LUND UNIVERSITY

Early parasympathetic neuropathy associated with elevated fasting plasma C-peptide concentrations and late parasympathetic neuropathy with hyperglycaemia and other microvascular complications.

Gottsäter, Anders; Kangro, M; Sundkvist, Göran

Link to publication

Early parasympathetic neuropathy associated with elevated fasting plasma C-peptide concentrations and late parasympathetic neuropathy with hyperglycaemia and other microvascular complications

A. Gottsäter, M. Kangro* and G. Sundkvist†

Departments of Vascular Diseases, *Opthalmology and †Endocrinology, University of Lund, University Hospital MAS, Malmö, Sweden

Abstract

Aims   To examine the relationship between parasympathetic neuropathy, hyperinsulinaemia, glycaemic control (HbA_{1c}), and future diabetic complications.

Methods   We assessed parasympathetic nerve function [expiration/inspiration (E/I) ratio], glomerular filtration rate (GFR), glycaemic control (HbA_{1c}), fasting plasma (f-p-) C-peptide in 82 Type 2 diabetic patients (age 61 ± 1 years) 5 and 12–15 years after diagnosis. Diabetic retinopathy was assessed 15 years after diagnosis.

Results   High HbA_{1c} values in the first study were associated with retinopathy (with 8.6 ± 2.0 vs. without retinopathy $6.2 \pm 1.9\%$; $P < 0.0001$) and disturbed parasympathetic nerve function (low E/I ratio; $r_s = -0.41$; $P = 0.0061$) in the second study, as well as with deteriorations in GFR between the first and second study ($r_s = 0.62$; $P < 0.0001$). Patients with parasympathetic neuropathy in the first study had significantly higher f-p-C-peptide concentrations than those without 3 years (1.20 ± 0.43 vs. 0.86 ± 0.40 nmol/l; $P = 0.0015$) and 5 years (1.20 ± 0.44 vs. 0.82 ± 0.33 nmol/l; $P < 0.0001$), but not 15 years after diagnosis.

Conclusion   High HbA_{1c} values 5 years after diagnosis of Type 2 diabetes were associated with retinopathy, disturbed parasympathetic nerve function, and deterioration in GFR 7–10 years later. Parasympathetic neuropathy 5 years after diagnosis was associated with high C-peptide concentrations. Parasympathetic nerve function has to be considered when β-cell function is evaluated. Hyperglycaemia is an important factor for the development of complications in Type 2 diabetes.

Diabet. Med. 21, 1304–1309 (2004)

Keywords   Type 2 diabetes mellitus, HbA_{1c}, complications, autonomic neuropathy

Introduction

Autonomic neuropathy occurs frequently in diabetes [1] and is associated with a serious prognosis [2,3]. Both sympathetic and parasympathetic nerves may be affected. In Type 2 diabetes, parasympathetic neuropathy may predominate [4,5]. To evaluate the association between autonomic neuropathy and cardiovascular risk factors, we have previously assessed autonomic nerve function in a well-defined group of Type 2 diabetic patients 5 years after diagnosis of diabetes. The evaluation demonstrated that parasympathetic neuropathy was associated with features of the metabolic syndrome, including hyperinsulinaemia [6] and has been confirmed by others [7,8]. We have now re-evaluated our patients for retinopathy, nephropathy, and parasympathetic neuropathy in a second study

Correspondence to: Dr Anders Gottsäter, Department of Vascular Diseases, University of Lund, University Hospital MAS, S-205 02 Malmö, Sweden. E-mail: anders.gottsater@skane.se

performed 12–15 years after diagnosis of Type 2 diabetes, i.e. 7–10 years after the first study.

The main aim of the new study was to explore whether the presence of parasympathetic neuropathy in the first study influenced future complications. We also evaluated whether the hyperinsulinaemia shown in those with parasympathetic neuropathy 5 years after diagnosis was still present after 12–15 years.

