Both Prediabetes and Type 2 Diabetes Are Associated With Lower Heart Rate Variability: The Maastricht Study

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OBJECTIVE

Low heart rate variability (HRV), a marker for cardiac autonomic dysfunction, is a known feature of type 2 diabetes, but it remains incompletely understood whether this also applies to prediabetes or across the whole glycemic spectrum. Therefore, we investigated the association among prediabetes, type 2 diabetes, and measures of glycemia and HRV.

RESEARCH DESIGN AND METHODS

In the population-based Maastricht Study ($n = 2,107$; mean ± SD age 59 ± 8 years; 52% men; normal glucose metabolism [$n = 1,226$], prediabetes [$n = 331$], and type 2 diabetes [$n = 550$, oversampled]), we determined 24-h electrocardiogram-derived HRV in time and frequency domains (individual *z*-scores, based upon seven and six variables, respectively). We used linear regression with adjustments for age, sex, and major cardiovascular risk factors.

RESULTS

After adjustments, both time and frequency domain HRV were lower in prediabetes and type 2 diabetes as compared with normal glucose metabolism (standardized β [95% CI] for time domain: −0.15 [−0.27; −0.03] and −0.34 [−0.46; −0.22], respectively, *P* for trend <0.001; for frequency domain: −0.14 [−0.26; −0.02] and −0.31 [−0.43; −0.19], respectively, *P* for trend <0.001). In addition, 1-SD higher glycated hemoglobin, fasting plasma glucose, and 2-h postload glucose were associated with lower HRV in both domains (time domain: −0.16 [−0.21; −0.12], −0.16 [−0.21; −0.12], and −0.15 [−0.20; −0.10], respectively; frequency domain: −0.14 [−0.19; −0.10], −0.14 [−0.18; −0.09], and −0.13 [−0.18; −0.08], respectively).

CONCLUSIONS

Both prediabetes and type 2 diabetes were independently associated with lower HRV. This is further substantiated by independent continuous associations between measures of hyperglycemia and lower HRV. These data strongly suggest that cardiac autonomic dysfunction is already present in prediabetes.

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Cardiovascular disease in type 2 diabetes is clinically diverse, ranging from myocardial infarction, stroke, and intermittent claudication (1) to sudden death, arrhythmia, silent ischemia, and heart failure (2–4). Diabetes is associated with atherothrombosis (5), arterial stiffening (6), and microvascular dysfunction (7), and these are major pathways explaining this clinical diversity. Another mechanism that is thought to contribute is diabetes-associated cardiac autonomic dysfunction (8).

Cardiac autonomic function can be noninvasively assessed by calculation of heart rate variability (HRV), which reflects the interaction of the sympathetic and parasympathetic parts of the autonomic nervous system on the sinus node (9). Low HRV is a validated measure of cardiac autonomic dysfunction and is associated with an increased risk of ventricular arrhythmias and sudden cardiac arrest (10).

Interestingly, evidence is accumulating that many abnormalities observed in type 2 diabetes are already present, although less severe, in prediabetes (7). Indeed, prediabetes is also associated with greater risk of cardiovascular disease (11). In addition, next to the glucose metabolism status classification, continuous measures of hyperglycemia have also been shown to be associated with cardiovascular disease (12,13). This raises the possibility that prediabetes, as compared with normal glucose metabolism, is also associated with cardiac autonomic dysfunction.

Some population-based studies on the associations of prediabetes and type 2 diabetes with HRV have suggested that this is the case (14–19). However, these studies have found inconsistent results using various individual HRV measures (14–19) or did not adjust for important potential confounders, such as physical activity (14,15,17,18). Importantly, all previous studies have only used short-term electrocardiogram (ECG) recordings (~5 min) (14–19), whereas HRV derived from 24-h ECG is a more accurate reflection of cardiac autonomic function (9,20).

In view of these considerations, we tested, in a population-based cohort study, the hypothesis that prediabetes and diabetes, as well as continuous measures of hyperglycemia (i.e., glycated hemoglobin [HbA_{1c}] and fasting and 2-h plasma glucose from an oral glucose tolerance test [OGTT]) are associated with cardiac autonomic dysfunction, as measured by time and frequency domain HRV derived from 24-h ECGs.

RESEARCH DESIGN AND METHODS

Study Population and Design

We used data from the Maastricht Study, an observational, prospective, population-based cohort study. The rationale and methods have been described previously (21). In brief, the study focuses on the etiology, pathophysiology, complications, and comorbidities of type 2 diabetes and is characterized by an extensive phenotyping approach. Eligible participants were individuals between 40 and 75 years of age and living in the southern part of the Netherlands. Participants were recruited through mass media campaigns and from the municipal registries and the regional Diabetes Patient Registry via mailings. Recruitment was stratified according to known type 2 diabetes status, with an oversampling of individuals with type 2 diabetes, for reasons of efficiency. The present report includes cross-sectional data from the first 3,541 participants, who completed the baseline survey between November 2010 and September 2013. The examinations of each participant were performed within a time window of 3 months. The study was approved by the institutional medical ethics committee (NL31329.068.10) and the Minister of Health, Welfare and Sport of the Netherlands (permit 131088–105234-PG) and was conducted in accordance with the Declarations of Helsinki. All participants gave written informed consent.

