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Autonomic function is not associated with the incidence of type 2 diabetes mellitus in a population at high risk of diabetes: the Hoorn study

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ABSTRACT

Aim

Impaired autonomic function is a complication of type 2 diabetes mellitus (DM2), but may also be involved in its development. For this reason, this study looked at the association of autonomic function with the incidence of DM2 in a homogeneous Caucasian population.

Methods

The Hoorn study is a prospective population-based study of individuals aged 50–75 years. For the 631 participants, the standard deviation of all normal-to-normal intervals (SDNN) and eight other parameters of autonomic function were calculated at baseline. Fasting and 2-h glucose were measured during follow-up by oral glucose tolerance test (OGTT). DM2 at baseline and follow-up was ascertained by questionnaire and OGTT. After excluding participants with DM2 at baseline, the association of parameters of autonomic function with incident diabetes was examined using logistic-regression analysis while adjusting for possible confounders.

Results

After excluding those with known ($n = 67$) or newly diagnosed ($n = 126$) DM2 at baseline and those missing follow-up data ($n = 140$), 298 participants were eligible for the study (182 with normal glucose tolerance, 19 with impaired fasting glucose and 97 with impaired glucose tolerance). During a median follow-up of 9.2 (range 4.5–11.1) years, 94 incident cases of DM2 were observed. After adjusting for confounding variables, the DM2 odds ratio was 1.12 (95% CI: 0.77, 1.64) per SDNN increase. Results for other parameters of autonomic function were similar.

Conclusion

The present study found no evidence for an association of autonomic function with the incidence of DM2 in a population at high risk of diabetes. This implies that previously observed associations between autonomic function and glucose metabolism in cross-sectional settings may reflect reverse causation.

INTRODUCTION

Type 2 diabetes mellitus (DM2) is an increasing public health problem[1] and autonomic dysfunction is one of its complications.[2] The autonomic nervous system is an involuntary nervous system with a sympathetic and a parasympathetic branch. Its purpose is to control homeostasis and regulate visceral functions. Autonomic dysfunction is characterized by less adaptive changes and relative sympathetic overdrive and is associated with an increased risk of morbidity and mortality.[3, 4] Previous cross-sectional studies showed that impaired autonomic function is associated with increased glucose levels and reduced glucose tolerance in various populations.[5-9]

Although altered autonomic function is considered a consequence of DM2, there are also indications that it may be involved in its development. First, autonomic function has been associated with glucose tolerance in normoglycemic individuals.[10] Second, several organs involved in the glucose metabolism, such as the liver, pancreas, adrenal and skeletal muscles are autonomically innervated. The parasympathetic autonomic nervous system is responsible for the release of insulin from the pancreas and the insulin sensitivity of several organs. The decreased parasympathetic modulation in autonomic dysfunction may therefore play a role in the development of insulin resistance.[11] Third, non-diabetic offspring of DM2 patients, who are at high risk of DM2, have a worse autonomic function than individuals of the same age without a family history of DM2, independent of other risk factors.[12] These findings suggest that autonomic dysfunction may not only be a complication of DM2, but may also play a role in its development. Thus, the aim of the present study was to prospectively investigate the association between autonomic function and the incidence of DM2 in a middle-aged population.

METHODS

Study design and participants

The Hoorn study is a population-based cohort study of glucose tolerance and cardiovascular risk factors in a Caucasian population aged 50 to 75 years. Baseline data were collected from 1989 to 1991. Two follow-up visits were performed to record incident diagnoses of DM2: the first was between 1996 and 1998, and the second was between 2000 and 2001. Details on the Hoorn study have been described elsewhere.[13] In brief, a random sample of men and women aged 50 to 75 years was selected from the municipal registry of the town of Hoorn, the Netherlands. At baseline, 2484 persons participated in a study visit that included a 75-g oral glucose tolerance test (OGTT). Within 3 to 5 weeks of the initial baseline visit, a subset of 631 individuals was invited for additional

baseline measurements. Selection of this subset was stratified by the 2-h glucose values of the first OGTT as well as age and gender. Because of the stratification by 2-h glucose values, the subset included 259 people with normal glucose tolerance (NGT), 28 with impaired fasting glucose (IFG), 151 with impaired glucose tolerance (IGT) and 193 with DM2. The additional baseline visit included measurements of autonomic function. The present study included all participants with available data for at least one parameter of autonomic function. Excluded were those with DM2 at baseline and with missing follow-up data. The Hoorn study had the approval of the ethics committee of the VU University Medical Centre, and all study participants gave their informed consent.

Data collection

During the first baseline visit to the Hoorn study centre, extensive information on demographic characteristics, smoking behaviour, medical history and use of medication was obtained by questionnaire. Physical activity was measured by a sum score of nine equally weighted yes/no questions about the regular performance of the following: sports, bicycling, gardening, walking, doing odd jobs, climbing stairs, household activities, daily food shopping and working.[14] During the physical examination, weight and height were measured with the participants barefoot and wearing light clothing. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m^2). Blood pressure was assessed twice on the right arm while sitting with a random-zero sphygmomano-meter (Hawksley, Lancing, Sussex, UK), and the mean of the two measurements was used for the analyses. Hypertension was defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg.