Patients and methods

Subjects

Between September 1985 and August 1987, all (n = 244) new consecutively diagnosed diabetic patients in the city of Malmö (230 056 inhabitants, 1986), Sweden, were classified as having Type 1 (clinical need for insulin treatment 1 week after diagnosis [9]) or Type 2 diabetes, and tested for islet cell antibodies (ICA) as well as fasting C-peptide [9]. Of the 244 patients, 184 were classified as having Type 2 diabetes without ICA, 18 as Type 2 diabetes with ICA [latent autoimmune diabetes in adulthood (LADA)], and 42 as having Type 1 diabetes. The design of this prospective study was originally focused on the aetiopathogenesis of adult-onset diabetes [9,10], and features related to diabetic complications were therefore not primarily included in the protocol. Since Type 2 diabetic patients with ICA at diagnosis comprise a subgroup of diabetes that within 3 years after diagnosis are insulin deficient and insulin dependent [10,11], these as well as those with Type 1 diabetes were excluded.

All surviving Type 2 diabetic patients (n = 153) were invited to a follow-up study 5 years after diagnosis [6,12]. Out of 153 Type 2 diabetic individuals without ICA at diagnosis (age 61 ± 1 years), 82 (54%) accepted; with regard to age and gender these were representative of all 153 Type 2 diabetic patients. Twelve years after diagnosis, all surviving individuals were invited to a new follow-up study including anthropometrical data, measurements of blood pressures (BP), glycaemic control (HbA$_{1c}$), and residual β-cell function (fasting plasma C-peptide, f-p-C-peptide). Of the 82 examined 5 years after diagnosis, 62 completed this new examination, seven had died, four had moved from the area, and nine refused or were unable to participate. Fifteen years after diagnosis, all surviving patients were invited to an evaluation of autonomic nerve function comprising the heart rate reaction to deep breathing, an established test of parasympathetic nerve function [13–15], urinary albumin excretion (UAE), and glomerular filtration rate (GFR; ^{51}Cr-EDTA clearance). Of the 62 evaluated 12 years after diagnosis, 52 completed this examination, seven had died, and three refused or were unable to participate. Diabetic retinopathy was assessed 15 years after diagnosis in 63 of the original 82 subjects. The Ethics Committee of the University of Lund approved the study. Informed consent was obtained from all subjects.

Analytical methods

Body mass index (BMI) was calculated as weight (kg)/height (m^2). Blood pressures were measured with a sphygmomanometer in the right upper arm, with the patient in the supine position and after 1 min in the upright position. HbA$_{1c}$ was measured by high-performance liquid chromatography [16]; reference values 3.90–5.30%. Radioimmunoassay was used in the assessment of fasting C-peptide concentrations [17]; reference values 0.25–0.75 nmol/l. Comparisons with HbA$_{1c}$ and C-peptide data from diagnosis and 3 years after diagnosis [6,11] were also made in this study. U-albumin was determined by rate nephelometry. At our hospital, the method used for the evaluation of albuminuria changed between the first and second study. In the first study, urine was collected during 24 h and the albumin excretion was expressed as g/24 h. In the second study, urine was collected during the night and the albumin excretion was expressed as μg/min. Microalbuminuria in the first study was therefore defined as an albumin excretion of 0.03–0.3 g/24 h and in the second study 20–200 μg/min [18]. Concentrations above these levels defined macroalbuminuria. As two different methods for quantitative albumin excretion were used, we could not compare the albumin excretion directly between the two studies (g/24 h vs. μg/min) because of the differences in urine collection. Therefore, we could only use micro- and macroalbuminuria as dichotomized variables whereas the concentration of albumin (g/l) had to be used as a continuous variable in comparisons between the first and second studies. GFR was evaluated by the ^{51}Cr-EDTA plasma clearance method [19]. To age adjust GFR, standard deviation scores (z-scores) were calculated using age-related normal GFR values [20]. Age-adjusted deterioration of GFR was calculated as the GFR deterioration index [21].

Parasympathetic nerve function

The deep breathing test assessed parasympathetic nerve function. In the deep breathing test, six maximal expirations and inspirations are performed continuously during 1 min in the supine position during the recording of a continuous electrocardiogram (ECG). The expiration/inspiration (E/I) ratio was calculated from the mean value of the longest R–R interval during expiration (E) and the shortest during inspiration (I) [14]. The E/I ratio is not affected by gender, but decreases with advancing age [22], and is the most informative test of parasympathetic nerve function also when compared with 24-h spectral analysis [23]. As in our previous study [6], the E/I ratio was expressed in age-adjusted standard deviation scores, and an abnormal autonomic nerve function test was defined as a value > 1.5 SD below the age corrected value [24].