Assessment of Glucose Metabolism Status

In order to determine glucose metabolism status, all participants (except those who used insulin) underwent a standardized 2-h 75-g OGTT after an overnight fast. For safety reasons, participants with a fasting glucose level >11.0 mmol/L, as determined by finger prick, did not undergo the OGTT. For these individuals, fasting glucose level and information about diabetes medication were used to determine glucose metabolism status. Glucose metabolism status was defined according to the World Health Organization 2006 criteria as normal glucose metabolism, prediabetes (impaired fasting glucose and/or impaired glucose tolerance), and type 2 diabetes (22).

Assessment of HRV

All ECGs were recorded by use of a 12-lead Holter system (Fysiologic ECG Services, Amsterdam, the Netherlands) over a 24-h period. During recording time, participants were asked to follow their normal daily activities, except that they were asked not to take a shower or a bath. Recordings were analyzed with proprietary Holter Analysis Software at Fysiologic ECG Services with an algorithm that excluded nonsinus cardiac cycles (e.g., artifacts and premature/ectopic beats), validated by manual inspection afterward. The software from Fysiologic provided the intervals between the individual R waves of sinus beats (i.e., interbeat intervals [IBIs], in milliseconds). From the obtained IBIs, HRV was calculated by use of the publicly available free GNU Octave software (23), according to the standard time and frequency domain measures defined by the 1996 Task Force document on HRV (9) and recently updated recommendations (24). The minimum duration of the recording for HRV analysis was 18 h after exclusion of nonsinus cardiac cycles. As time domain measures, we calculated the SD of all normal-to-normal (NN) intervals (SDNN, in milliseconds); the SD of the averages of NN intervals in all 5-min segments of the entire recording (SDANN, in milliseconds); the square root of the mean of the sum of squares of differences between adjacent NN intervals (RMSSD, in ms); the mean of the SDs of all NN intervals for all 5-min segments of the entire recording (SDNN index, in milliseconds); the SDs of differences between adjacent NN intervals (SDSD, in milliseconds); the number of pairs of adjacent NN intervals differing by >50 ms in the entire recording (NN50 count); and the NN50 count divided by the total number of all NN intervals (pNN50, a percentage). As frequency domain measures, we used the Fast Fourier Transform to calculate the variance of all NN intervals ≤0.4 Hz (total power [TP], in milliseconds squared); power in the ultralow-frequency range (ULF, in milliseconds squared) (≤0.003 Hz); power in the very-low-frequency range (VLF, in milliseconds squared) (0.003–0.04 Hz); power in the low-frequency range (LF, in milliseconds squared) (0.04–0.15 Hz); power in the high-frequency range (HF, in milliseconds squared) (0.15–0.4 Hz); and the LF/HF ratio. Individual *z*-scores were calculated for the time and frequency

domain measures and combined in an overall time domain variable ([SDNN + RMSSD + SDANN + SDNN index + pNN50]/5) and an overall frequency domain variable ([TP + ULF + VLF + LF + HF]/5). SDSD, NN50, and LF/HF were excluded from the *z*-score calculation due to the fact that these variables were derived from variables already included in the overall score (i.e., SDNN, SDANN, LF, and HF).

Measurement of Covariates

We assessed history of cardiovascular disease, duration of diabetes, education level (low, intermediate, and high), smoking status (never, former, and current), alcohol use (none, low, and high) by questionnaire, and physical activity by questionnaire (total activity hours per week) and accelerometer (mean stepping time per hour; activPAL3). We assessed use of medication during an interview in which we registered generic name, dose, and frequency. We measured body weight, height, BMI, waist circumference, ambulatory 24-h blood pressure, plasma glucose levels, HbA_{1c}, plasma insulin, HOMA of insulin resistance (HOMA2-IR; based on fasting plasma glucose and insulin), the Matsuda index of insulin sensitivity (based on plasma glucose and insulin levels during an OGTT), and plasma lipid profile as described elsewhere (21). Estimated glomerular filtration rate (in mL/min/1.73 m^2) was calculated with the Chronic Kidney Disease Epidemiology Collaboration equation based on both serum creatinine and serum cystatin C (25).

Analytical Sample

From the initial 3,451 participants included, we excluded individuals with other types of diabetes than type 2 diabetes ($n = 41$). Of the remaining sample, we excluded those in whom HRV could not be adequately assessed (no 24-h ECG available due to logistics [$n = 856$], ECG registration <18 h [$n = 16$], or arrhythmia [$n = 22$]). Of the remaining 2,506 participants, 2,107 had complete data on potential confounders and were included in the final study population (Supplementary Fig. 1). The characteristics of individuals excluded from the analysis compared with those included were largely similar, but individuals excluded from the analysis had a somewhat more adverse cardiovascular risk profile (Supplementary Table 1).