During both the first and additional baseline visit, blood samples were taken after overnight fasting, and a 75-g OGTT administered to those with no previously diagnosed diabetes. Fasting and 2-h glucose concentrations were measured in plasma (mmol/L) using the glucose dehydrogenase method (Merck, Darmstadt, Germany). Baseline glucose concentrations were defined as the mean of the first and additional baseline measurements. At baseline, participants were classified into categories of glucose tolerance according to World Health Organization (WHO) criteria.[15] NGT was defined as fasting plasma glucose (FPG) < 6.1 mmol/L and 2-h plasma glucose (2-hPG) < 7.8 mmol/L. IFG was defined as FPG 6.1–6.9 mmol/Land 2-hPG < 7.8 mmol/L; IGT with or without IFG was defined as FPG < 7.0 mmol/L and 2-hPG 7.8–11.1 mmol/L; and DM2 was defined as FPG ≥ 7.0 mmol/L or 2-hPG ≥ 11.1 mmol/L.

Fasting serum insulin levels were quantified by insulin-specific double-antibody radioimmunoassay (antibody SP21, EMD Millipore, Billerica, MA, USA).

Autonomic function tests

Participants were asked to refrain from smoking and drinking coffee for 2 hours prior to the additional baseline visit. Tests were performed at a temperature of 19–22°C between 08.30 and 16.00 at least 1 hour after a light meal and with participants in supine position. The tests were preceded by a rest period of at least 10 minutes. During the tests, heart rate and blood pressure were continuously recorded on a PC-based data-acquisition system. RR intervals were obtained by a bipolar electrocardiography (ECG) chest lead and a QRS detector device with an accuracy of 1 ms. Blood pressure was recorded continuously using the Finapres (finger arterial blood pressure) method (model BP2000, GE Datex-Ohmeda, Madison, WI, USA), digitally sampled at 200 Hz, and offline low-pass-filtered and down-sampled to 100 Hz. Systolic blood pressure values were obtained by an automated procedure verified by visual inspection.

Cardiac cycle duration (RR interval) and continuous finger arterial pressure were measured under three conditions: (1) spontaneous breathing for 3 min; (2) six deep breaths over 1 min; and (3) active change in position from supine to standing. The breathing frequency of six breaths/min was dictated by the investigator. When offline spectral analysis showed that the participants failed to breathe at the appropriate frequency, the recording was discarded. After each test, a rest period of 1 min was included to prevent any effects from previous tests.

During spontaneous breathing, the mean of the normal-to-normal RR intervals (NN intervals) and standard deviation of all NN intervals (SDNN) were calculated. Furthermore, spectral analysis was used to assess the power of the low-frequency (LF; 0.04–0.12 Hz) and high-frequency (HF; 0.12–0.40 Hz) bands. Impaired autonomic function leads to lower values of these four measurements. From the deep-breathing recordings, it was possible to measure the difference in maximum and minimum RR intervals during expiration and inspiration as averaged over six breaths (EI difference). Baroreflex sensitivity (BRS) was also calculated from the deep-breathing recordings, defined as the change in RR intervals caused by changes in systolic blood pressure (ms/mmHg) and estimated as the gain in transfer function between blood pressure and RR-interval changes. Only spectral components between 0.05 and 0.15 Hz were used, together with a squared coherence (χ^2) of 0.5 or higher. Lower EI difference and BRS sensitivity are a sign of impaired autonomic function. During the active change in position from supine to standing, the difference between the mean RR interval during 1 min of rest prior to standing and the minimum RR interval within 15 s of standing (RRmax) was calculated. In addition, the maximum RR interval 15–30 s after standing divided by the minimum RR interval at around 15 s after standing (RRmax/min), and the systolic blood pressure difference (SBP difference) after standing, defined as the mean SBP over 30 s

within 90–120 s of standing minus the mean over 30 s prior to standing, were calculated. Impaired autonomic function is reflected by lower RRmax and RRmax/min, and a larger (more negative) SBP difference. In some cases, data were missing because the test schedule was not completed, the quality of data was inadequate for processing or non-sinus beats constituted > 10% of the total number of recorded beats.

In addition to individual parameters of autonomic function, a summary score of autonomic function was constructed, as described elsewhere.[16] The results for each parameter of autonomic function were divided into quartiles. Each participant was assigned 0 points if the result was in the most abnormal quartile, 1 point if in the second quartile, 2 points if in the third quartile, and 3 points if in the best quartile of autonomic function. For all parameters except SBP difference, participants in the highest quartile had the best autonomic function. If all nine parameters were available, the scores for each were added together to construct a summary score. If one or two results were missing (41/298), these were replaced by the median value for that score. If three or more results were missing, the summary score was not calculated (59/298). Summary scores ranged from 0 (very poor) to 27 (very good).