Assessment of diabetic retinopathy

Diabetic retinopathy was assessed by an experienced ophthalmologist (M.K.) from fundus photography (n = 46) or by clinical examination (n = 17). Results were graded as no retinopathy, non-proliferative (NPDR) or proliferative (PDR) diabetic retinopathy.

Statistical analysis

Differences between groups were evaluated with the Mann–Whitney U-test and the χ^2 test, whereas Wilcoxon's signed rank test was used within groups. Spearman's correlation coefficient (r_s) was calculated for correlations and regression

coefficients were calculated in a simple regression model. All tests were two-tailed and $P < 0.05$ was considered significant. Results are presented as mean ± SD or *n* (%). StatView 4.5 (SAS Institute, Cary, NC, USA) was used for the statistical calculations.

Results

All patients

Patient characteristics are shown in Table 1. This survivor analysis showed that those with parasympathetic neuropathy 5 years after diagnosis of diabetes were slightly younger and more obese than those without. Fifteen years after diagnosis of Type 2 diabetes, parasympathetic neuropathy (abnormal E/I ratio) was found in 14/52 (27%) subjects, retinopathy in 39/63 (62%; five had proliferative retinopathy), microalbuminuria in 19/52 (37%), macroalbuminuria in 7/52 (13%), and abnormally low (< 1.5 SD) GFR in 3/52 (6%). Table 2 shows the development of complications in relation to early parasympathetic neuropathy. Amongst the 24 patients with parasympathetic neuropathy in the first study, seven had normal parasympathetic nerve function in the second study, six had died and five were not followed up. Amongst the 58 individuals without parasympathetic neuropathy in the first study, eight had developed this complication in the second. These patients showed significantly higher HbA$_{1c}$ values 5 years after diagnosis compared with those remaining without parasympathetic neuropathy 10 years later (10.4 ± 2.1% vs. 6.9 ± 1.9%; $P = 0.0012$).

Table 1 Age, body mass index (BMI) and HbA$_{1c}$ at, and 3, 5, and 12 years after diagnosis in Type 2 diabetic patients with and without parasympathetic neuropathy [abnormal age-adjusted expiration/inspiration (E/I) ratio, value < 1.5 SD [31]] 5 years after diagnosis (mean ± SD)

	With parasympathetic neuropathy at 5 years	Without parasympathetic neuropathy at 5 years	*P*-value
At diagnosis	*n* = 24	*n* = 58	
Age (years)	48 ± 10	56 ± 11	0.0011
Gender (male/female)	15/9	36/22	0.9708
Body mass index (kg/m^2)	30.9 ± 4.7	28.2 ± 3.5	0.0068
HbA$_{1c}$ (%)	7.4 ± 1.7	7.5 ± 2.3	0.6465
After 3 years	*n* = 24	*n* = 58	
Body mass index (kg/m^2)	29.9 ± 3.8	27.4 ± 3.6	0.0117
HbA$_{1c}$ (%)	7.3 ± 1.9	7.1 ± 1.6	0.4145
After 5 years	*n* = 24	*n* = 58	
Body mass index (kg/m^2)	29.1 ± 3.5	27.0 ± 3.8	0.0151
HbA$_{1c}$ (%)	8.2 ± 2.2	7.7 ± 2.2	0.3719
After 12 years	*n* = 20	*n* = 42	
Body mass index (kg/m^2)	28.7 ± 3.6	26.4 ± 3.1	0.0117
HbA$_{1c}$ (%)	7.5 ± 1.8	7.4 ± 2.0	0.4845
Antihypertensive treatment (%)	14 (70)	28 (67)	0.7930
β-Blocker treatment (%)	4 (20)	5 (12)	0.3980

Table 2 Parasympathetic nervous function [age-adjusted expiration/inspiration (E/I) ratio], glomerular filtration (Cr-EDTA clearance and age adjusted z-score), and U-albumin 5 and 15 years after diagnosis in Type 2 diabetic patients without and with parasympathetic neuropathy (abnormal age-adjusted E/I ratio, value < 1.5 SD [31]) 5 years after diagnosis (mean ± SD or *n* (%))

