Autonomic Neuropathy in Nondiabetic Offspring of Type 2 Diabetic Subjects Is Associated With Urinary Albumin Excretion Rate and 24-h Ambulatory Blood Pressure

The Fredericia Study

C. Hauerslev Foss,[1,2,3] E. Vestbo,[1] A. Frøland,[3] H.J. Gjessing,[3] C.E. Mogensen,[1] and E.M. Damsgaard[2]

The aim of this study was to examine the impact of parental type 2 diabetes on the autonomic nervous system and to determine whether autonomic neuropathy is present and associated with changes in 24-h ambulatory blood pressure (AMBP) and urinary albumin excretion rate (UAER) in nondiabetic subjects with parental type 2 diabetes. We examined 223 nondiabetic offspring of type 2 diabetic subjects and a control group of 258 offspring of nondiabetic subjects. The autonomic nervous system was assessed by three cardiovascular reflex tests, 24-h AMBP was measured with an oscillometric recorder (90207; Spacelabs, Redmond, WA), and UAER was determined through three overnight urine samples. The subjects with parental type 2 diabetes had significantly lower heart rate variation in all three bedside tests ($P < 0.01$) than subjects without parental diabetes. The prevalence of autonomic neuropathy in the nondiabetic offspring with parental type 2 diabetes (6.7%) was significantly ($P < 0.01$) higher compared with the control group (1.6%). Autonomic neuropathy was associated with a higher fasting insulin level ($P < 0.05$), higher UAER ($P < 0.001$), higher 24-h mean AMBP ($P < 0.01$), and reduced diurnal blood pressure variation ($P < 0.001$) after adjustment for age, sex, and BMI. In conclusion, parental type 2 diabetes was found to be associated with alterations in the autonomic nervous system in nondiabetic subjects. The presence of autonomic neuropathy in subjects with parental type 2 diabetes was associated with higher UAER, fasting insulin level, and 24-h AMBP and a reduced diurnal blood pressure variation. This study indicates that parental type 2 diabetes has an impact on the cardiac autonomic function in nondiabetic subjects. *Diabetes* 50:630–636, 2001

From the [1]Medical Department M, Diabetes and Endocrinology, Aarhus Kommunehospital, Aarhus University Hospital; the [2]Geriatric Department C, Marselisborg Hospital, Aarhus University Hospital, Aarhus; and the [3]Medical Department, Fredericia Hospital, Fredericia, Denmark.

Address correspondence and reprint requests to C. Hauerslev Foss, Medical Department M, Diabetes and Endocrinology, Aarhus Kommunehospital, Aarhus University Hospital, DK-8000 Aarhus C, Denmark. E-mail: chf@dadlnet.dk.

Received for publication 23 March 2000 and accepted in revised form 27 November 2000.

AMBP, ambulatory blood pressure; CVD, cardiovascular disease; dBP, diastolic blood pressure; ELISA, enzyme-linked immunosorbent assay; IGT, impaired glucose tolerance; sBP, systolic blood pressure; UAER, urinary albumin excretion rate.

Reduced heart rate variation is used as a marker of cardiac autonomic neuropathy (1,2). It is known that type 2 diabetic subjects have increased cardiovascular morbidity and mortality that is associated with microalbuminuria (3–6), hypertension (7–9), and cardiac autonomic neuropathy detectable by cardiovascular reflex tests (10–12). The association among microalbuminuria, cardiac autonomic neuropathy, and diurnal blood pressure profile has been studied in type 2 diabetic subjects in the recent years, and the question of a causative relationship has arisen. Studies in type 2 diabetes have shown that abnormal diurnal variation in blood pressure is associated with microalbuminuria and autonomic neuropathy (13,14). Recent studies have shown the urinary albumin excretion rate (UAER) to be related to subclinical autonomic neuropathy in type 2 diabetic subjects (15,16), and a study demonstrated that the presence of autonomic neuropathy was associated with hyperinsulinemia and hypertriglyceridemia (17). Results from the Hoorn Study demonstrated an association between autonomic neuropathy and albuminuria in elderly subjects with impaired glucose tolerance (IGT) or type 2 diabetes (18).

Type 2 diabetes is a familial disease with a lifetime risk of 40% if one parent has type 2 diabetes (19,20). Several studies have shown that nondiabetic family members of type 2 diabetic subjects are characterized by the features of the metabolic syndrome, and longitudinal studies have detected cardiovascular risk factors in subjects who later developed type 2 diabetes (21,22). Parasympathetic neuropathy has been observed in subjects with IGT (23), and the results from a recent study demonstrated altered autonomic response in insulin-resistant offspring of type 2 diabetic subjects (24). These latter observations are compatible with the hypothesis that autonomic neuropathy may be present in nondiabetic subjects with parental type 2 diabetes.

In this study, the aim was to examine the impact of parental type 2 diabetes on the autonomic nervous system and to determine whether autonomic neuropathy was present and associated with changes in 24-h ambulatory blood pressure (AMBP) and UAER in nondiabetic subjects with parental type 2 diabetes. The study includes data from 223 nondiabetic offspring with parental type 2 diabe-