Statistical Analyses

We used multivariable linear regression analysis to determine the associations of glucose metabolism (normal glucose metabolism, prediabetes, and type 2 diabetes), fasting plasma glucose, 2-h postload plasma glucose levels from the OGTT, and HbA_{1c} with time and frequency domain HRVs. For linear trend analyses, the categorical variable glucose metabolism status (normal glucose metabolism = 0, prediabetes = 1, and type 2 diabetes = 2) was used in the regression models. To assess regression coefficients per glucose metabolism group, we used pairwise analyses with dummy variables for prediabetes and type 2 diabetes. To test for a linear relationship of lower HRV across deteriorating glucose metabolism status, we compared the aforementioned models with a likelihood ratio test (in which the null hypothesis states that there is a linear response).

We adjusted for the following covariates: model 1 adjusted for age, sex, and education level; model 2 additionally adjusted for cardiovascular risk factors that have previously been associated with lower HRV (26) (i.e., BMI, alcohol consumption, smoking behavior, self-reported physical activity, systolic blood pressure, total-to-HDL cholesterol levels, and use of antihypertensive and lipid-modifying drugs); and model 3 additionally adjusted for history of cardiovascular disease and estimated glomerular filtration rate.

To test whether associations differed by sex, interaction terms (prediabetes * sex, type 2 diabetes * sex, and measures of hyperglycemia * sex) were added to the regression models.

We performed several additional analyses to test the robustness of our findings: *1*) we repeated the analyses with individual measures of HRV as the outcome; *2*) we additionally adjusted for heart period (i.e., mean IBI) and markers of insulin resistance (i.e., fasting plasma insulin, HOMA-IR, and the Matsuda index of insulin sensitivity), although this may cause overadjustment: heart period may be intrinsically linked to HRV, but is also a reflection of autonomic function (27), and insulin resistance has been suggested to be associated with lower HRV (17), but may lie on the causal pathway of deteriorating glucose metabolism to autonomic dysfunction; *3*) we excluded individuals who used antihypertensive medication, as it has been suggested that the use of β-blockers, nondihydropiridine calcium channel blockers, diuretics, and renin-angiotensin system blockers may influence HRV (28); and *4*) we adjusted for objectively measured physical activity by an accelerometer instead of physical activity based on questionnaires, for waist circumference instead of BMI, and for 24-h systolic blood pressure instead of office systolic blood pressure. We did this in additional rather than the main analyses because participants more often had missing data on these variables (analyses done in $n = 1{,}610$ for objectively measured physical activity, $n = 2{,}105$ for waist circumference, and $n = 1{,}944$ for 24-h blood pressure).

All statistical analyses were performed with IBM SPSS version 25.0 for Windows (IBM Corp., Somers, NY). A two-sided *P* value of <0.05 was considered statistically significant, except for interaction analyses, in which $P_{interaction} < 0.10$ was considered statistically significant.

RESULTS

Characteristics of Study Population

The study population consisted of 2,107 individuals with a mean ± SD age of 59.7 ± 8.2 years, of whom 51.3% were men (Table 1). Individuals with type 2 diabetes were, by design, oversampled (26.1%). From normal glucose metabolism to type 2 diabetes, age, BMI, blood pressure, and use of antihypertensive and lipid-modifying medication were higher. In addition, individuals with prediabetes or type 2 diabetes were, as compared with normal glucose metabolism, more often men and current smokers, more often had a history of cardiovascular disease, and had lower amounts of physical activity, lower estimated glomerular filtration rate, and lower estimates of HRV (Table 1).

Glucose Metabolism Status and HRV

The composite scores for time and frequency domain HRV were lower in individuals with prediabetes and type 2 diabetes as compared with normal glucose metabolism (Table 2 [crude model]). Adjustment for age, sex, and education level attenuated the differences between individuals with type 2 diabetes or prediabetes and normal glucose metabolism, but the associations remained statistically significant (*P* for trend <0.001).

Table 1—General characteristics and HRV measures of the study population according to glucose metabolism status