	Parasympathetic neuropathy			
	Without		With	
Time after diagnosis	5 years (*n* = 58)	15 years (*n* = 38)	5 years (*n* = 24)	15 years (*n* = 14)
E/I ratio (SD)	−0.69 ± 0.74	−1.04 ± 0.7*	−1.82 ± 0.25	−1.43 ± 0.51*
Cr-EDTA clearance (ml/min)	93 ± 23	73 ± 27‡	97 ± 24	89 ± 28*§
Age adjusted z-score (SD)	0.52 ± 1.56	−0.52 ± 1.94‡	0.38 ± 1.82	0.30 ± 2.19
U-albumin (g/l)	0.04 ± 0.10	0.06 ± 0.11†	0.10 ± 0.27	0.24 ± 0.45*

*$P < 0.05$ compared with same group 5 years after diagnosis.
†$P < 0.001$ compared with same group 5 years after diagnosis.
‡$P < 0.0001$ compared with same group 5 years after diagnosis.
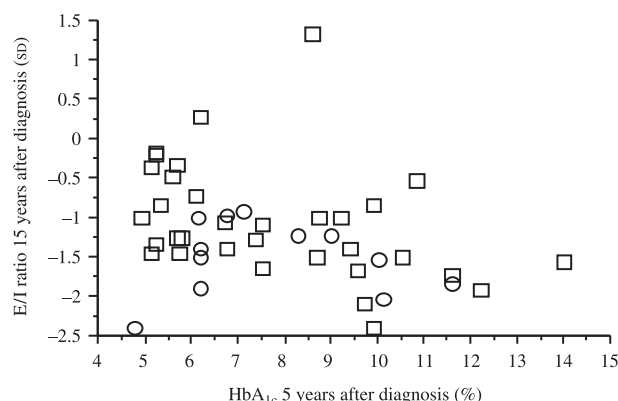§$P < 0.05$ compared with Type 2 diabetic patients without parasympathetic neuropathy.

Figure 1 Relation between metabolic control (HbA_{1c}) in the first study and age adjusted expiration/inspiration (E/I ratio) 10 years later in Type 2 diabetic patients in patients with (○) and without (□) parasympathetic neuropathy 5 years after diagnosis of diabetes ($r_s = -0.41$; $P = 0.0061$).
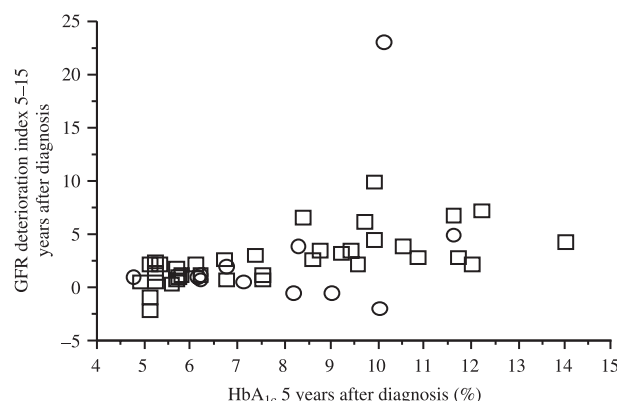

Figure 2 Relation between metabolic control (HbA_{1c}) in the first study and deterioration in glomerular filtration rate (GFR deterioration index) 10 years later in Type 2 diabetic patients in patients with (○) and without (□) parasympathetic neuropathy 5 years after diagnosis of diabetes ($r_s = 0.62$; $P < 0.0001$).

Factors affecting the development of complications

Neuropathy

Figure 1 shows that high HbA_{1c} values in the first study were associated with disturbed parasympathetic nerve function (low E/I ratios) in the second study 10 years later ($n = 52$, $r_s = -0.41$; $P = 0.0061$) in all patients. Those with parasympathetic neuropathy in the second study had significantly ($n = 38$, $9.8 \pm 2.6\%$ vs. $n = 14$, $7.0 \pm 1.7\%$; $P = 0.0016$) higher HbA_{1c} values in the first study. A similar difference for HbA_{1c} was not found in the second study ($8.5 \pm 2.3\%$ vs. $7.4 \pm 1.8\%$; $P = 0.1014$). Amongst those with parasympathetic neuropathy in the first study, three of five with parasympathetic neuropathy in the second study had high ($> 10\%$) HbA_{1c} values in the first compared with none of those without ($0/7$; $P = 0.0180$). Hence, hyperglycaemia in the first study was associated with parasympathetic neuropathy 10 years later.