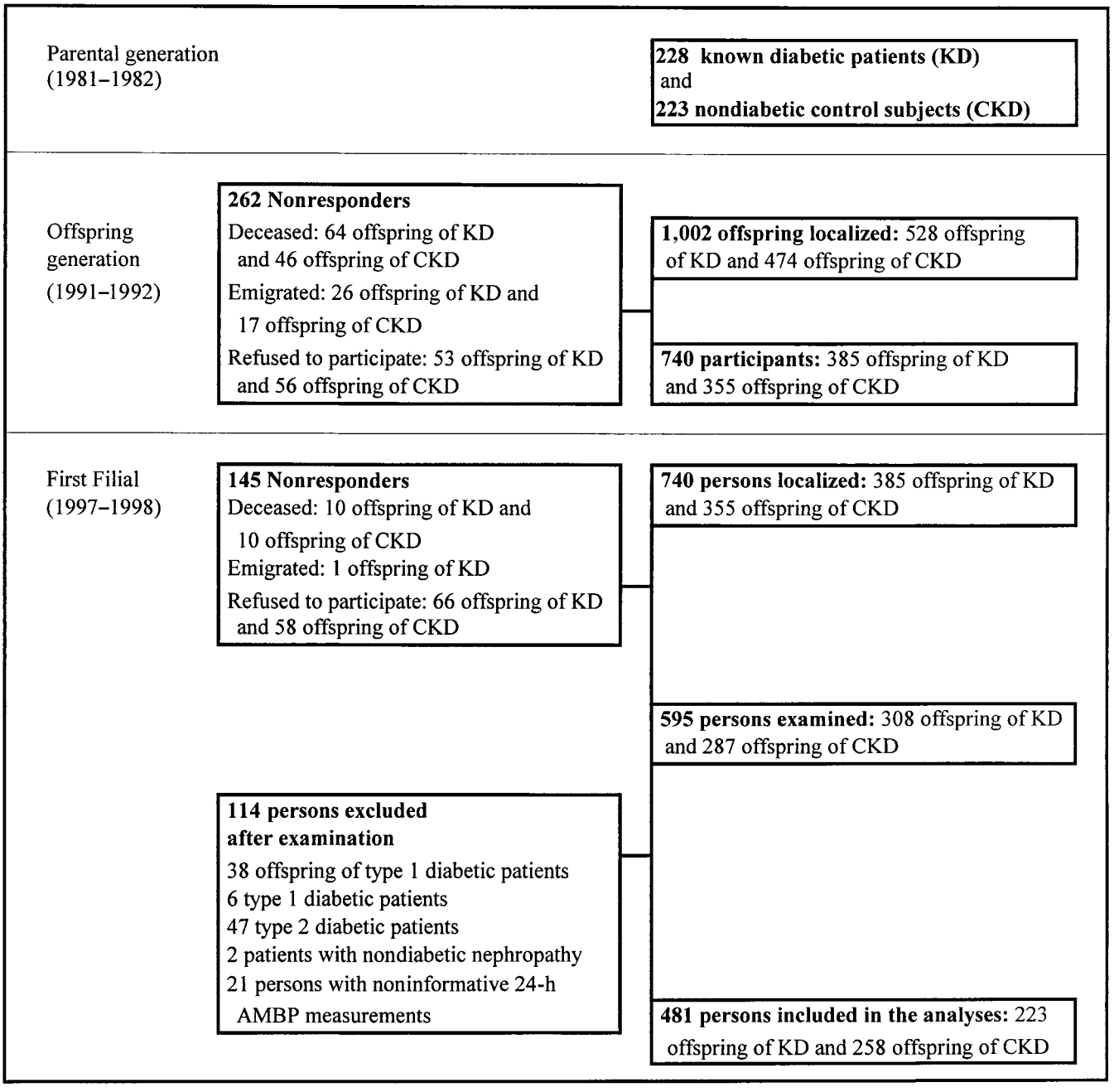

FIG. 1. Flow chart for the Fredericia Study. CKD, nondiabetic control subjects; KD, offspring of known diabetic subjects.

tes and 258 nondiabetic offspring without parental diabetes.

RESEARCH DESIGN AND METHODS

Subjects. In 1997 and 1998, 308 offspring of known diabetic subjects and 287 offspring of control subjects participated in this study, which is part of the Fredericia Study. In 1981–1982, an epidemiological study of 93% (5,292) of all subjects in the age-group 60–74 years took place in the municipality of Fredericia, Denmark (25). From this study, a subgroup of 228 subjects with known diabetes and 223 control subjects underwent a clinical and biochemical examination. The known-diabetes group consisted of 20 type 1 and 208 type 2 diabetic patients diagnosed according to the World Health Organization criteria (26), and the control group consisted of sex- and age-matched control subjects with no history of diabetes and one fasting whole-blood glucose level <7 mmol/l.

As part of an interview, the two groups provided information about their children. The known-diabetes and control groups had 1,029 children, and 1,002 of these offspring were localized in 1991–1992 (27). They were examined for the first time in 1991–1992 and again in 1997–1998 in the present study. This article is based on data from 223 nondiabetic offspring of known diabetic subjects and 258 nondiabetic offspring of control subjects (Fig. 1). The regional ethics committees approved the study, which was conducted according to the principles of the Helsinki Declaration.

Methods. All participants were examined by the same investigator with help from an assistant. Height and weight were measured without the participants wearing shoes, and BMI was calculated as the weight in kilograms divided by height in meters squared. After an overnight fast (10–12 h), blood samples were obtained for determination of whole-blood glucose, plasma C-peptide, serum insulin, HbA_{1c}, total serum cholesterol, and serum HDL cholesterol. Blood glucose was measured with a glucose dehydrogenase method (Cobas Mira; Merck, Rahway, NJ); C-peptide was measured with an enzyme-linked immunosorbent assay (ELISA) method (Dako A/S, Santa Barbara, CA); serum insulin was measured with an ELISA method (Dako A/S) that was highly specific for biologically active insulin but did not detect proinsulin or split [31,32] or des- [32,33] proinsulin (28). HbA_{1c} was measured with a high-

performance liquid chromatographic assay. Total cholesterol, HDL cholesterol, and triglycerides were measured with an enzymatic method (717; Hitachi, Nakakuyo, Japan).

Participants were interviewed about medication, general health (hypertension, diabetes, and nephropathy), and smoking habits. Smokers were defined as subjects smoking cigarettes, cigars, or a pipe every day during the last year.