Characteristic	Normal glucose metabolism (n = 1,226)	Prediabetes (n = 331)	Type 2 diabetes (n = 550)
Demographics			
Age, years	57.9 ± 8.1	61.4 ± 7.7	62.5 ± 7.6
Men	534 (43.6)	180 (54.4)	367 (66.7)
Education level			
Low	297 (24.2)	118 (35.6)	250 (45.5)
Intermediate	351 (28.6)	93 (28.1)	159 (28.9)
High	578 (47.1)	120 (36.3)	141 (25.6)
Lifestyle variables			
Smoking behavior			
Never	483 (39.4)	98 (29.6)	152 (27.6)
Former	595 (48.5)	194 (58.6)	323 (58.7)
Current	148 (12.1)	39 (11.8)	75 (13.6)
Alcohol consumption			
None	171 (13.9)	51 (15.4)	162 (29.5)
Low	703 (57.3)	178 (53.8)	281 (51.5)
High	352 (28.7)	102 (30.8)	107 (19.5)
Physical activity, h/week	15.2 ± 8.2	14.3 ± 7.7	12.7 ± 8.2
Clinical characteristics			
BMI, kg/m^2	25.5 ± 3.6	27.8 ± 4.1	29.7 ± 5.1
History of cardiovascular disease	141 (11.7)	43 (13.0)	154 (28.0)
eGFR, mL/min/1.73 m^2	90.2 ± 13.2	86.9 ± 13.9	84.8 ± 17.3
Fasting plasma glucose, mmol/L*	5.2 ± 0.4	5.9 ± 0.6	7.9 ± 2.1
2-h postload glucose, mmol/L*	5.4 ± 1.1	8.2 ± 1.8	14.5 ± 3.8
HbA_{1c}, %*	5.5 ± 0.3	5.7 ± 0.4	6.9 ± 1.1
HbA_{1c}, mmol/mol*	36.1 ± 3.7	38.8 ± 4.4	51.8 ± 11.5
Diabetes duration, years	—	—	5.0 (3.0–11.0)
Office systolic blood pressure, mmHg	130.9 ± 17.2	138.1 ± 17.7	142.3 ± 17.5
Office diastolic blood pressure, mmHg	75.5 ± 9.8	78.2 ± 9.5	77.5 ± 9.6
Office heart rate, bpm	65.9 ± 9.6	68.3 ± 11.1	71.4 ± 12.5
Total-to-HDL cholesterol ratio	3.6 ± 1.2	3.9 ± 1.3	3.8 ± 1.2
Total cholesterol, mmol/L	5.5 ± 1.0	5.5 ± 1.2	4.5 ± 1.0
HDL cholesterol, mmol/L	1.7 ± 0.5	1.5 ± 0.4	1.3 ± 0.4
LDL cholesterol, mmol/L	3.4 ± 0.9	3.3 ± 1.1	2.5 ± 0.9
Triglycerides, mmol/L	1.1 (0.8–1.4)	1.3 (1.0–1.8)	1.5 (1.1–2.2)
Use of lipid-modifying medication	209 (17.0)	113 (34.1)	400 (72.7)
Use of antihypertensive medication	261 (21.3)	145 (43.8)	404 (73.5)
β-Blockers	104 (8.5)	74 (22.4)	193 (35.1)
Diuretics	83 (6.8)	62 (18.7)	188 (34.2)
Calcium antagonists	46 (3.8)	22 (6.6)	118 (21.5)
ACE inhibitors	73 (6.0)	28 (8.5)	142 (25.8)
Angiotensin II inhibitors	98 (8.0)	75 (22.7)	180 (32.7)
Use of glucose-lowering medication	—	—	418 (76.0)
Insulin	—	—	101 (18.4)
Metformin	—	—	372 (67.6)
Sulfonylureas	—	—	114 (20.7)
Thiazolidinediones	—	—	5 (0.9)
GLP-1 analogs	—	—	4 (0.7)
DPP-4 inhibitors	—	—	33 (6.0)
HRV			
Time domain			
Composite score, SD	0.15 ± 0.93	−0.10 ± 0.84	−0.33 ± 0.95
SDNN, ms	141.6 ± 37.3	131.3 ± 37.5	118.6 ± 35.0
SDANN, ms	127.8 ± 35.8	118.7 ± 36.8	107.0 ± 32.9
RMSSD, ms	29.9 ± 13.9	27.8 ± 13.1	27.4 ± 16.9
SDNN index, ms	56.4 ± 16.8	51.9 ± 14.6	47.2 ± 17.6
SDSD, ms	26.9 ± 15.0	27.9 ± 13.2	24.8 ± 16.5
NN50, count	6,697 (3,174–12,304)	5,266 (2,546–10,283)	4,244 (1,777–8,952)
pNN50, %	7.1 (3.2–13.4)	5.5 (2.6–11.1)	4.4 (1.7–9.8)
Frequency domain			
Composite score, SD	0.16 ± 0.99	−0.10 ± 0.80	−0.32 ± 0.91
TP, ms^2	12,579 (8,776–17,228)	10,627 (7,072–15,876)	8,678 (5,763–13,019)
ULF, ms^2	10,798 (7,409–14,881)	8,961 (5,867–13,847)	7,379 (4,731–11,092)

Continued on p. 1130

Table 1—Continued

Characteristic	Normal glucose metabolism (n = 1,226)	Prediabetes (n = 331)	Type 2 diabetes (n = 550)
VLF, ms^2	1,164 (812–1,718)	1,015 (714–1,477)	795 (539–1,228)
LF, ms^2	413 (242–657)	327 (204–515)	246 (139–413)
HF, ms^2	94 (54–162)	75 (47–134)	62 (34–121)
LF/HF	4.7 ± 2.5	4.7 ± 2.5	4.4 ± 2.6

Data are reported as mean ± SD, median (interquartile range), or number (percentage). bpm, beats per minute; DPP-4, dipeptidyl peptidase 4; eGFR, estimated glomerular filtration rate; GLP-1, glucagon-like peptide 1. *Data available for fasting plasma glucose, n = 2,105; for 2-h postload glucose, n = 1,982; and for HbA_{1c}, n = 2,102.