Ascertainment of type 2 diabetes during follow-up

At the follow-up visits during 1996–1998 and 2000–2001, examinations including OGTTs were performed. New diagnoses of DM2 were ascertained during a follow-up visit using three methods: (1) by asking 'Have you been diagnosed with DM2 since the last study visit?' and 'Which doctor monitors your DM2?'; (2) by recording the use of glucose-lowering medication; and (3) by OGTTs in participants with no known diabetes. DM2 was defined as FPG ≥ 7.0 mmol/L or 2-hPG ≥ 11.1 mmol/L. At the follow-up visits, both fasting and 2-h glucose concentrations were measured by the hexokinase method (Boehringer Ingelheim Pharma, Ingelheim, Germany) at follow-up.

Statistical analyses

Descriptive statistics were calculated as mean ± standard deviation (SD) or percentage for the total population and stratified by tertiles of SDNN. Also, differences in baseline characteristics between tertiles of autonomic function were tested by analysis of variance (ANOVA) for continuous variables and by chi-square test for categorical variables. As most cases of diabetes were detected at follow-up study visits and the exact dates of onset were unknown, it was not possible to use time-to-event analyses, so logistic regression was performed instead to study the association between the nine parameters of autonomic function at baseline and the incidence of DM2 at follow-up. The nine parameters were standardized to a mean of zero with 1 SD, and crude odds ratios (ORs) were calculated for these standardized parameters with 95% confidence intervals (CI),

while ORs were also adjusted for age (continuous), gender, BMI (continuous), hypertension (yes/no), prevalent cardiovascular disease (CVD; yes/no), cardiac medication (yes/no), smoking (no smoking (reference), current smoking or former smoking), physical activity score (continuous), follow-up duration (continuous) and family history of DM (yes/no). In addition, baseline fasting glucose and insulin concentrations and 2-h glucose concentrations (continuous) were added to the model to correct for the effects of glucose and insulin concentrations on autonomic function. These analyses were also performed in a subgroup of individuals with NGT at baseline. For these participants, a composite endpoint consisting of the development of IFG, IGT or DM2 was calculated, using data from the last available follow-up. The same logistic regression analyses were performed with this composite endpoint as outcome.

Also, linear regression analysis was performed to examine the association of autonomic function with continuous fasting and 2-h glucose concentrations at the follow-up visits, using glucose concentrations from the last available follow-up visit. Statistical analyses were performed with Stata statistical soft-ware, version 12 (StataCorp LP, College Station, TX, USA).

RESULTS

Baseline characteristics

A total of 631 participants had measurements of autonomic function at baseline. Excluded were those with known (n = 67) or newly diagnosed (n = 126) DM2 at baseline. Of the 438 remaining participants, 140 had missing follow-up data. Also, 51 participants died, 30 moved to another region and, for 59 participants, the reason for not attending the follow-up visit was unknown. Those with missing follow-up data were somewhat older (SD) at 66 (7) years compared with 63 (7) years, had higher 2-h glucose levels at 7.1 (2) mmol/L compared with 6.6 (2.0) mmol/L and more often used cardiac medication, with 27% using compared with 19% not. All parameters of autonomic function were slightly lower in those lost to follow-up: their mean (SD) NN interval was 929 (133) ms compared with 974 (152) ms, while BRS was 8 (4) ms/mmHg compared with 9 (6) ms/mmHg.

The present study ultimately included 298 participants: 182 with NGT; 19 with IFG; and 97 with IGT. Their mean age (SD) was 63 (7) years, 51% were male and their mean (SD) BMI was 26 (3) kg/m^2. Baseline characteristics for the entire study population (n = 298) by tertiles of SDNN (n = 272) are shown in Table1.

Table 1: Baseline characteristics for the total study population (n=298) and stratified by tertiles of SDNN (n=272)

	Total	SDNN (ms) in tertiles		
		11-29 ms	30-42 ms	43-118 ms
N	298	91	91	90
Sex (% men)*	51	48	42	62
Age (years)*	63 (7)	64 (7)	63 (7)	61 (6)
BMI (kg/m^2)	26 (3)	27 (3)	26 (3)	26 (3)
Smoking				
- Current (%)	26	23	24	28
- Former (%)	35	32	37	34
Fasting glucose (mmol/L)	5.5 (0.6)	5.6 (0.6)	5.5 (0.5)	5.5 (0.5)
2-hour glucose (mmol/L)	6.7 (2.0)	7.0 (2.0)	6.5 (1.9)	6.5 (2.0)
Activity score (scale 0-9)	6 (2)	5 (2)	6 (1)	6 (2)
Hypertension (%)*	30	42	24	20
Prevalent CVD (%)*	13	18	8	9
Use of cardiac medication (%)*	19	30	13	11
Family history of DM (%)	26	24	25	27
IFG or IGT at baseline (%)	39	42	40	33

Data are presented as mean (SD) or percentage
*p-value<0.05

Those in the highest tertile of SDNN were younger, more often male, less often hypertensive and less often used cardiac medication than those in the lowest SDNN tertile. The prevalence of CVD was highest in the lowest tertile of SDNN. All participants had at least one parameter of autonomic function available, the values of which are presented in Table 2. The first follow-up visit included 286 participants and, of these, 230 attended the second follow-up visit whereas nine participants only attended the second follow-up visit. For one participant the exact date of the follow-up visit was unknown. The median follow-up duration was 9.2 (range 4.5–11.1) years.