Diabetes. Diabetes was diagnosed according to World Health Organization criteria (26). Subjects with fasting blood glucose ≥4.4 mmol/l were asked to come back for an oral glucose tolerance test. They were classified as having type 2 diabetes according to their fasting C-peptide level (>0.30 nmol/l) (25,29). Information about the time of diagnosis, medication, and levels of C-peptide, fasting serum insulin, and blood glucose was reviewed in subjects with known diabetes.

Autonomic neuropathy. The following cardiovascular heart rate reflex tests were performed after at least 5 min of rest: heart rate variation to deep breathing, heart rate response to standing up (30:15 ratio), and heart rate response to the Valsalva maneuver. The tests were performed and evaluated according to the procedure described by Ewing et al. (30). The heart rate response to the procedures was assessed; low values indicated low heart rate variation. Subjects with more than one borderline test or at least one abnormal test were classified as having autonomic neuropathy. The borderline and abnormal values used are empirically based on data from both diabetic and healthy control subjects (31,32).

Heart rate variation to deep breathing. In the supine position, the subject was asked to draw six deep breaths over 1 min. The maximum and minimum R-R intervals during each breathing cycle were measured and converted to beats per minute. The mean of the difference in heart rate during three successive breathing cycles was calculated.

Valsalva maneuver. The subjects were asked to blow into a mouthpiece connected to a mercury sphygmomanometer and to produce a constant pressure of 40 mmHg for 15 s. The Valsalva ratio is the ratio between the longest R-R interval after relief of pressure and the shortest R-R interval during sustained pressure. The Valsalva ratio is expressed as the mean ratio from three consecutive maneuvers performed with 1 min of rest between each one.

Heart rate response to standing up (30:15 ratio). After 5 min of rest in the supine position, the subject was asked to stand up. The 30:15 ratio was calculated as the ratio between the longest R-R interval near the 30th beat and the shortest R-R interval near the 15th beat.

Cutoff points for the diagnosis of autonomic neuropathy. For deep breathing, the cutoffs defined by Ewing et al. (30) were normal ≥15, borderline 11–14, and abnormal ≤10. For the Valsalva maneuver, the cutoffs were normal ≥1.21, borderline 1.11–1.20, and abnormal ≤1.10. For the 30:15 ratio, the cutoffs were normal ≥1.04, borderline 1.01–1.03, and abnormal ≤1.00.

UAER. The UAER was calculated from analyses of three overnight urine samples collected in 1 week. The urinary albumin concentration was measured by radioimmunoassay (33), and the UAER was calculated as the geometric mean of three samples. The samples were examined for urinary tract infection, and the subjects with infection collected urine samples again after relevant treatment. Normoalbuminuria was defined as at least two of the three overnight urine collections with UAER <15 μg/min and microalbuminuria as at least two of the three overnight urine collections with UAER ≥15 μg/min and <150 μg/min (34).

24-h AMBP. The 24-h AMBP was measured by oscillometric technique, using a portable lightweight noninvasive monitor with a self-inflating cuff (90207; Spacelabs). This system is validated by the British Hypertension Society (35). Readings were obtained at 20-min intervals throughout a 24-h period with normal daily activities at work and at home. Systolic blood pressure (sBP) and diastolic blood pressure (dBP) were measured. Individual self-reported sleeping time was taken into account when daytime and nighttime values were calculated. The night/day ratio (systolic and diastolic) was defined as the mean value at nighttime divided by mean value during the daytime.

Statistical analysis. Statistical analyses were carried out using SPSS version 8.0 (SPSS, Chicago) and Stata version 5.0 (College Station, TX). The difference in frequencies of clinical characteristics between groups was compared with Pearson's χ^2 test. The mean values (±SD) were calculated, and the difference in mean values between the groups was tested by regression analysis. The generalized estimation equation was used to adjust for correlation between siblings. Before analysis, UAER, plasma C-peptide, serum insulin, and serum triglycerides were log-transformed to approximate normal distribution. For these variables, the results are reported as the geometric mean (antilog of arithmetic mean) multiplied and/or divided by the tolerance factor (antilog of SD of log-transformed data), and the difference estimates express the ratio between the geometric mean values in the two groups. The 95% CIs were calculated for the difference estimates, and two-tailed P values ≥0.05 are considered nonsignificant. P values ≤0.2 are reported as exact values.

RESULTS

Diabetic offspring with parental type 2 diabetes compared with diabetic offspring without parental diabetes. There were 30 subjects with type 2 diabetes and 3 subjects with type 1 diabetes in the group of offspring with parental diabetes. In the group of offspring without parental diabetes, there were 17 subjects with type 2 diabetes and 3 subjects with type 1 diabetes. The diabetic subjects were excluded from further analyses (Fig. 1).

Nondiabetic offspring with parental type 2 diabetes versus nondiabetic offspring without parental diabetes.

Clinical and laboratory data. There was a significantly lower prevalence (χ^2 test, $P = 0.04$) of men versus women in the group of offspring with parental type 2 diabetes (106 men and 117 women) than in the group of offspring without parental diabetes (147 men and 111 women), so difference estimates and P values were corrected for sex (Table 1). There was no significant difference in UAER ($P = 0.22$), but the offspring with parental type 2 diabetes had higher values of BMI ($P < 0.001$), fasting glucose ($P < 0.001$), fasting serum insulin ($P < 0.001$), C-peptide ($P < 0.001$), HbA_{1c} ($P < 0.05$), and triglycerides ($P < 0.01$), and they had lower HDL ($P < 0.01$). There were no significant differences in the 24-h AMBP level or night/day ratio between the groups (data not shown).