GFR

Figure 2 shows that deteriorations in GFR during the 10-year follow-up period were clearly associated with high HbA_{1c} ($r_s = 0.62$; $P < 0.0001$) but not with parasympathetic neuropathy in the first study.

Retinopathy

Retinopathy in the second study was associated with high HbA_{1c} values in the first [i.e. 5 years after diagnosis (Fig. 3): retinopathy $8.6 \pm 2.0\%$ vs. without retinopathy $6.2 \pm 1.9\%$; $P < 0.0001$] and second study ($8.2 \pm 2.0\%$ vs. $6.3 \pm 1.2\%$; $P = 0.0004$). HbA_{1c} at diagnosis ($7.8 \pm 1.9\%$ vs. $6.8 \pm 1.8\%$; $P = 0.0176$) and 3 years later ($7.4 \pm 1.6\%$ vs. $6.5 \pm 1.4\%$; $P = 0.0180$) were also significantly higher in those with retinopathy in the second study. Retinopathy at follow-up was more frequent in those with parasympathetic neuropathy in the first study than in those without [17 of 21 (81%) vs. 22 of 42 (52%); $P = 0.0179$]. In a logistic regression analysis with BMI, parasympathetic
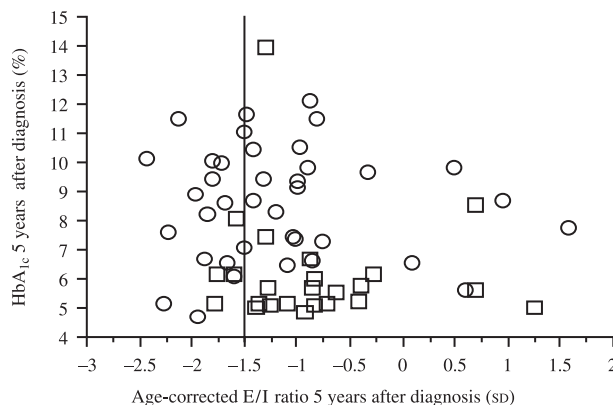

Figure 3 HbA_{1c} and age-corrected expiration/inspiration (E/I) ratio 5 years after diagnosis of diabetes in patients with (○) and without (□) diabetic retinopathy 15 years after diagnosis of diabetes. Vertical line at E/I ratio -1.5 sᴅ marks definition of parasympathetic neuropathy.

nerve function (E/I ratio), HbA_{1c}, s-triglycerides, s-LDL-cholesterol, age, sex and diastolic blood pressure 5 years after diagnosis as predictive variables and retinopathy 15 years after diagnosis as outcome variable, only high HbA_{1c} ($\beta = 1.815 \pm 0.616$; $P = 0.003$) and low BMI ($\beta = -0.354 \pm 0.175$; $P = 0.043$) 5 years after diagnosis were independent predictors of retinopathy 15 years after diagnosis.

The development of f-p-C-peptide

Patients with parasympathetic neuropathy in the first study showed higher f-p-C-peptide concentrations than those without 3 years ($P = 0.0015$), and 5 years ($P = 0.0002$) after diagnosis. Fifteen years after diagnosis f-p-C-peptide had significantly decreased ($P = 0.0242$) and the concentration was not different ($P = 0.6055$) compared with those without parasympathetic neuropathy initially (Fig. 4). There were significant negative correlations between the E/I ratio in the first study vs.
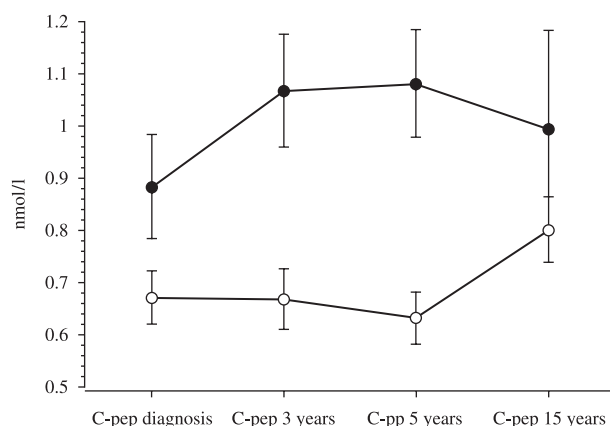
1308   Neuropathy, HbA$_{1c}$, and GFR in Type 2 diabetes • A. Gottsäter et al.