Additional adjustment for cardiovascular risk factors (model 2), history of cardiovascular disease, and estimated glomerular filtration rate (model 3) further attenuated the differences in HRV between individuals with type 2 diabetes or prediabetes and normal glucose metabolism, but the associations remained statistically significant. Regression coefficients in prediabetes were consistently approximately one-half of the type 2 diabetes coefficients (P for trend <0.001) (Table 2 [model 3] and Fig. 1). Possible confounders, in the fully adjusted model, that were associated with HRV in the time domain were age (β = −0.02 [95% CI −0.03; −0.02]), female sex (β = −0.27 [−0.36; −0.19]), BMI (β = −0.014 [−0.024; −0.004]), total-to-HDL cholesterol ratio (β = −0.07 [−0.10; −0.03]), alcohol use (β = −0.28 [−0.41; −0.16]), and smoking behavior (β = −0.25 [−0.38; −0.13]); β values indicate the SD change in HRV per unit higher age (years), BMI (kilograms per meter squared), or total-to-HDL cholesterol ratio, being female or a current (vs. never) smoker, or being a high alcohol consumer (vs. none). Similar results were found for HRV in the frequency domain (data not shown). Continuous measures of hyperglycemia (i.e., HbA_{1c}, fasting plasma glucose, and 2-h postload glucose) were all linearly inversely associated with HRV in both time and frequency domains (P < 0.001) (Table 2 [model 3] and Fig. 2).

Additional Analyses

Because we did not observe consistent patterns of interaction with sex, we did not stratify analyses by sex. When we analyzed the individual measures of time and frequency domain HRV as the outcome, associations were similar to those of the analyses based on the composite HRV scores, except for the LF/HF ratio, in which associations were not statistically significant (Supplementary Table 3 and Supplementary Fig. 2). When we additionally adjusted for heart period (i.e., mean IBI), associations were attenuated but remained statistically significant (Supplementary Table 4). When we additionally adjusted for markers of insulin resistance, results did not materially change. Exclusion of individuals who used antihypertensive medication also did not materially change the results (Supplementary Table 5). When we adjusted for stepping time (i.e., objectively measured physical activity) instead of physical activity by questionnaire, for waist circumference instead of BMI, or for 24-h systolic blood pressure instead of office systolic blood pressure, results again remained similar (Supplementary Table 6).

CONCLUSIONS

The results of the current study demonstrate that both prediabetes and type 2 diabetes were associated with lower

Table 2—Associations among glucose metabolism status and measures of glycemia (i.e., HbA_{1c}, fasting plasma glucose, and 2-h postload glucose) and HRV

HRV measure	Prediabetes	Type 2 diabetes	P for trend	HbA_{1c}*	P	Fasting plasma glucose*	P	2-h postload glucose*	P
Time domain†									
Crude	−0.26 (−0.37; −0.14)	−0.44 (−0.53; −0.34)	<0.001§	−0.22 (−0.26; −0.18)	<0.001	−0.21 (−0.25; −0.17)	<0.001	−0.20 (−0.24; −0.16)	<0.001
Model 1	−0.23 (−0.35; −0.11)	−0.42 (−0.52; −0.32)	<0.001§	−0.20 (−0.24; −0.16)	<0.001	−0.20 (−0.24; −0.16)	<0.001	−0.17 (−0.21; −0.13)	<0.001
Model 2	−0.16 (−0.28; −0.04)	−0.35 (−0.47; −0.23)	<0.001§	−0.16 (−0.21; −0.12)	<0.001	−0.16 (−0.21; −0.12)	<0.001	−0.15 (−0.20; −0.11)	<0.001
Model 3	−0.15 (−0.27; −0.03)	−0.34 (−0.46; −0.22)	<0.001§	−0.16 (−0.21; −0.12)	<0.001	−0.16 (−0.21; −0.12)	<0.001	−0.15 (−0.20; −0.10)	<0.001
Frequency domain‡									
Crude	−0.26 (−0.38; −0.15)	−0.44 (−0.54; −0.34)	<0.001§	−0.21 (−0.25; −0.17)	<0.001	−0.20 (−0.24; −0.16)	<0.001	−0.20 (−0.24; −0.15)	<0.001
Model 1	−0.23 (−0.34; −0.11)	−0.41 (−0.51; −0.31)	<0.001§	−0.19 (−0.23; −0.15)	<0.001	−0.19 (−0.23; −0.15)	<0.001	−0.16 (−0.20; −0.12)	<0.001
Model 2	−0.15 (−0.27; −0.03)	−0.31 (−0.43; −0.19)	<0.001§	−0.14 (−0.19; −0.10)	<0.001	−0.14 (−0.19; −0.10)	<0.001	−0.13 (−0.18; −0.08)	<0.001
Model 3	−0.14 (−0.26; −0.02)	−0.31 (−0.43; −0.19)	<0.001§	−0.14 (−0.19; −0.10)	<0.001	−0.14 (−0.18; −0.09)	<0.001	−0.13 (−0.18; −0.08)	<0.001