Autonomic neuropathy. In the offspring of type 2 diabetic parents, we found the prevalence of autonomic neuropathy to be 6.7% (15 of 223) in contrast to a prevalence of 1.6% (4 of 258) in the offspring of nondiabetic parents (Table 1). After adjustment for age and sex with multiple regression analysis, the difference in prevalence remained significant ($P < 0.01$). There were significant differences in mean values of all three bedside tests ($P < 0.01$) after adjustment for age, sex, and correlation between siblings. The data were further adjusted (data not shown) for the following factors: antihypertensive treatment, 24-h mean systolic and diastolic AMBP, angina pectoris or previous myocardial infarction, UAER, BMI, cholesterol, fasting insulin, fasting glucose, HbA_{1c}, age, sex, and correlation between siblings. After this adjustment, the nondiabetic offspring with parental type 2 diabetes still had lower heart rate variation to deep breathing ($P < 0.05$), heart rate response to standing up ($P < 0.001$), and heart rate response to Valsalva maneuver ($P < 0.001$) than the offspring of nondiabetic parents.

Definition of autonomic neuropathy. For the group of subjects with no parental diabetes, the following values represent the 2.5th percentile of the autonomic test results: 14, deep breathing test; 1.18, Valsalva maneuver; and 1.10, heart rate response to standing. These values are similar to the cutoff points for borderline values defined by Ewing et al. (30).

Nondiabetic offspring with parental type 2 diabetes: those with autonomic neuropathy versus those without autonomic neuropathy. There were only four subjects with autonomic neuropathy in the group of offspring without parental type 2 diabetes. We chose to concentrate on the subjects with parental type 2 diabetes in the

TABLE 1
Clinical and laboratory data of nondiabetic offspring with parental type 2 diabetes compared with nondiabetic offspring with no parental diabetes

	Unadjusted values		Values adjusted for age, sex, and correlation between siblings	
	Parental type 2 diabetes	No parental diabetes	Difference	*P*
n	223	258		
Deep breathing test (beats/min)*	18.82 ± 6.98	20.96 ± 8.07	–2.07 (–3.44, –0.70)	0.003
Valsalva maneuver (Valsalva ratio)*	1.48 ± 0.16	1.55 ± 0.20	–0.07 (–0.10, –0.03)	0.000
Heart rate response to standing up (30:15 ratio)*	1.26 ± 0.14	1.32 ± 0.14	–0.05 (–0.08, –0.03)	0.000
Autonomic neuropathy (yes/no)	15/208 (6.7)	4/254 (1.6)	0.05 (0.02, 0.09)	0.004
Age (years)*	53.80 ± 6.89	52.83 ± 7.40	0.85 (–0.74, 2.44)	NS
BMI (kg/m^2)*	27.77 ± 4.64	26.25 ± 4.11	1.61 (0.78, 2.44)	0.000
Glucose (mmol/l)*	4.38 ± 0.62	4.14 ± 0.46	0.26 (0.15, 0.37)	0.000
HbA_{1c}*	4.76 ± 0.42	4.67 ± 0.04	0.09 (0.01, 0.17)	0.029
C-peptide (pmol/l)†	692 ×/÷ 1.49	603 ×/÷ 1.42	1.15 (1.07, 1.23)	0.000
Fasting insulin (pmol/l)†	47.6 ×/÷ 1.80	39.4 ×/÷ 1.70	1.23 (1.10, 1.35)	0.000
Cholesterol (mmol/l)*	6.21 ± 1.19	5.92 ± 1.30	0.24 (–0.03, 0.51)	0.077
HDL cholesterol (mmol/l)*	1.52 ± 0.38	1.60 ± 0.43	–0.11 (–0.19, –0.04)	0.003
UAER (μg/min)†	3.55 ×/÷ 2.01	3.31 ×/÷ 2.04	1.12 (0.97, 1.32)	0.115
Triglycerides (mmol/l)†	1.58 ×/÷ 1.78	1.35 ×/÷ 1.74	1.20 (1.07, 1.35)	0.002
Smoking (yes/no)	82/191 (36.8)	91/167 (35.3)	0.02 (–0.07, 0.10)	NS
Angina pectoris and/or previous myocardial infarction (yes/no)	12/211 (5.4)	11/247 (4.3)	0.01 (–0.03, 0.05)	NS
Antihypertensive treatment (yes/no)	19/204 (8.5)	23/235 (8.9)	0.00 (–0.05, 0.05)	NS

Data are means ± SD, geometric means ×/÷ tolerance factors, or *n* (%). Estimates of difference between the two groups (95% CIs) were calculated as *{[Parental diabetes]$_{mean}$ − [No parental diabetes]$_{mean}$} or † {[Parental diabetes]$_{mean}$/[No parental diabetes]$_{mean}$}. Values are adjusted for age, sex, and correlation between siblings with a generalized estimation equation model. $P \geq 0.05$ was not significant. $P \leq 0.2$ is reported as an exact value.

characterization of subjects with autonomic neuropathy compared with those without autonomic neuropathy.

There was no significant difference between the two groups with regard to age, sex, BMI, fasting glucose, HbA_{1c}, C-peptide, and lipid profile, but there was a significant difference between values for UAER ($P < 0.001$) and fasting insulin concentration ($P < 0.05$), even after adjustment for age, BMI, sex, and correlation between siblings (Table 2). The 15 subjects with autonomic neuropathy and parental type 2 diabetes were all normoalbuminuric.