DMI

Figure 4 The development of fasting plasma C-peptide (C-pep) during 15 years in Type 2 diabetic patients with (●) and without parasympathetic neuropathy (○) 5 years after diagnosis. Data are mean ± SEM.

f-p-C-peptide at diagnosis ($r_s = -0.27$; $P = 0.0105$), 3 years ($r_s = -0.38$; $P = 0.0002$), 5 years ($r_s = -0.38$; $P < 0.0001$) after diagnosis and a lack of association 15 years after diagnosis ($r_s = -0.05$; $P = 0.7150$).

Discussion

This study showed that glycaemic control 5 years after diagnosis of Type 2 diabetes was related to deterioration in GFR, disturbed parasympathetic nerve function, and the development of retinopathy during the following decade, but unrelated to parasympathetic neuropathy 5 years after diagnosis. However, our study is a survivor analysis, and this limits interpretation of the data.

In those with parasympathetic neuropathy in the first study, the median E/I ratio was unchanged 10 years later. This might suggest that once parasympathetic neuropathy has developed, it does not progress because of structural and irreversible changes in parasympathetic nerves. However, this was not the case here. Seven patients with parasympathetic neuropathy in the first study did not fulfil the criteria for this diagnosis 10 years later. This indicates that early parasympathetic neuropathy may improve. It is possible that a reversible neuropathy may be related to hyperinsulinaemia.

Hyperinsulinaemia is associated with parasympathetic dysfunction in Type 2 diabetic patients [6–8], and might explain why obese subjects, with [4] and without [25] diabetes, often have impaired parasympathetic nerve function. Our previous finding that parasympathetic neuropathy occurs in impaired glucose tolerance [26] supports the concept of hyperinsulinaemia-related parasympathetic dysfunction. Although parasympathetic neuropathy may be of primary importance for hyperinsulinaemia [6,27], insulin resistance in itself or its consequence (hyperinsulinaemia) may also cause parasympathetic nerve dysfunction [28]. Indeed, experimental studies have demonstrated that insulin increases the heart rate [29], a feature of

parasympathetic neuropathy [30]. Hyperglycaemia in combination with insulinopenia promotes progression of peripheral neuropathy [31]. This may explain the later development of parasympathetic neuropathy in patients lacking parasympathetic neuropathy in the first study. Patients who had developed parasympathetic neuropathy in the second study showed high HbA$_{1c}$ and low f-p-C-peptide concentrations initially.

Recently, autonomic neuropathy has been proposed as a predictor of severe retinopathy [32–36]. In keeping with this, parasympathetic neuropathy in the first study was associated with an increased prevalence of retinopathy 10 years later. However, logistic regression showed that high HbA$_{1c}$ values rather than parasympathetic neuropathy were associated with retinopathy. That HbA$_{1c}$ values in the first study were associated with later retinopathy might indicate that the development of both neuropathy and retinopathy is related to hyperglycaemia [33]. It is also possible, however, that parasympathetic neuropathy might itself aggravate the risk for retinopathy [36]. Alternatively, increased nocturnal blood pressure, a feature of parasympathetic neuropathy [37], might favour the development of retinopathy.

A fall in GFR was related to high HbA$_{1c}$ values 5 years after diagnosis. We have previously shown that sympathetic neuropathy [21] may promote deterioration in GFR. The current study confirms that hyperglycaemia is associated with a fall in GFR. This indicates that both blood pressure and glycaemic control contribute to diabetic nephropathy.

In conclusion, high HbA$_{1c}$ values 5 years after diagnosis of Type 2 diabetes were associated with retinopathy, disturbed parasympathetic nerve function, and deterioration in GFR 7–10 years later. Parasympathetic neuropathy 5 years after diagnosis, often reversible in patients with good glycaemic control (low HbA$_{1c}$ values), was associated with high C-peptide concentrations.