Data are β (95% CI) unless otherwise indicated. For glucose metabolism status, regression coefficients (β) represent mean difference in HRV measure with normal glucose metabolism as reference. For measures of glycemia, regression coefficients represent SD difference in HRV measure per 1-SD increment in glucose measure (1 SD is equivalent to, for HbA_{1c}, 0.87%; for fasting plasma glucose, 1.65 mmol/L; and for 2-h postload glucose, 4.20 mmol/L). Model 1 was adjusted for age, sex, and education level; model 2 was additionally adjusted for BMI, total-to-HDL cholesterol ratio, alcohol consumption, smoking behavior, physical activity, systolic blood pressure, and use of lipid-modifying and antihypertensive medication; and model 3 was additionally adjusted for history of cardiovascular disease and estimated glomerular filtration rate. *Analyses performed in, for HbA_{1c}, n = 2,070; for fasting plasma glucose, n = 2,073; and for 2-h postload glucose, n = 1,955. †Time domain z-score combines SDNN, SDANN, RMSSD, SDNN index, SDSD, NN50, and pNN50. ‡Frequency domain z-score combines TP, ULF, VLF, LF, and HF. §P value for likelihood ratio test >0.05, which indicates that there is a linear response of lower HRV across deteriorating glucose metabolism status.

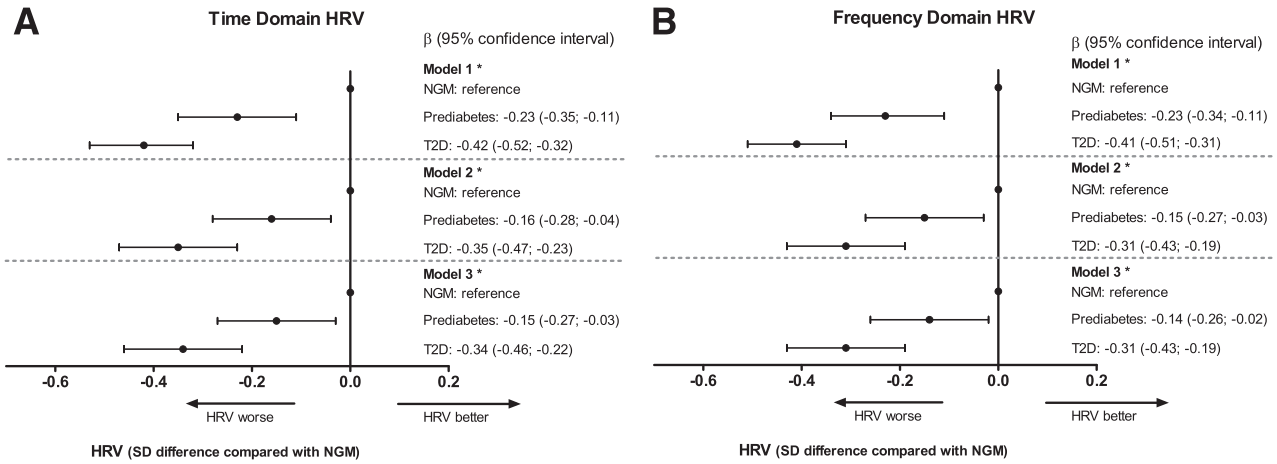

Figure 1—Multivariable adjusted differences in time domain (*A*) and frequency domain HRV (*B*) between individuals with prediabetes and type 2 diabetes (T2D) compared with individuals with normal glucose metabolism (NGM). Point estimates represent SD difference with 95% CIs in time domain and frequency domain HRV for prediabetes and type 2 diabetes compared with NGM. Model 1 was adjusted for age, sex, and education level. Model 3 was additionally adjusted for BMI, total-to-HDL cholesterol ratio, alcohol consumption, smoking behavior, physical activity, systolic blood pressure, use of lipid-modifying and antihypertensive medication, history of cardiovascular disease, and estimated glomerular filtration rate. *P for trend <0.001.