The subjects with autonomic neuropathy had higher 24-h AMBP than subjects without autonomic neuropathy, but the difference was not significant for daytime sBP ($P = 0.054$) and daytime dBP ($P = 0.068$) after adjustment for

TABLE 2
Clinical and laboratory data of nondiabetic offspring with parental type 2 diabetes

	Unadjusted values		Values adjusted for age, sex, BMI, and correlation between siblings	
	AN$^+$	AN$^-$	Difference	*P*
n	15	208		
Age (years)*	55.28 ± 8.55	53.69 ± 6.77	1.58 (–2.05, 5.22)	NS
Sex (M/F)	8/7 (53)	98/110 (47)	0.06 (–0.20, 0.32)	NS
BMI (kg/m^2)*	29.60 ± 5.52	27.64 ± 4.55	1.96 (–0.48, 4.39)	0.113
UAER (μg/min)†	7.41 ×/÷ 1.94	3.39 ×/÷ 2.00	2.10 (1.48, 3.02)	0.000
Cholesterol (mmol/l)*	6.31 ± 1.33	6.21 ± 1.18	–0.06 (–0.63, 0.51)	NS
HDL cholesterol (mmol/l)*	1.53 ± 0.46	1.52 ± 0.38	–0.10 (–0.28, 0.77)	NS
Triglycerides (mmol/l)†	1.70 ×/÷ 2.19	1.58 ×/÷ 1.74	0.99 (0.74, 1.31)	NS
HbA_{1c}*	4.83 ± 0.39	4.75 ± 0.42	0.07 (–0.14, 0.28)	NS
Glucose (mmol/l)*	4.47 ± 0.59	4.38 ± 0.63	0.01 (–0.30, 0.32)	NS
Fasting insulin (pmol/l)†	72.4 ×/÷ 2.45	45.7 ×/÷ 1.74	1.36 (1.05, 1.76)	0.024
C-peptide (pmol/l)†	759 ×/÷ 1.78	676 ×/÷ 1.48	1.01 (0.83, 1.21)	NS
Smoker (yes/no)	6/9 (40)	76/132 (37)	0.05 (–0.20, 0.31)	NS
Antihypertensive treatment (yes/no)	2/13 (13)	17/191 (8)	0.05 (–0.12, 0.23)	NS
IGT (yes/no)	2/13 (13)	15/193 (7)	0.06 (–0.11, 0.24)	NS

Data are means ± SD, geometric means ×/÷ tolerance factors, or *n* (%). Subjects with autonomic neuropathy (AN+) are compared with subjects without autonomic neuropathy (AN−). Difference estimates (95% CIs) were calculated as *(AN$^+_{mean}$ − AN$^-_{mean}$) or †(AN$^+_{mean}$/AN$^-_{mean}$). Values are adjusted for age, sex, BMI, and correlation between siblings with a generalized estimation equation model. $P \geq 0.05$ was not significant. $P \leq 0.2$ is reported as an exact value.

TABLE 3
24-h AMBP of nondiabetic offspring with parental type 2 diabetes

	Unadjusted values		Values adjusted for age, sex, BMI, and correlation between siblings	
	AN^+	AN^-	Difference	*P*
n	15	208		
24-h mean sBP	138 ± 17	127 ± 12	8.2 (2.0, 14.3)	0.009
24-h mean dBP	84 ± 9	78 ± 8	5.2 (1.3, 9.1)	0.009
Daytime sBP	142 ± 18	133 ± 13	6.4 (–0.1, 12.8)	0.054
Daytime dBP	87 ± 9	83 ± 8	3.8 (–0.3, 8.0)	0.068
Nighttime sBP	131 ± 15	115 ± 13	13.1 (6.8, 19.3)	0.000
Nighttime dBP	79 ± 9	68 ± 8	10.0 (5.9, 14.0)	0.000
Night/day ratio sBP	0.93 ± 0.06	0.87 ± 0.06	0.06 (0.02, 0.09)	0.001
Night/day ratio dBP	0.90 ± 0.06	0.82 ± 0.07	0.08 (0.04, 0.12)	0.000

Data are means ± SD, unless otherwise indicated. Subjects with autonomic neuropathy (AN+) were compared with subjects without autonomic neuropathy (AN−). Difference estimates (95% CIs) were calculated as ($AN^+_{mean} - AN^-_{mean}$). Values are adjusted for age, sex, BMI, and correlation between siblings with a generalized estimation equation. $P \geq 0.05$ was not significant. $P \leq 0.2$ was reported as an exact value.

age, sex, BMI, and correlation between siblings (Table 3). The night/day ratio was significantly higher for both sBP ($P < 0.01$) and dBP ($P < 0.001$) in the subjects with autonomic neuropathy compared with the subjects without autonomic neuropathy.

DISCUSSION

We found significantly lower mean values for the three cardiovascular reflex tests in nondiabetic subjects with parental type 2 diabetes compared with those without parental diabetes. The results indicate that heart rate variation is reduced in the nondiabetic subjects with parental type 2 diabetes compared with those without parental diabetes. We found a higher prevalence of cardiac autonomic neuropathy in the nondiabetic subjects with parental type 2 diabetes compared with those without parental diabetes. In the group of nondiabetic subjects with parental type 2 diabetes, we found that those with autonomic neuropathy had higher values for UAER, fasting insulin concentration, and 24-h AMBP, and they had reduced diurnal blood pressure variation.

The association we found among autonomic neuropathy, UAER, and 24-h AMBP levels and variation is compatible with previous findings in type 2 diabetes (13,14). Our findings of autonomic neuropathy in nondiabetic subjects with a predisposition to type 2 diabetes are supported by a recent study demonstrating parasympathetic neuropathy in subjects with IGT (23). Other studies found autonomic neuropathy in newly diagnosed type 2 diabetic subjects (36,37). It has generally been accepted that neuropathy is a consequence of long-term hyperglycemia (38,39), and studies have shown that dysregulation of diabetes affects the progression of autonomic neuropathy in a negative way (40,41). In our study, long-term hyperglycemia is not a plausible cause of autonomic neuropathy. The differences in mean values of the three cardiovascular reflex tests between nondiabetic offspring with parental type 2 diabetes and offspring without parental diabetes remained significant after adjustment for fasting glucose, fasting insulin, and HbA_{1c}. In addition, only 2 of the 15 subjects with autonomic neuropathy had IGT, and the subjects with autonomic neuropathy did not have elevated fasting glucose levels or HbA_{1c} fractions compared with the offspring without autonomic neuropathy. If the glucose level played a role in the pathogenesis of autonomic neuropathy in our study, the level influencing the development of neuropathy had to be lower than the cutoff values for IGT or type 2 diabetes.