References

1 O'Brien IAD, O'Hare JP, Levin IG, Corrall RJM. The prevalence of autonomic neuropathy in insulin-dependent diabetes mellitus: a controlled study based on heart rate variability. *QJ Med* 1986; **61**: 957–967.

2 Ewing DJ, Campbell IW, Clarke BF. The natural history of diabetic autonomic neuropathy. *QJ Med* 1980; **49**: 95–108.

3 Sampson MJ, Wilson S, Karagiannis P, Edmonds M, Watkins PJ. Progression of diabetic autonomic neuropathy over a decade in insulin-dependent diabetics. *QJ Med* 1990; **75**: 635–646.

4 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* 1990; **227**: 57–63.

5 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* 1995; **12**: 1015–1021.

6 Gottsäter A, Ahmed M, Fernlund P, Sundkvist G. Autonomic neuropathy in type 2 diabetic patients is associated with hyperinsulinemia and hypertriglyceridemia. *Diabet Med* 1999; **16**: 49–54.

7 Szelag B, Wroblewski M, Castenfors J, Henricsson M, Berntorp K, Fernlund P *et al.* Obesity, microalbuminuria, hyperinsulinemia, and increased plasminogen activator inhibitor 1 activity associated with parasympathethic neuropathy in type 2 diabetes. *Diabetes Care* 1999; **22**: 1907–1908.

8 Takayama S, Sakura H, Katsumori K, Wasada T, Iwamoto Y. A possible involvement of parasympathethic neuropathy on insulin resistance in patients with type 2 diabetes. *Diabetes Care* 2001; **24**: 968–969.

9 Landin-Olsson M, Nilsson K-O, Lernmark Å, Sundkvist G. Islet cell antibodies and fasting C-peptide predict insulin requirement at diagnosis of diabetes mellitus. *Diabetologia* 1990; **33**: 561–568.

10 Gottsäter A, Landin-Olsson M, Fernlund P, Lernmark Å, Sundkvist G. β-cell function in relation to islet cell antibodies (ICA) during the first three years after the clinical diagnosis of diabetes in non-insulin-dependent (type II) diabetic patients. *Diabetes Care* 1993; **16**: 902–910.

11 Gottsäter A, Landin-Olsson M, Lernmark Å, Fernlund P, Sundkvist G. Islet cell antibodies are associated with β-cell failure also in obese adult onset diabetic patients. *Acta Diabetol* 1994; **31**: 226–231.

12 Gottsäter A, Ahmed M, Lilja B, Fernlund P, Sundkvist G. Islet cell antibodies, but not leanness, relate to a better cardiovascular risk factor profile five years after diagnosis of non-insulin dependent diabetes mellitus. *Diabetes Care* 1996; **19**: 60–63.

13 Wheeler T, Watkins PJ. Cardiac denervation in diabetes. *Br Med J* 1973; **4**: 584–586.

14 Sundkvist G, Almér L-O, Lilja B. Respiratory influence of heart rate in diabetes mellitus. *Br Med J* 1979; **1**: 924–925.

15 Ewing DJ, Martyn CN, Yound RJ, Clarke BF. The value of cardiovascular autonomic tests: 10 years experience in diabetes. *Diabetes Care* 1985; **8**: 491–498.

16 Jeppsson J-O, Jerntorp P, Sundkvist G, Englund H. Nylund. Measurement of hemoglobin A1c by a new liquid-chromatographic assay: methodology, clinical utility, and relation to glucose tolerance evaluated. *Clin Chem* 1986; **32**: 1867–1872.

17 Heding LS. Radioimmunological determination of C-peptide in serum. *Diabetologia* 1975; **11**: 541–548.

18 Feldt-Rasmussen B, Mathiesen E, Hegedüs L, Deckert T. Kidney function during 12 months of strict metabolic control in insulin-dependent diabetic patients with incipient nephropathy. *N Engl J Med* 1986; **314**: 665–670.

19 Bröchner-Mortenson J. A simple method for determination of glomerular filtration rate. *Scand J Clin Lab Invest* 1972; **30**: 271–274.

20 Granerus G, Aurell M. Reference values for 51Cr-EDTA clearance as a measure of glomerular filtration rate. *Scand J Clin Lab Invest* 1981; **41**: 611–616.

