. those without (n = 32). Data presented as box and whisker plots showing the 10th, 25th, 50th (median), 75th and 90th percentiles, Values above the 90th and below the 10th percentile plotted as points. The decrease in GFR was significantly (P = 0.0074) higher in patients with an abnormal postural diastolic blood pressure reaction at baseline vs. those without.

been shown to be more efficient than 24-h ECG spectral analysis in the detection of parasympathetic neuropathy [19]. The acceleration and brake indices evaluate the immediate postural heart-rate reaction to tilt, that comprises an immediate acceleration (acceleration index) followed by a transient deceleration (brake index). A fast inhibition of parasympathetic control (acceleration) followed by re-institution of vagal nerve activity (brake) when postural adaptation has been resumed are the basis for this postural heart-rate reaction [12]. Hence, although the two manoeuvres (breathing and tilting) differ with regard to the afferent stimulation, both influence parasympathetic nerve activity. Recent studies indicate that the acceleration index reflects afferent parasympathetic function, whereas the E/I ratio reflects efferent parasympathetic function [20]. In addition to parasympathetic nerve activity, at least initially, the brake index seems to reflect sympathetic nerve function [11].

In agreement with Ziegler *et al.* [21], our study demonstrates that autonomic neuropathy is an early complication of diabetes found in half at baseline conducted after a median duration of Type 1 diabetes of 12 years. Our follow-up showed a deterioration in autonomic nerve function when the duration of Type 1 diabetes had increased over 14 further years. This deterioration was associated with impaired glycaemic control. High HbA_{1c} values were associated with future impaired autonomic nerve function test results. The progression of retinopathy was more dramatic. At baseline, 41% had retinopathy as compared with almost all (91%) 14 years later ($P < 0.0001$).

Autonomic neuropathy is associated with an increased risk for death [1], but in one prospective study, mortality was less than expected; 27% in those with autonomic neuropathy vs. 11% in those without [7]. Those who died in the current study had signs of autonomic neuropathy at the examination prior to their death, but the number of fatalities was low [three of 58 (5%) patients during 14 years].

We have previously suggested a causal connection between autonomic neuropathy and diabetic nephropathy [3,4], confirmed by other investigators [5,6]. In diabetic individuals with autonomic neuropathy, there is loss of the normal nocturnal decrease in blood pressure [22–24], suggesting that intraglomerular pressure is increased at night in autonomic neuropathy. This and the fall in intraglomerular pressure during the day due to postural falls in blood pressure may be a consequence of autonomic nephropathy [4]. The current study, supports the hypothesis that autonomic neuropathy precedes development of albuminuria. Disturbed parasympathetic nerve function (low E/I ratios) at baseline, not supine blood pressures, HbA_{1c} values, or albumin excretion, was associated with increased albumin excretion 14 years later. As parasympathetic neuropathy (disturbed E/I ratio) was predominant in our patients with albuminuria, it is possible that parasympathetic nerve damage disturbs the balance between the parasympathetic and sympathetic nervous system with a nocturnal predominance of sympathetic nerve activity explaining the loss of normal nocturnal drop in blood pressure [25]. This increases the intraglomerular pressure, causing an increased leakage of albumin [26,27]. Indeed, it was recently shown that autonomic neuropathy precedes microalbuminuria in Type 1 diabetic patients [28]. Autonomic neuropathy is also associated with albuminuria in subjects with impaired glucose tolerance [29] and non-diabetic relatives of Type 2 diabetic patients [30]. The recent observation that microalbuminuria is reversible in half of Type 1 diabetic patients [31] suggests that autonomic neuropathy may be a permissive factor for the development of constant and persistent microalbuminuria.

Because of intensified anti-hypertensive treatment during recent years, the prognosis of nephropathy has improved. The decline in GFR is nowadays not steeply progressive but is more variable and albuminuria is not the only progression promoter [32]. As in other work [3–6], our study suggests that autonomic neuropathy may be one such factor. As we have observed previously [3,4], a low brake index at baseline was associated with higher than expected decreases in GFR between the third and fourth examination, as was an abnormal postural diastolic blood pressure fall at baseline. This underlines the idea of sympathetic denervation as another promoter of nephropathy progression [4].

In conclusion, autonomic neuropathy is a frequent complication in Type 1 diabetic patients. Autonomic dysfunction is a progressive disorder associated with future albuminuria and deterioration in GFR.

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