Our observations indicate that subclinical autonomic neuropathy may develop without the presence of long-term hyperglycemia in family members of type 2 diabetic subjects; thus, it is not simply a complication of the hyperglycemia in these patients. An explanation could be that it is possible to inherit susceptibility genes for autonomic neuropathy, and that these genes could be expressed before—or maybe even without—the subjects developing diabetes. Different factors (including hyperglycemia) could subsequently affect the expression of the genes and influence the progression of neuropathy. In Pima Indians (42), sib-pair analyses of genes associated with retinopathy and nephropathy identified different loci on chromosomes that could affect susceptibility to these complications, and results from studies on clustering of microalbuminuria indicate that microalbuminuria might be an inherited trait in nondiabetic family members of patients with type 2 diabetes (43,44). These results suggest that genetic factors could play a role in the pathogenesis of the features known as complications of type 2 diabetes, and they also suggest that these features can be present without diabetes.

In parallel to our findings concerning autonomic neuropathy, we suggest that subclinical autonomic neuropathy may be part of a genetic syndrome that includes augmented risk for developing cardiovascular disease (CVD), type 2 diabetes, symptomatic autonomic neuropathy, hypertension, and possibly premature death. Whether such development takes place could depend on exogenous factors, such as nutrition, smoking, and physical activity. The escalating incidence of type 2 diabetes and CVD seen in the footsteps of the Westernized lifestyle is compatible with these considerations, and a recent study on twins suggests that nongenetic factors might play a predominant role in controlling whether a genetically predisposed individual progresses to overt type 2 diabetes (45). Hyperglycemia may aggravate CVD, but CVD may be considered more as a concomitant disease than a secondary compli-

cation of type 2 diabetes. The same could be the case with subclinical autonomic neuropathy. The findings of cardiac autonomic neuropathy in nondiabetic offspring of type 2 diabetic subjects could be attributed to the overlapping of genetic determinants for type 2 diabetes and autonomic neuropathy as hypothesized, but the possibility that these findings could be due to nongenetic factors should also be considered.

There are limitations in our study concerning the detection of cardiac autonomic neuropathy. In this study of 528 subjects, we used three cardiovascular reflex tests according to the procedures described by Ewing et al. (30) and combined them to a dichotomous variable (autonomic neuropathy: yes/no) using the cutoff values indicated by Ewing et al. (30). Normal values were not adjusted for age as proposed by other authors (46–48). In this nondiabetic population, we chose to use the cutoff points defined by Ewing et al. (30), who provided both borderline and abnormal values. This could give rise to misclassification of autonomic neuropathy because of low sensitivity in young subjects and low specificity in older subjects. In our study, there were no significant differences in age between the subjects with autonomic neuropathy and those without autonomic neuropathy. Despite these limitations, the group of subjects diagnosed as having cardiac autonomic neuropathy had higher levels of UAER, serum insulin, and blood pressure and reduced diurnal variation, as previously found in type 2 diabetic patients with autonomic neuropathy (13–15,17,49–51). The present study cannot provide information about causality or the temporal relationship among autonomic neuropathy, UAER, and changes in 24-h blood pressure, but this is the first epidemiological study to show increased prevalence of autonomic neuropathy and an association among autonomic neuropathy, UAER, and 24-h blood pressure in a group of nondiabetic offspring of parents with type 2 diabetes.

In conclusion, we have shown that nondiabetic subjects with parental type 2 diabetes have increased prevalence of cardiac autonomic neuropathy compared with nondiabetic subjects without parental type 2 diabetes. We found that in three cardiovascular reflex tests assessing heart rate variation, nondiabetic subjects with parental type 2 diabetes had significantly lower mean values than nondiabetic subjects without parental diabetes. The subjects with autonomic neuropathy had a significantly higher fasting insulin concentration, UAER, and 24-h AMBP and a reduced diurnal blood pressure variation as seen in type 2 diabetes. This cross-sectional study shows that autonomic dysfunction is a feature related to parental type 2 diabetes. The results indicate that early autonomic neuropathy may be present without the influence of long-term hyperglycemia, and we suggest that autonomic neuropathy may be part of a genetic syndrome rather than a secondary complication of diabetes. In future studies on family members of type 2 diabetic subjects, we will presumably gain important knowledge by following the trait of autonomic neuropathy as well as the diabetes trait itself.

ACKNOWLEDGMENTS

The following institutions provided financial support for this study: Sygekassernes Helsefond, Vejle Amts Foundation, the Danish Diabetes Association, Novo Nordisk Foundation, the Danish Heart Foundation, the Sehested Hansen Foundation, the Hede Nielsens Foundation, and Fonden Til Lægevidenskabens Fremme.

We acknowledge the skilled technical assistance of the staff members at the laboratory of Fredericia Hospital and the laboratory of Medical Department M, Aarhus Kommunehospital. We are grateful to Lene Andersen for her assistance in the data-collecting process.

