Cardiac Autonomic Neuropathy and Early Progressive Renal Decline in Patients with Nonmacroalbuminuric Type 1 Diabetes

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Abstract
Background and objectives Cardiac autonomic neuropathy predicts future adverse renal outcomes in the general population. This study sought to determine its relationship with early progressive renal decline in type 1 diabetes.

Design, setting, participants, & measurements A subset of participants with normoalbuminuria (n=204) or microalbuminuria (n=166) from the First Joslin Kidney Study underwent assessment for cardiac autonomic neuropathy using heart rate variability during baseline visits performed from January 1991 to April 1992. Cardiac autonomic neuropathy was defined as an R-R variation (mean circular resultant)<20. Participants also had baseline and follow-up measurement of eGFR. Early progressive renal decline was evaluated according to two definitions: early GFR loss (slope of eGFR estimated by cystatin C <-3.3%/year) and incident advanced CKD (stage ≥3, defined by eGFR [calculated by Modification of Diet in Renal Disease method]<60 ml/min per 1.73 m²). Association with baseline cardiac autonomic neuropathy was assessed by adjusted logistic regression and Cox proportional hazards.

Results Among the 370 participants, 47 (13%) had baseline cardiac autonomic neuropathy, 51 (14%) had early GFR loss, and 68 (18%) had incident advanced CKD over a median 14-year follow-up. Early GFR loss occurred in 15 (32%) of the 47 patients with baseline autonomic neuropathy and in 32 (10%) of the 323 without baseline autonomic neuropathy (P<0.001). Baseline autonomic neuropathy was strongly associated with odds of early GFR loss (adjusted odds ratio, 4.09; 95% confidence interval, 1.65 to 10.12; P=0.002). Incident advanced CKD was observed in 12 (47%) of those with baseline autonomic neuropathy and 46 (14%) of those without baseline autonomic neuropathy (P<0.001). Autonomic neuropathy was independently associated with incident advanced CKD (adjusted hazard ratio, 2.76; 95% confidence interval, 1.44 to 5.30; P=0.002).

Conclusions Cardiac autonomic neuropathy was a strong independent predictor of the long-term risk of early progressive renal decline in type 1 diabetes. Future research should explore the mechanisms by which autonomic neuropathy may be associated with renal function loss.


Introduction
Diabetic nephropathy (DN) is a common complication of diabetes, associated with increased risk of cardiovascular disease and death (1). Historically, nearly one quarter of patients with type 1 diabetes developed DN after 30 years of follow-up, as characterized by albuminuria or impairment in GFR (2). Although rates of complications appear to have declined substantially over the past 2 decades, such improvements may not apply to the rates of ESRD (3). Furthermore, despite advances aimed at improving glycemic control and inhibiting deleterious neurohormonal pathways, such as the renin-angiotensin-aldosterone system (RAAS), evidence from population databases and clinical trials imply that the incidence of DN has increased in proportion to the rising prevalence of diabetes (3,8). Thus, there is a need to discover novel mechanisms underlying renal function loss in type 1 diabetes, particularly at early stages when prevention is most feasible.

In addition to the critical role of the RAAS, the autonomic nervous system contributes to the regulation of renal hemodynamic function and activates the RAAS via sympathetic inputs to the renal vasculature and juxtaglomerular apparatus (9). Operationally, autonomic dysfunction can be assessed through heart rate variability (HRV)—the instantaneous beat-to-beat variation in heart rate during respiration. Abnormal HRV or other validated cardiovascular autonomic reflex tests (CARTs) are commonly used to identify cardiac autonomic neuropathy (10), a condition characterized by autonomic dysfunction of the cardiovascular system...
This condition is independently associated with cardiovascular morbidity and mortality among patients with diabetes (12–14). Autonomic dysfunction may play a role in the pathogenesis of DN through an increase in sympathetic tone relative to parasympathetic tone, leading to proteinuria, impaired nocturnal BP dipping, and declining renal function (15,16).

To date, several studies have explored the causal relationship between cardiac autonomic neuropathy and renal impairment (6,12,17–19). In the nondiabetic population, the Atherosclerosis Risk in Communities study— which followed 13,241 adults, 12% of whom had baseline type 2 diabetes—demonstrated that low HRV was associated with the subsequent development of ESRD and CKD-related hospitalization after 16 years of follow-up (17). Meanwhile, in a smaller study of patients with type 1 diabetes, low HRV was associated with increased albuminuria after 14 years, although its association with advanced CKD could not be fully reconciled (18).

The First Joslin Kidney Study of the Natural History of Microalbuminuria was designed to identify the determinants of DN in type 1 diabetes. Previous results have identified factors affecting the incidence of microalbuminuria (20), its progression to macroalbuminuria, and its regression to normoalbuminuria (21). Although it was not the primary objective, baseline HRV data were measured in a subset of patients who have had subsequent long-term follow-up for renal outcome measures; this cohort design provides a unique opportunity to reconcile the relationship between HRV and future DN risk. In the current study, we aimed to determine the relationship between cardiac autonomic neuropathy and early progressive renal decline (EPRD), including the development of early GFR loss (22) and advanced CKD (23), among normoalbuminuric patients with type 1 diabetes.