Autonomic function and incidence of DM2

Of the 298 participants included in the analyses, 94 developed DM2 at one of the follow-up visits: 24 from the NGT group; 10 from the IFG group; and 60 from the IGT group. At the first follow-up visit (1996–1998), there were 67 cases of DM2 and, at the second follow-up visit (2000–2001), 27 cases of DM2. The ORs (95% CI) for DM2 associated with parameters of autonomic function are shown in Table 3. After adjusting for confounding factors and glucose and insulin levels, ORs ranged from 0.88 (0.59, 1.31) to 1.36 (0.93, 1.98) and were all non-significant. In addition to individual parameters of autonomic

Table 2: Parameters of autonomic function

Parameter of autonomic function	N	Mean (SD)
Mean NN (ms)	272	974 (152)
SDNN (ms)	272	38 (17)
LF power (ms^2)	272	264 (121-547)*
HF power (ms^2)	272	206 (94-451)*
EI-difference (ms)	278	193 (108)
BRS (ms/mmHg)	260	9.4 (5.6)
RR max (ms)	279	260 (98)
RR max/min (ms)	279	1.3 (0.2)
SBP difference (mmHg)	256	–5.5 (14.8)
Summary score	234	14 (6)

*Median (25^{th}-75^{th} percentile)

Table 3: Odds ratio and 95% CI for the incidence of type 2 diabetes per SD of parameters of autonomic function

	N	Events	SD	Model 1	Model 2	Model 3	Model 4
Mean NN (ms)	272	85	152	0.87 (0.67, 1.13)	0.87 (0.67, 1.14)	0.86 (0.65, 1.14)	1.01 (0.69, 1.47)
SDNN (ms)	272	85	17	0.89 (0.68, 1.16)	0.93 (0.71, 1.22)	0.92 (0.69, 1.23)	1.12 (0.77, 1.64)
Ln LF power (ms^2)	272	85	1	1.00 (0.78, 1.30)	1.08 (0.82, 1.41)	1.09 (0.81, 1.47)	1.21 (0.82, 1.77)
Ln HF power (ms^2)	272	85	1	0.95 (0.74, 1.23)	0.99 (0.76, 1.29)	0.93 (0.71, 1.23)	1.17 (0.81, 1.68)
EI-difference (ms)	278	88	108	1.06 (0.82, 1.36)	1.13 (0.87, 1.47)	1.13 (0.85, 1.51)	1.36 (0.93, 1.98)
BRS (ms/mmHg)	260	82	5.6	0.98 (0.75, 1.28)	1.00 (0.76, 1.31)	1.03 (0.77, 1.36)	1.23 (0.83, 1.83)
RR max (ms)	279	90	98	0.79 (0.60, 1.04)	0.82 (0.62, 1.08)	0.82 (0.60, 1.09)	0.92 (0.62, 1.35)
RR max/min (ms)	279	90	0.2	1.03 (0.80, 1.32)	1.11 (0.84, 1.46)	1.17 (0.88, 1.57)	1.35 (0.94, 1.95)
SBP difference (mmHg)	256	82	14.8	0.91 (0.70, 1.18)	0.92 (0.70, 1.20)	0.90 (0.67, 1.20)	0.88 (0.59, 1.31)
Summary score	262	84	6	0.95 (0.73, 1.23)	0.99 (0.75, 1.31)	1.04 (0.78, 1.39)	1.36 (0.92, 2.01)

Model 1: crude
Model 2: adjusted for age and sex
Model 3: adjusted for age, sex, BMI, hypertension, prevalent CVD, cardiac medication, smoking, physical activity, follow-up duration and family history of DM
Model 4: adjusted for age, sex, BMI, hypertension, prevalent CVD, cardiac medication, smoking, physical activity, follow-up duration, family history of DM, and baseline glucose and insulin concentrations

function, a summary score of autonomic function was calculated. For every SD of the summary score, the adjusted OR for DM2 was 1.36 (0.92, 2.01).