HRV. The amount by which HRV was lower in prediabetes (as compared with normal glucose metabolism) was approximately half of that in type 2 diabetes in both time and frequency domains. In addition, continuous measures of glycemia (HbA_{1c}, fasting, or 2-h postload plasma glucose levels) were linearly associated with HRV, which suggests a graded decline in HRV with worsening glucose tolerance. These associations were independent of major cardiovascular risk factors. Therefore, our results support the concept that cardiac autonomic dysfunction occurs before the diagnosis of type 2 diabetes and may play a role in the development of cardiovascular diseases in prediabetes and early in the course of type 2 diabetes.

This is the first population-based study to consistently show that cardiac autonomic dysfunction is a feature of prediabetes and type 2 diabetes. Importantly, and in contrast to previous population-based studies (14–19), we found that virtually all time and frequency domain measures of HRV, either in a composite score or as individual measures, were associated with worsening glucose tolerance. This may be explained by the fact that we used the more accurate 24-h ECG-derived HRV as opposed to HRV derived from short-term ECG recordings. In addition, we were able to adjust for a large series of potential confounders, including (objectively measured) physical activity.

We found a graded decline in cardiovascular autonomic function from normal glucose metabolism to type 2 diabetes, as supported by linear trends that worsening glucose tolerance was associated with lower HRV. In addition, individuals with prediabetes had statistically significantly lower HRV compared with individuals with normal glucose metabolism status, which indicates that cardiac autonomic dysfunction precedes the clinical diagnosis of type 2 diabetes. We observed such associations across the whole spectrum of hyperglycemia (i.e., we found statistically significant associations of higher HbA_{1c} and fasting and 2-h postload plasma glucose with lower HRV).

We used composite scores of time and frequency domain HRV measures to assess cardiac autonomic function. The advantage of such an approach is that, if all measures reflect similar underlying pathophysiology, the influence of biological variability of its components is reduced (29), and the chance of a type 1 error is lower. Nonetheless, when we investigated HRV measures individually, we found that worsening glucose tolerance showed directionally similar associations with lower HRV for every individual measure, except for the LF/HF ratio. However, the use of the LF/HF ratio as a marker of sympathetic-parasympathetic (im)balance has been criticized due to its oversimplified reflection of a nonlinear phenomenon (30).

Hyperglycemia may cause cardiac autonomic dysfunction via increased oxidative stress, endothelial dysfunction, and the formation of advanced glycation end products, all of which may lead to neuronal damage and subsequent autonomic dysfunction (31). In addition, the association between hyperglycemia and autonomic dysfunction may be bidirectional. It has been suggested that autonomic dysfunction causes hyperglycemia via impaired insulin release by the pancreas, increased glucose production by the liver, and impaired glucose uptake and insulin resistance in skeletal muscles (32). Indeed, several studies have shown that cardiac autonomic dysfunction measured by high heart rate or low HRV is associated with incident type 2 diabetes (33,34). Hence, it is possible that a vicious cycle of hyperglycemia and cardiac autonomic dysfunction exists. Note, however, that results remained similar after adjustment for markers of insulin resistance (i.e., plasma insulin, HOMA2-IR, and the Matsuda index). After adjustment for heart period, the associations remained statistically significant but were attenuated. This may be partially due to overadjustment, because mean heart period is also a reflection of cardiac autonomic function (27).

Strengths of this study include the population-based approach with an oversampling of type 2 diabetes, which enabled a more accurate comparison of individuals with and without type 2 diabetes, and the use of the more accurate 24-h ECGs to calculate HRV. In addition, we assessed a large series of potential