**Materials and Methods**

**Selection of Study Participants**

The study cohort was derived from the First Joslin Kidney Study of the Natural History of Microalbuminuria (National Institutes of Health grant no. DK41526) (20). Details of the selection criteria, methods, and follow-up have been previously published (20–22,24), with an abbreviated description provided here. From January 1991 to April 1992, a 50% sample of patients with type 1 diabetes at the Joslin Diabetes Center, aged 15–44 years, were screened for albuminuria using the urinary albumin excretion rate (AER). In the 2-year baseline interval, 1602 patients were categorized as having normoalbuminuria (AER <30 μg/min), macroalbuminuria (AER 30–299 μg/min), or microalbuminuria (AER ≥300 μg/min; called "proteinuria" in previous publications) (13,20–22,25,26). Patients with microalbuminuria were not systematically assessed by HRV and were excluded from this analysis. Within 4 years of the baseline assessment, new-onset microalbuminuria developed in 109 of 943 patients with normoalbuminuria at baseline, providing a unique subgroup to follow for long-term renal outcomes. Assessments of cardiac autonomic nerve function were performed for a subset of 370 (29%) patients during the last 12 months of the 2-year accrual period and are the focus of the current study. According to AER, the study cohort was divided into three distinct subgroups: 204 of 834 (24%) patients with long-standing normoalbuminuria, 27 of 109 (25%) patients with new-onset microalbuminuria, and 139 of 312 (45%) patients with prevalent microalbuminuria (22,23). Compared with those not examined by HRV, participants in this analysis did not differ according to age, diabetes duration, sex, systolic BP, hemoglobin A1c (HbA1c), lipid profile, or AER. However, those included in analysis had slightly lower diastolic BP (72±9 mmHg; P=0.003) and higher eGFR (119±24 compared with 113±30 ml/min per 1.73m²; P=0.01). Ascertainment of cardiac autonomic nerve function was nondifferential relative to the early GFR loss and advanced CKD outcome measures. The Committee on Human Studies of the Joslin Diabetes Center approved the study protocol and consent procedures.

**Baseline Entry Examination and Measurement of Exposure Variables**

A comprehensive medical and diabetes history was obtained for all patients via questionnaire administered by a trained study recruiter. BP, serum lipid profiles, serum cystatin C, and HbA1c were also assessed at baseline using previously published assay parameters (20). Seated BP measurements for the first three clinic visits were averaged to establish baseline BP. Lipid profiles were measured by an enzymatic timed end point method. Serum cystatin C was measured using the BN ProSpec System nephelometer (Dade Behring, Newark, DE). Baseline HbA1c was calculated as the mean of all HbA1c measurements in the year before study entry, including the HbA1c measured on entry examination. The baseline AER was estimated on the basis of the albumin-to-creatinine ratio in multiple random urine samples, as previously described (20).

**Assessment of Cardiac Autonomic Nerve Function**

Cardiac autonomic nervous function was assessed using HRV on the Monitor One nDX device (QMed Inc., Eton-town, NJ). The device obtains an electrocardiographic recording via three chest electrodes and provides audio prompts to ensure standardized measurements. Patients avoided caffeinated beverages, smoking, alcohol, and large meals for at least 2 hours before the assessment (27). The absence of hypoglycemia or marked hyperglycemia was confirmed with capillary blood glucose measurements. Recordings were preceded by a minimum of 10 minutes in the supine position. A total of four HRV parameters were obtained using standard CARTs. Three were measured during deep breathing paced at 6 breaths/min (5 seconds of inspiration and 5 seconds of expiration), including the expiration/inspiration (E/I) index, mean circular resultant (MCR), and SD. The E/I index was calculated as the longest R-R interval (in milliseconds) during expiration divided by the shortest R-R interval during inspiration, averaged for the six respiration cycles. The MCR was computed as a dimensionless circular mean vector of R-R intervals during paced breathing (25,28) and was the main HRV parameter used in the Diabetes Control and Complications Trial (DCCT). The SD of the mean R-R interval is a statistical estimate of the HRV during paced breathing. All three of
these measures aimed to assess the magnitude of sinus arrhythmia, a physiologic response mediated predominantly by the parasympathetic nervous system. The posture index was calculated based on R-R intervals during a transition from 3 minutes of the supine position to 60 seconds of the standing position. More specifically, it calculated the longest R-R interval over 30 ± 5 beats after standing divided by the shortest R-R interval over 15 ± 5 beats after standing (29).

The presence of cardiac autonomic neuropathy was defined as an MCR < 20. Low MCR was one of the primary case definitions for cardiac autonomic neuropathy used in the DCCT (26), and an MCR < 20 was identified as an abnormal threshold by the manufacturers of the Monitor One nDX device.