Autonomic function and fasting and 2-h glucose concentrations at follow-up

The linear regression analyses used glucose concentrations from the last available follow-up visit (104 from the first follow-up visit and 194 from the second). The Figure shows the crude associations of SDNN (ms) at baseline with fasting (A) and 2-h (B)

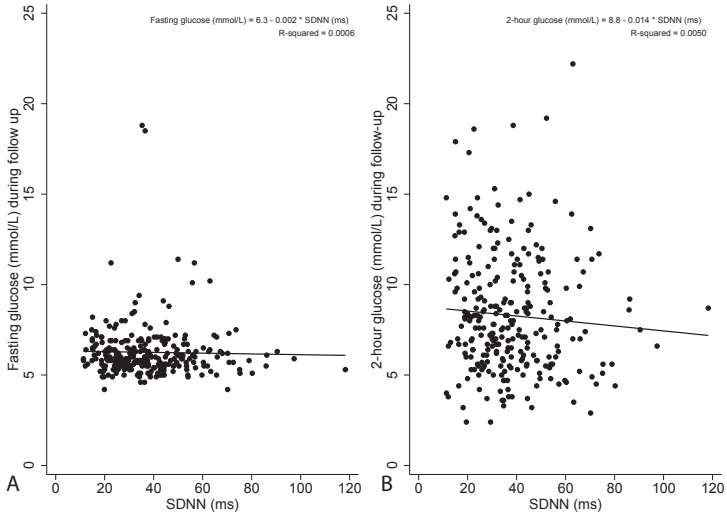

Figure. Scatter plots of the standard deviation of all normal-to-normal intervals (SDNN; ms) at baseline, and fasting (A) and 2-h (B) glucose concentrations (mmol/L) at the last available follow-up study visits.

plasma glucose concentrations (mmol/L) at the time of the last available follow-up. The associations between parameters of autonomic function and glucose concentrations were small, and only the relationship between the EI difference and fasting glucose was statistically significant after adjustment (mean increase was 0.20 mmol/L (95% CI: 0.03, 0.36) per SD increase in EI difference). All other associations were not significant after adjusting for age, gender, BMI, hypertension, prevalent CVD, cardiac medication, smoking, physical activity, follow-up duration, and baseline glucose and insulin concentrations. The summary score of the autonomic function tests also showed no relationship. For every SD of the summary score, the adjusted difference in fasting glucose concentration at follow-up was 0.09 (−0.09, 0.27) mmol/L, with a 2-h glucose concentration of 0.09 (−0.25, 0.43) mmol/L (Table 4).

Autonomic function and incidence of DM2 in participants with normal glucose tolerance at baseline

In the subgroup of 182 participants with NGT at baseline, 24 developed DM2. Sixteen cases were detected at the first follow-up visit and eight at the second follow-up (Table 5). ORs were close to 1 and comparable to those for the total study population. In addition, there was no association between the summary score of autonomic function and DM2 incidence in participants with NGT at baseline (adjusted OR: 1.13, 95% CI: 0.60, 2.15). For participants with IFG or IGT at baseline, the OR for DM2 per SD of the summary score was 1.43 (0.72, 2.85), indicating that reverse causation may have been involved. Of the 182 participants who had NGT at baseline, 72 reached the composite

Table 4: Associations between parameters of autonomic function with fasting and 2-hour glucose concentrations at follow-up

		Difference in fasting glucose (mmol/L) (95% CI)[1]		Difference in 2-hour glucose (mmol/L) (95% CI)[1]	
	SD	Crude	Adjusted[2]	Crude	Adjusted[2]
Mean NN (ms)	153	−0.08 (−0.26, 0.10)	0.03 (−0.14, 0.20)	−0.11 (−0.53, 0.28)	0.18 (−0.15, 0.50)
SDNN (ms)	17	−0.04 (−0.22, 0.14)	0.08 (−0.08, 0.25)	−0.23 (−0.63, 0.16)	0.12 (−0.20, 0.45)
Ln LF power (ms^2)	1	−0.01 (−0.19, 0.17)	0.08 (−0.10, 0.25)	−0.24 (−0.64, 0.15)	−0.06 (−0.39, 0.27)
Ln HF power (ms^2)	1	0.10 (−0.08, 0.28)	0.14 (−0.02, 0.31)	−0.14 (−0.54, 0.26)	0.18 (−0.14, 0.50)
EI-difference (ms)	108	0.19 (0.01, 0.36)	0.20 (0.03, 0.36)	−0.29 (−0.68, 0.11)	0.00 (−0.33, 0.33)
BRS (ms/mmHg)	6	0.09 (−0.09, 0.28)	0.14 (−0.04, 0.31)	−0.28 (−0.69, 0.14)	0.06 (−0.27, 0.40)
RR max (ms)	98	−0.19 (−0.37,−0.02)	−0.04 (−0.20, 0.12)	−0.32 (−0.71, 0.07)	0.00 (−0.32, 0.31)
RR max/min (ms)	0.2	−0.10 (−0.27, 0.08)	0.00 (−0.17, 0.17)	−0.19 (−0.57, 0.21)	0.13 (−0.19, 0.46)
SBP difference (mmHg)	15	0.02 (−0.16, 0.21)	0.01 (−0.16, 0.18)	−0.23 (−0.64, 0.20)	−0.04 (−0.37, 0.29)
Summary score	6	−0.08 (−0.27, 0.10)	0.09 (−0.09, 0.27)	−0.25 (−0.66, 0.16)	0.09 (−0.25, 0.43)