REFERENCES

1. Ewing DJ, Neilson JM, Shapiro CM, Stewart JA, Reid W: Twenty four hour heart rate variability: effects of posture, sleep, and time of day in healthy controls and comparison with bedside tests of autonomic function in diabetic patients. *Br Heart J* 65:239–244, 1991
2. Ziegler D: Diabetic cardiovascular autonomic neuropathy: prognosis, diagnosis and treatment. *Diabetes Metab Rev* 10:339–383, 1994
3. Damsgaard EM, Froland A, Jorgensen OD, Mogensen CE: Microalbuminuria as predictor of increased mortality in elderly people. *BMJ* 300:297–300, 1990
4. Yudkin JS, Forrest RD, Jackson CA: Microalbuminuria as predictor of vascular disease in non-diabetic subjects: Islington Diabetes Survey. *Lancet* 2:530–533, 1988
5. Mogensen CE: Microalbuminuria predicts clinical proteinuria and early mortality in maturity-onset diabetes. *N Engl J Med* 310:356–360, 1984
6. Jarrett RJ, Viberti GC, Argyropoulos A, Hill RD, Mahmud U, Murrells TJ: Microalbuminuria predicts mortality in non-insulin-dependent diabetics. *Diabet Med* 1:17–19, 1984
7. Stamler J, Vaccaro O, Neaton JD, Wentworth D, for the Multiple Risk Factor Intervention Trial Research Group: Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. *Diabetes Care* 16:434–444, 1993
8. U.K. Prospective Diabetes Study Group: Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. *BMJ* 317:703–713, 1998
9. Turner RC, Millns H, Neil HA, Stratton IM, Manley SE, Matthews DR, Holman RR: Risk factors for coronary artery disease in non-insulin dependent diabetes mellitus: United Kingdom Prospective Diabetes Study (UKPDS: 23). *BMJ* 316:823–828, 1998
10. Ewing DJ, Campbell IW, Clarke BF: The natural history of diabetic autonomic neuropathy. *Q J Med* 49:95–108, 1980
11. Rathmann W, Ziegler D, Jahnke M, Haastert B, Gries FA: Mortality in diabetic patients with cardiovascular autonomic neuropathy. *Diabet Med* 10:820–824, 1993
12. Forsblom CM, Sane T, Groop PH, Totterman KJ, Kallio M, Saloranta C, Laasonen L, Summanen P, Lepantalo M, Laatikainen L, Matikainen E, Teppo AM, Koskimies S, Groop L: Risk factors for mortality in type II (non-insulin-dependent) diabetes: evidence of a role for neuropathy and a protective effect of HLA-DR4. *Diabetologia* 41:1253–1262, 1998
13. Nakano S, Uchida K, Kigoshi T, Azukizawa S, Iwasaki R, Kaneko M, Morimoto S: Circadian rhythm of blood pressure in normotensive NIDDM subjects: its relationship to microvascular complications. *Diabetes Care* 14:707–711, 1991
14. Nielsen FS, Rossing P, Bang LE, Svendsen TL, Gall MA, Smidt UM, Parving HH: On the mechanisms of blunted nocturnal decline in arterial blood pressure in NIDDM patients with diabetic nephropathy. *Diabetes* 44:783–789, 1995
15. Wirta OR, Pasternack AI, Mustonen JT, Laippala PJ, Reinikainen PM: Urinary albumin excretion rate is independently related to autonomic neuropathy in type 2 diabetes mellitus. *J Intern Med* 245:329–335, 1999
16. Rutter MK, McComb JM, Brady S, Marshall SM: Autonomic neuropathy in asymptomatic subjects with non-insulin-dependent diabetes mellitus and microalbuminuria. *Clin Auton Res* 8:251–257, 1998
17. Gottsater A, Ahmed M, Fernlund P, Sundkvist G: Autonomic neuropathy in type 2 diabetic patients is associated with hyperinsulinaemia and hypertriglyceridaemia. *Diabet Med* 16:49–54, 1999
18. Smulders YM, Jager A, Gerritsen J, Dekker JM, Nijpels G, Heine RJ, Bouter LM, Stehouwer CD: Cardiovascular autonomic function is associated with (micro-)albuminuria in elderly Caucasian subjects with impaired glucose tolerance or NIDDM: the Hoorn Study. In *Microalbuminuria and Cardiovascular Risk Factors in Type 2 Diabetes Mellitus*. Thesis. Amsterdam, the Netherlands, University of Amsterdam, 1999, p. 103–116
19. Pierce M, Keen H, Bradley C: Risk of diabetes in offspring of parents with non-insulin-dependent diabetes. *Diabet Med* 12:6–13, 1995