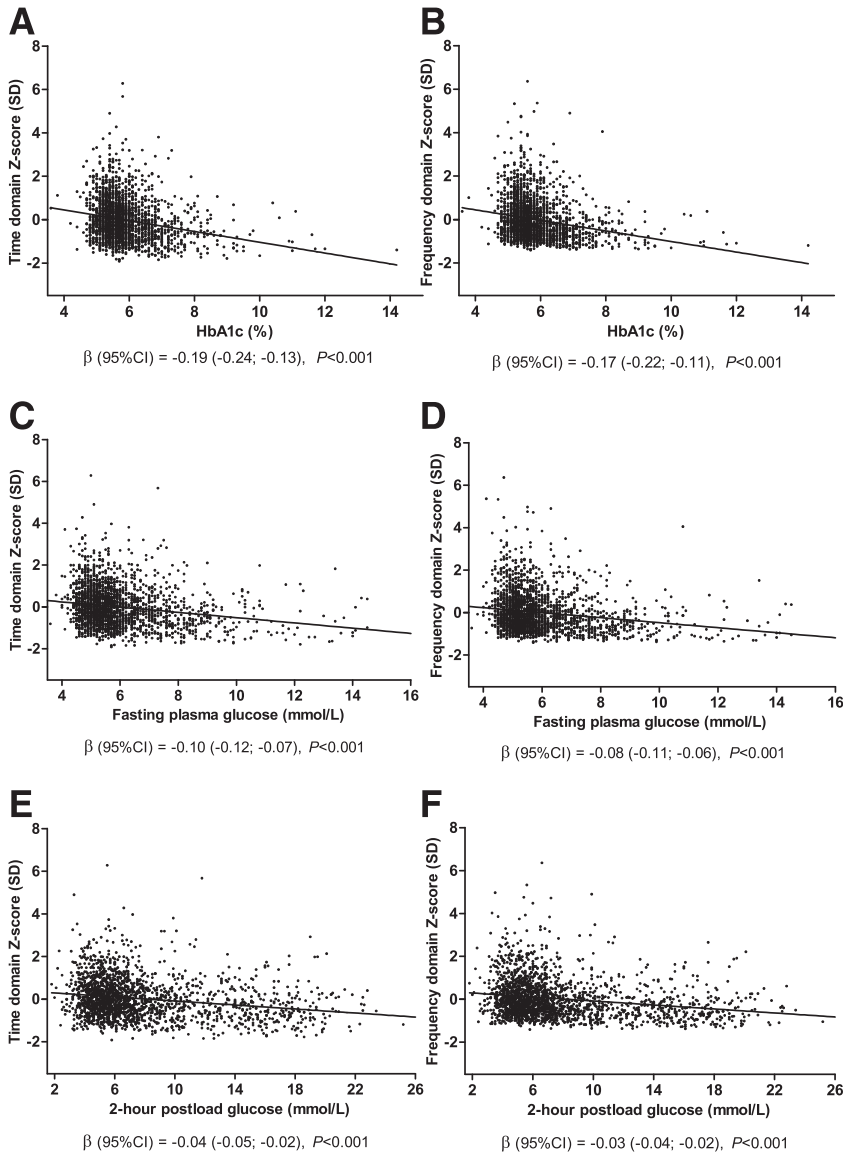

Figure 2—Associations of HbA_{1c} (*A* and *B*), fasting plasma glucose (*C* and *D*), and 2-h postload glucose (*E* and *F*) with time domain HRV (*A*, *C*, and *E*) and frequency domain HRV (*B*, *D*, and *F*). *A*: Association between HbA_{1c} and time domain HRV. *B*: Association between HbA_{1c} and frequency domain HRV. *C*: Association between fasting plasma glucose and time domain HRV. *D*: Association between fasting plasma glucose and frequency domain HRV. *E*: Association between 2-h postload glucose and time domain HRV. *F*: Association between 2-h postload glucose and frequency domain HRV. Regression coefficients (β) indicate SD difference in time domain and frequency domain *z*-scores per 1-unit increment in HbA_{1c} (%), fasting plasma glucose (mmol/L), and 2-h postload glucose (mmol/L).

confounders, including objectively measured physical activity (next to questionnaires) and 24-h blood pressures (next to office blood pressures).

Our study had some limitations. First, due to the cross-sectional design of our study, any causal inference should be made with caution. As mentioned previously, it is possible that a bidirectional association exists. Second, the use of antihypertensive and glucose-lowering medication may have influenced the results. However, when we excluded individuals who used antihypertensive medication, results remained similar. Removal of the influence of glucose-lowering medication was not feasible due to the observational nature of this study, and this may have caused an underestimation of our results. Of note, despite relatively well-controlled glycemic levels, we still observed significant differences in HRV in type 2 diabetes compared with normal glucose metabolism. Third, results from the fully adjusted model (i.e., model 3) may have been overadjusted. For example, model 3 considers prior cardiovascular disease as a potential confounder, but it should be realized that cardiovascular disease may lie on the pathway between hyperglycemia and lower HRV (i.e., may be a mediator instead of a confounder). Nonetheless, regression coefficients remained similar to those of model 2. Fourth, 399 participants were excluded from the analyses due to missing confounders, but we suggest that the risk of selection bias was minimal, because variable values were similar between the included and excluded individuals. Fifth, our study population consisted of middle-aged Caucasians with access to high-quality diabetes care, so results may not be generalizable to other populations.

In conclusion, we showed, in a population-based study, that both prediabetes, type 2 diabetes, and measures of hyperglycemia are associated with cardiac autonomic dysfunction, as measured by low HRV, independently of major cardiovascular risk factors. These findings support the concept that cardiac autonomic dysfunction precedes the clinical diagnosis of type 2 diabetes and may play a role in the development of various cardiovascular diseases, such as myocardial infarction and sudden cardiac death. The current study gives further support to the notion that prediabetes may be considered as a potential therapeutic target.

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Author Contributions. C.C. and T.L.Z. performed data collection and statistical analysis and wrote the manuscript. J.H. performed data analysis. R.M.A.H., J.H., N.C.S., A.K., M.T.S., C.J.H.v.d.K., and A.W. critically reviewed the manuscript. R.J.A.d.E. contributed to data analysis. H.J.G.M.C. and C.D.A.S. conceived the study and cowrote the manuscript. T.L.Z. and C.D.A.S. are the guarantors of this work

and, as such, had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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