[1] Difference (95% CI) in glucose concentrations per SD increase of parameters of autonomic function
[2] Adjusted for age, sex, BMI, hypertension, prevalent CVD, cardiac medication, smoking, physical activity, follow-up duration, family history of DM, and baseline glucose and insulin concentrations

Table 5: Odds ratios and 95% CI for the incidence of type 2 diabetes per SD of parameters of autonomic function in the individuals with normal glucose tolerance at baseline

	N	Events	SD	Model 1	Model 2	Model 3	Model 4
Mean NN (ms)	168	23	154	0.95 (0.61, 1.48)	0.95 (0.60, 1.48)	0.78 (0.46, 1.33)	0.79 (0.39, 1.58)
SDNN (ms)	168	23	18	1.01 (0.64, 1.57)	1.02 (0.65, 1.61)	0.98 (0.59, 1.61)	0.99 (0.55, 1.78)
Ln LF power (ms^2)	168	23	1	1.16 (0.75, 1.80)	1.19 (0.75, 1.90)	1.26 (0.75, 2.11)	1.39 (0.75, 2.58)
Ln HF power (ms^2)	168	23	1	0.91 (0.58, 1.42)	0.91 (0.58, 1.44)	0.84 (0.52, 1.36)	0.87 (0.48, 1.59)
EI-difference (ms)	173	24	108	0.95 (0.61, 1.48)	0.95 (0.59, 1.50)	1.01 (0.60, 1.70)	1.16 (0.65, 2.08)
BRS (ms/mmHg)	159	22	5	0.91 (0.56, 1.46)	0.87 (0.53, 1.42)	0.81 (0.46, 1.43)	0.89 (0.43, 1.84)
RR max (ms)	172	24	99	0.77 (0.46, 1.27)	0.76 (0.45, 1.27)	0.69 (0.39, 1.22)	0.74 (0.38, 1.45)
RR max/min (ms)	172	24	0.2	1.04 (0.69, 1.59)	1.05 (0.67, 1.65)	1.05 (0.64, 1.73)	1.08 (0.63, 1.84)
SBP difference (mmHg)	156	21	15	1.29 (0.82, 2.05)	1.29 (0.81, 2.06)	1.11 (0.62, 1.98)	0.89 (0.44, 1.79)
Summary score	162	23	6	0.99 (0.64, 1.55)	1.00 (0.63, 1.59)	1.03 (0.61, 1.73)	1.13 (0.60, 2.15)

Model 1: crude
Model 2: adjusted for age and sex
Model 3: adjusted for age, sex, BMI, hypertension, prevalent CVD, cardiac medication, smoking, physical activity, follow-up duration and family history of DM
Model 4: adjusted for age, sex, BMI, hypertension, prevalent CVD, cardiac medication, smoking, physical activity, follow-up duration, family history of DM, and baseline glucose and insulin concentrations

endpoint of IFG/IGT/DM2 (24 cases at the first follow-up and 48 at the second). All ORs were non-significant (Table 6). Linearregression analyses with parameters of autonomic

function as determinants, and fasting and 2-h glucose at the last available follow-up visit as outcomes, also showed no significant associations (data not shown).

Table 6: Odds ratios and 95% CI for the incidence of IFG, IGT or DM2 per SD of parameters of autonomic function in individuals with normal glucose tolerance at baseline

	N	Events	SD	Model 1	Model 2	Model 3	Model 4
Mean NN (ms)	168	66	154	0.98 (0.72, 1.34)	0.98 (0.72, 1.34)	1.08 (0.77, 1.51)	1.16 (0.80, 1.69)
SDNN (ms)	168	66	18	0.92 (0.67, 1.27)	0.91 (0.66, 1.26)	0.90 (0.63, 1.28)	0.90 (0.62, 1.31)
Ln LF power (ms^2)	168	66	1	0.79 (0.58, 1.09)	0.76 (0.54, 1.07)	0.75 (0.52, 1.07)	0.71 (0.48, 1.05)
Ln HF power (ms^2)	168	66	1	1.01 (0.74, 1.38)	1.01 (0.73, 1.40)	0.98 (0.69, 1.38)	1.04 (0.72, 1.50)
EI-difference (ms)	173	71	108	0.91 (0.67, 1.24)	0.91 (0.66, 1.26)	0.80 (0.56, 1.15)	0.78 (0.53, 1.14)
BRS (ms/mmHg)	159	68	5	0.93 (0.68, 1.29)	0.96 (0.69, 1.33)	0.88 (0.61, 1.26)	0.94 (0.63, 1.38)
RR max (ms)	172	69	100	0.87 (0.63, 1.20)	0.86 (0.62, 1.20)	0.93 (0.67, 1.31)	1.01 (0.71, 1.44)
RR max/min (ms)	172	69	0.2	0.84 (0.61, 1.16)	0.83 (0.59, 1.17)	0.84 (0.57, 1.23)	0.86 (0.59, 1.27)
SBP difference (mmHg)	156	63	15	1.02 (0.74, 1.40)	1.01 (0.73, 1.39)	0.97 (0.66, 1.43)	0.92 (0.61, 1.39)
Summary score	162	63	6	0.75 (0.54, 1.03)	0.72 (0.51, 1.02)	0.72 (0.50, 1.04)	0.75 (0.50, 1.12)