20. Kobberling J, Tillil H: Empirical risk figures for first-degree relatives of non-insulin-dependent diabetics. In *Genetics of Diabetes Mellitus*. Kobberling J, Tattersall R, Eds. London, London Academic Press, 1982, p. 201–209
21. Haffner SM: Cardiovascular risk factors and the prediabetic syndrome. *Ann Med* 28:363–370, 1996
22. Mykkänen L, Haffner SM, Kuusisto J, Pyorälä K, Laakso M: Microalbuminuria precedes the development of NIDDM. *Diabetes* 43:552–557, 1994
23. Eriksson KF, Nilsson H, Lindgarde F, Osterlin S, Dahlin LB, Lilja B, Rosen I, Sundkvist G: Diabetes mellitus but not impaired glucose tolerance is associated with dysfunction in peripheral nerves. *Diabet Med* 11:279–285, 1994
24. 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
25. Damsgaard EM, Faber OK, Frøland A, Green A, Hauge M, Holm NV, Iversen S: Prevalence of fasting hyperglycemia and known non-insulin-dependent diabetes mellitus classified by plasma C-peptide: Fredericia survey of subjects 60–74 yr old. *Diabetes Care* 10:26–32, 1987
26. World Health Organization: *Diabetes Mellitus*: *Report of a WHO Study Group*. Geneva, World Health Org., 1985 (Tech. Rep. Ser., no. 727)
27. Vestbo E, Damsgaard EM, Froland A, Mogensen CE: Urinary albumin excretion in a population based cohort. *Diabet Med* 12:488–493, 1995
28. Andersen L, Dinesen B, Jørgensen PN, Poulsen F, Røder ME: Enzyme immunoassay for intact human insulin in serum or plasma. *Clin Chem* 39: 578–582, 1993
29. Gjessing HJ, Matzen LE, Faber OK, Froland A: Fasting plasma C-peptide, glucagon stimulated plasma C-peptide, and urinary C-peptide in relation to clinical type of diabetes. *Diabetologia* 32:305–311, 1989
30. Ewing DJ, Martyn CN, Young RJ, Clarke BF: The value of cardiovascular autonomic function tests: 10 years experience in diabetes. *Diabetes Care* 8:491–498, 1985
31. Ewing DJ, Campbell IW, Clarke BF: Heart-rate response to standing as a test for automatic neuropathy (Letter). *Br Med J* 1:1700, 1978
32. Clarke BF, Ewing DJ, Campbell IW: Diabetic autonomic neuropathy. *Diabetologia* 17:195–212, 1979
33. Christensen CK, Ørskov C: Rapid screening PEG radioimmunoassay for quantification of pathological microalbuminuria. *Diabet Nephropathy* 3: 92–94, 1984
34. Feldt RB, Jensen JS, Borch-Johnsen K: Value of screening for microalbuminuria. In *The Kidney and Hypertension in Diabetes Mellitus*. 3rd ed. Mogensen CE, Ed. Boston, MA, Kluver Academic Publishers, 1998, p. 151–161
35. O'Brien E, Mee F, Atkins N, O'Malley K: Accuracy of the Spacelabs 90207 determined by the British Hypertension Society protocol. *J Hypertens* 9:573–574, 1991
36. Ratzmann KP, Raschke M, Gander I, Schimke E: Prevalence of peripheral and autonomic neuropathy in newly diagnosed type II (noninsulin-dependent) diabetes. *J Diabetes Complications* 5:1–5, 1991
37. Wirta OR, Pasternack AI, Oksa HH, Mustonen JT, Koivula TA, Helin HJ, Lahde YE: Occurrence of late specific complications in type II (non-insulin-dependent) diabetes mellitus. *J Diabetes Complications* 9:177–185, 1995
38. Understanding diabetic neuropathy (Editorial). *Lancet* 338:1496–1497, 1991
39. Greene DA, Sima AA, Stevens MJ, Feldman EL, Lattimer SA: Complications: neuropathy, pathogenetic considerations (Review). *Diabetes Care* 15:1902–1925, 1992
40. Toyry JP, Niskanen L, Mantysaari MJ, Lansimies EA, Uusitupa MI: Occurrence, predictors, and clinical significance of autonomic neuropathy in NIDDM: ten-year follow-up from the diagnosis. *Diabetes* 45:308–315, 1996
41. Gaede P, Vedel P, Parving HH, Pedersen O: Intensified multifactorial intervention in patients with type 2 diabetes mellitus and microalbuminuria: the Steno type 2 randomised study. *Lancet* 353:617–622, 1999
42. Imperatore G, Hanson RL, Pettitt DJ, Kobes S, Bennett PH, Knowler WC: Sib-pair linkage analysis for susceptibility genes for microvascular complications among Pima Indians with type 2 diabetes: Pima Diabetes Genes Group. *Diabetes* 47:821–830, 1998
43. Forsblom CM, Kanninen T, Lehtovirta M, Saloranta C, Groop LC: Heritability of albumin excretion rate in families of patients with type II diabetes. *Diabetologia* 42:1359–1366, 1999
44. Faronato PP, Maioli M, Tonolo G, Brocco E, Noventa F, Piarulli F, Abaterusso C, Modena F, de Bigontina G, Velussi M, Inchiostro S, Santeusanio F, Bueti A, Nosadini R: Clustering of albumin excretion rate abnormalities in Caucasian patients with NIDDM: the Italian NIDDM Nephropathy Study Group. *Diabetologia* 40:816–823, 1997
45. Poulsen P, Kyvik KO, Vaag A, Beck-Nielsen H: Heritability of type II (non-insulin-dependent) diabetes mellitus and abnormal glucose tolerance: a population-based twin study. *Diabetologia* 42:139–145, 1999
46. Bergstrom B, Lilja B, Osterlin S, Sundkvist G: Autonomic neuropathy in non-insulin dependent (type II) diabetes mellitus: possible influence of obesity. *J Intern Med* 227:57–63, 1990
47. Ziegler D, Laux G, Dannehl K, Spuler M, Muhlen H, Mayer P, Gries FA: Assessment of cardiovascular autonomic function: age-related normal ranges and reproducibility of spectral analysis, vector analysis, and standard tests of heart rate variation and blood pressure responses. *Diabet Med* 9:166–175, 1992
48. Low PA, Denq JC, Opfer-Gehrking TL, Dyck PJ, O'Brien PC, Slezak JM: Effect of age and gender on sudomotor and cardiovagal function and blood pressure response to tilt in normal subjects. *Muscle Nerve* 20:1561–1568, 1997
49. Jermendy G, Ferenczi J, Hernandez E, Farkas K, Nadas J: Day-night blood pressure variation in normotensive and hypertensive NIDDM patients with asymptomatic autonomic neuropathy. *Diabetes Res Clin Pract* 34:107–114, 1996
50. Cohen JA, Jeffers BW, Faldut D, Marcoux M, Schrier RW: Risks for sensorimotor peripheral neuropathy and autonomic neuropathy in non-insulin-dependent diabetes mellitus (NIDDM). *Muscle Nerve* 21:72–80, 1998
51. Neil HA, Thompson AV, John S, McCarthy ST, Mann JI: Diabetic autonomic neuropathy: the prevalence of impaired heart rate variability in a geographically defined population. *Diabet Med* 6:20–24, 1989