21 Sundkvist G, Lilja B. Autonomic neuropathy predicts deterioration in glomerular filtration rate in patients with IDDM. *Diabetes Care* 1993; **16**: 773–779.

22 Bergström B, Lilja B, Rosberg K, Sundkvist G. Autonomic nerve function tests. Reference values in healthy subjects. *Clin Phys* 1986; **6**: 523–528.

23 May O, Arildsen H. Assessing cardiovascular autonomic neuropathy in diabetes mellitus: how many tests to use? *J Diabetes Complications* 2000; **14**: 7–12.

24 Bergström B, Lilja B, Österlin S, Sundkvist G. Autonomic neuropathy in type I diabetes; influence of duration and other diabetic complications. *Acta Med Scand* 1987; **222**: 147–154.

25 Rossi M, Marti G, Ricordi L, Fornasari G, Finardi G, Frationo P *et al.* Cardiac autonomic dysfunction in obese subjects. *Clin Sci* 1989; **76**: 567–572.

26 Eriksson KF, Nilsson H, Lindgärde F, Österlin S, Dahlin LB, Lilja B *et al.* Diabetes mellitus but not impaired glucose tolerance is associated with dysfunction in peripheral nerves. *Diabet Med* 1994; **11**: 279–285.

27 Latour MG, Lautt WW. The hepatic vagus nerve in the control of insulin sensitivity in the rat. *Auton Neurosci* 2002; **95**: 125–130.

28 Töyry JP, Niskanen LK, Mäntysaari MJ, Länsimies EA, Haffner SM, Miettinen HJJ *et al.* Do high proinsulin and C-peptide levels play a role in autonomic nervous dysfunction? Power spectral analysis in patients with non-insulin-dependent diabetes and nondiabetic subjects. *Circulation* 1997; **96**: 1185–1191.

29 Jacobsen F, Christensen NJ. Stimulation of heart rate by insulin: uninfluenced by beta-adrenergic receptor blockade in rabbits. *Scand J Clin Lab Invest* 1979; **39**: 253–256.

30 Ewing DJ, Campbell IW, Clarke BF. Heart rate changes in diabetes mellitus. *Lancet* 1981; **1**: 183–185.

31 Partanen J, Niskanen L, Lehtinen J, Mervaala E, Sitonen O, Uusitupa M. Natural history of peripheral neuropathy in patients with non-insulin-dependent diabetes mellitus. *N Engl J Med* 1995; **333**: 89–94.

32 Henricsson M, Nyström L, Blohmé G, Östman J, Cullberg K, Svensson M *et al.* The incidence of retinopathy 10 years after diagnosis in young adult people with diabetes. Results from the nationwide, population-based Diabetes Incidence Study in Sweden (DISS). *Diabetes Care* 2003; **26**: 349–354.

33 Krolewski AS, Barzilay J, Warram JH, Martin BC, Pfeifer M, Rand LI. Risk of early-onset proliferative retinopathy in IDDM is closely related to cardiovascular autonomic neuropathy. *Diabetes* 1992; **41**: 430–437.

34 Fong DS, Warram JH, Aiello LM, Rand LI, Krolewski AS. Cardiovascular autonomic neuropathy and proliferative diabetic retinopathy. *Am J Ophthalmol* 1995; **120**: 317–321.

35 Schmid H, Schaan B, Cecconello F, Maestri T, Neumann C. Proliferative diabetic retinopathy is related to cardiovascular autonomic neuropathy in non-insulin-dependent diabetes mellitus. *Diab Res Clin Pract* 1995; **29**: 163–168.

36 Zander E, Heinke P, Herfurth S, Reindel J, Ostermann FE, Kerner W. Relations between diabetic retinopathy and cardiovascular neuropathy—a cross-sectional study in IDDM and NIDDM patients. *Exp Clin Endocrinol Diabetes* 1997; **105**: 319–326.

37 Spallone V, Bernardi L, Ricordi L, Soldà P, Rosaria Maiello M, Calciati A *et al.* Relationship between the circadian rhytms of blood pressure and sympathovagal balance in diabetic autonomic neuropathy. *Diabetes* 1993; **42**: 1745–1752.