Model 1: crude
Model 2: adjusted for age and sex
Model 3: adjusted for age, sex, BMI, hypertension, prevalent CVD, cardiac medication, smoking, physical activity, follow-up duration and family history of DM
Model 4: adjusted for age, sex, BMI, hypertension, prevalent CVD, cardiac medication, smoking, physical activity, follow-up duration, family history of DM, and baseline glucose and insulin concentrations

DISCUSSION

The present study aimed to examine the association between autonomic function and the incidence of DM2 in a homogeneous Caucasian population aged 50–75 years. Non-significant associations were observed between nine parameters of autonomic function (mean NN interval, SDNN, LF power, HF power, EI difference, BRS, RRmax, RRmax/min and SBP difference) and DM2 after a median follow-up duration of 9.2 (range: 4.5–11.1) years. In addition, a summary score of all nine functions was not associated with incident DM2. The associations between parameters of autonomic function and fasting and 2-h glucose concentrations at the last available follow-up visit were small and non-significant, with the exception of an unexpected positive association between EI difference and fasting glucose during follow-up. To exclude the influence of higher glucose concentrations on autonomic function, two analyses were performed in individuals with NGT at baseline, one with DM2 and another with a composite endpoint including IFG, IGT and DM2 as outcomes. There were no significant associations between any of these parameters of autonomic function and DM2 incidence.

An important strength of the present study was its prospective design. Cross-sectional studies fail to provide information on causal relationships between autonomic function and glucose tolerance, particularly because glucose and insulin concentrations can also influence autonomic function.[17-20] The present prospective study was able to investigate the temporal relationship between autonomic function and the development of DM2. By adjusting the analyses for baseline glucose and insulin concentrations and performing the analyses in individuals with NGT at baseline, it was possible to correct for the possible effect of baseline glucose and insulin levels on autonomic function (reverse causation).

Another strength was the use of OGTTs for the diagnosis of DM2 at follow-up, as this test is more reliable than self-reporting for diagnosis of DM2 and also identifies diabetics who have not yet been diagnosed by their physicians.[21] Furthermore, we used an extensive set of parameters of autonomic function that can be divided in three categories. Four parameters (EI-difference, RRmax, RRmax/min and SBP difference) are part of the Ewing test battery.[22] These tests evaluate cardiovascular autonomic reflexes and are indicative of sympathetic (EI-difference and RRmax) or parasympathetic (EI-difference, RRmax, RRmax/min and SBP difference) integrity. The value of Ewing tests has been extensively evaluated and the tests are used in clinical practice for the assessment of (diabetic) neuropathy.[23] The second category of autonomic function tests are the heart rate (variability) parameters: mean NN, SDNN, LF power and HF power. The mean NN interval is the reciprocal of mean heart rate and is indicative of the sympathovagal balance, with shorter NN intervals representing more sympathetic activation.[24] Heart rate variability is the result of autonomic modulations of the sympathetic and parasympathetic nervous system, with the purpose to buffer blood pressure.[25, 26] Finally, baroreflex sensitivity is defined as the reflex-induced change in interbeat interval in milliseconds per millimeter of Hg blood pressure change and describes the functioning of the baroreflexes in short term regulation of arterial blood pressure.[27] The use of three categories of autonomic function parameters enabled us to evaluate several aspects of the autonomic nervous system and is therefore an important strength of our study.

On the other hand, one limitation of the present study was the small sample size of 298 participants, which may have resulted in insufficient power. The proportion of IFG and IGT cases at baseline was high because of oversampling of such individuals in the study population, and this contributed to the 94 cases of DM2 found at follow-up. Yet, despite the small sample size, overall there were no indications of any association between autonomic function and incidence of DM2. The associations with fasting and 2-h glucose concentrations were small, and the limits of the 95% CI excluded large effects. For this reason, it is not believed that associations would be detected with larger

sample sizes and power. The present limited sample size was in large part the result of non-participation in the follow-up visits (140 of the 438 non-diabetic participants at baseline did not participate, 51 died, 29 moved and, for 59 the reason for not attending was unknown). In fact, the 298 participants included in our analyses were healthier and had better autonomic function than the 140 who were lost to follow-up. The association between autonomic function and DM2 among non-participants may have been stronger than among participants, and this may have led to underestimation of the true association between autonomic function and DM2 in our analyses. However, even with a hypothetical worst-case scenario in which all participants who did not return for follow-up developed DM2, analysis would still not have found a significant association between parameters of autonomic function and incidence of DM2 (data not shown).

Several cross-sectional studies have shown an association between autonomic function and glucose tolerance.[5-9] Four previous studies prospectively investigated the association between autonomic function and incident DM2. Shigetoh et al found a significant OR (5.39, 95% CI: 1.34, 21.8) for the development of DM2 in individuals with a heart rate ≥80 beats/min as compared with a heart rate <60 beats/min (n=614, number of incident cases of DM2 not reported).[28] However, it is unclear whether individuals with known DM2 at baseline were excluded from this study and therefore the results may be biased. An analysis on the Chicago Heart Association Detection Project in Industry showed an association for 1 SD increase in heart rate (12 beats/min) and diabetes mortality (n=14992, 400 cases of diabetes mortality): OR=1.21, 95% CI: 1.03-1.41) in individuals aged 35-49 years. Heart rate was also associated with non-fatal DM2, but this association attenuated after adjustment for BMI and post load glucose concentrations at baseline.[29] Since we only investigated non-fatal DM2 and not diabetes mortality, these results are comparable to our study. The ARIC study (n=8185, 1063 incident cases of DM2) showed an association of resting heart rate with DM2 after adjustment for confounding including baseline glucose (relative risk per SD (9.7 beats/min) = 1.06 (95% CI 1.00, 1.13). A comparable association was observed in individuals with normal fasting glucose at baseline (RR=1.13, 95% CI: 1.04, 1.22). In line with our study, there were no associations between LF and HF power and SDNN with DM2.[30] The ARIC study is comparable with the Hoorn study in design. An important difference is the inclusion of more ethnic groups (mainly black, 19%) in the ARIC study. The influence of ethnicity on autonomic function is unknown, but differences in glucose metabolism have been shown between ethnic groups.[31] The CARDIA study (n=3295, 98 incident cases of DM2) showed an association between low heart rate recovery and incident DM2 in individuals with poor fitness (OR=3.27, 95% CI 1.34, 7.94), but not in fit persons.[32] In our study, there was no association in either fit or unfit participants after stratification for regular physical activity (defined as at least five days a week moderately active) (data not shown). However, the population of the

CARDIA study is much younger than the population of the Hoorn study (18-30 years versus 50-75 years). It is possible that physical activity is an important effect modifier in young individuals, but is less important in older age due to an entirely different risk profile. Slow heart rate recovery may reflect decreased parasympathetic activity.[33] Our study did not investigate heart rate recovery, which is defined as the rapid decrease in heart rate following cessation of exercise, but a previous study showed a significant association between heart rate variability and heart rate recovery.[34] Therefore it would be expected that the association of heart rate recovery and heart rate variability with DM2 would be comparable. However, the results of the CARDIA study are not adjusted for glucose concentrations at baseline. Since glucose concentrations at baseline are a strong predictor for the development of DM2, results may suffer from reverse causation.[35] Overall, the results from previous prospective studies are inconsistent. Furthermore, our study used more parameters of autonomic function than the previous studies. It has been shown that relying on one parameter may result in over- or underestimation of the autonomic function.[22] This may explain the incidental significant results that were found earlier. In our study we examined several aspects of the autonomic nervous system and we consistently found no evidence for an association with DM2.

The results of cross-sectional studies have identified an association between autonomic function and glucose tolerance that may be bidirectional. Some studies suggested that autonomic dysfunction may not only be a complication of DM2, but may also be involved in its development. However, in our prospective analysis there was no association between autonomic function and DM2. This suggests that autonomic dysfunction is solely a consequence of glucose concentrations and is not involved in the development of DM2. Such a link was proposed in 1986 by Landsberg, who postulated that the sympathetic activation associated with obesity is mediated by insulin resistance and concomitant hyperinsulinaemia.[36] Experimental studies in humans showed that hyperinsulinaemia caused by the infusion of insulin enhanced sympathetic activation and depressed vagal activation.[17, 18] This decreased autonomic function may be the result of sympathetic activation caused by insulin acting in the hypothalamus.[37] Also hyperglycaemia has been associated with sympathetic activation.[19, 38] These studies indicate that hyperinsulinaemia and hyperglycaemia may be the underlying factor of the previously reported cross-sectional association between impaired glucose metabolism and sympathetic activation.

In conclusion, there is no evidence from the present study of any association between autonomic function and the incidence of DM2 and fasting glucose concentrations in the follow-up of a homogeneous Caucasian population aged 50–75 years. These results suggest that previously observed associations between autonomic function and glucose metabolism in cross-sectional settings may have simply reflected reverse causation.

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