**Assessment of EPRD**

EPRD, defined by renal decline in those with renal function in the normal ranges at baseline, was measured in two ways: an abnormally rapid quantitative loss of filtration, termed "early GFR loss," and the observation that an individual's renal function crossed the threshold to CKD stage ≥ 3, termed "incident advanced CKD." These are in contrast to "late progressive renal decline," which occurs when GFR is already abnormally low (< 60 ml/min per 1.73 m²).

**Determination of Early GFR Loss.** To measure change in filtration over time, GFR was estimated by serum cystatin C using the formula eGFR\textsubscript{CYSTATIN} C = (8.67/cystatin C) – 4.2 because cystatin C-based estimates have been validated to be more accurate than creatinine-based estimates when renal function is in the normal range (30–32). eGFR\textsubscript{CYSTATIN} C values were transformed to the logarithmic scale, and slopes were estimated by the regression coefficient for the time variable and then converted from the logarithmic scale to produce the annual percentage change in eGFR\textsubscript{CYSTATIN} C. Early GFR loss (previously referred to as early renal function decline) was defined as an annual decline in eGFR\textsubscript{CYSTATIN} C > 3.3% (22, 31).

**Determination of Incident Advanced CKD.** The four-variable Modification of Diet in Renal Disease (MDRD) equation (22, 33) was used to establish incident advanced CKD. Serum creatinine was measured by a modified picate method of Jaffe on a Ciba Corning Express Plus Chemistry Analyzer. Advanced CKD was defined as at least one serum measurement of an eGFR\textsubscript{MDRD} < 60 ml/min per 1.73 m², corresponding to CKD stage ≥ 3.

Patients were followed prospectively on an annual basis with serial measurements of AER and eGFR\textsubscript{MDRD} for a median of 14 (interquartile range [IQR], 11, 15) years, with minimum follow-up of 7 years and maximum follow-up of 16 years.

**Statistical Analyses**

Analyses were performed using SAS software, version 9.3 for Windows (SAS Institute, Inc., Cary, NC). Clinical characteristics were compared between those with and without baseline cardiac autonomic neuropathy using a t test, Wilcoxon rank-sum test, or chi-square test, depending on the type and distribution of the variable. Normality was assessed by the Shapiro–Wilk test and by visual inspection of the corresponding histograms. Non-normally distributed variables are indicated in Table 1 by the presence of median (IQR) values. Logistic regression models were used to determine the unadjusted odds ratios for early GFR loss, and Cox proportional-hazards models were used to determine the unadjusted hazard ratios for incident advanced CKD over the duration of follow-up for the entire cohort. Multivariable models were generated for both outcome measures using variables that were significant in the univariable models for early GFR loss or incident advanced CKD (sex, diabetes duration, diastolic BP, HbA1c, triglycerides, eGFR, AER, and presence of microalbuminuria). We also forced systolic BP into the adjusted models. Age and total cholesterol were not included in adjusted models owing to their association with diabetes duration and triglyceride level, respectively, and the fact that these latter variables had a stronger association with the outcomes. In a sensitivity analysis, the annual percentage change in eGFR\textsubscript{CYSTATIN} C was analyzed as the outcome measure, in place of the dichotomized variable for early GFR loss. This was done using a multivariable linear regression model with the same model structure as explained above. Significance was set at an α-level < 0.05.

**Results**

Of the 370 participants with type 1 diabetes studied, 204 had long-standing normoalbuminuria, 27 developed new onset microalbuminuria, and 139 had prevalent microalbuminuria. Forty-seven (13%) had baseline cardiac autonomic neuropathy, 51 (14%) had early GFR loss, and 68 (18%) developed incident advanced CKD. Participants with new onset or prevalent microalbuminuria compared with those with long-standing normoalbuminuria had higher prevalence of baseline cardiac autonomic neuropathy (31 [19%] compared with 16 [8%]; P = 0.002), developed early GFR loss at a greater frequency (37 [22%] compared with 14 [7%]; P < 0.001), and developed advanced CKD (stage ≥ 3) more frequently (38 [23%] compared with 30 [15%]; P < 0.001). The clinical characteristics of the cohort according to the presence or absence of baseline cardiac autonomic neuropathy are detailed in Table 1. Compared with those without neuropathy, participants with neuropathy at baseline were older; had longer diabetes duration, higher HbA1c values, lower eGFR\textsubscript{MDRD}, and higher urinary albumin excretion; and consisted of a greater proportion of individuals with microalbuminuria.

The details of HRV parameters are also shown in Table 1. By definition, because cardiac autonomic neuropathy is defined by abnormality in this parameter, the mean circular resultant was abnormally lower in patients with neuropathy, as was the E/I index, the SD of the HRV, and the posture index. Early GFR loss was more frequently observed among those with baseline neuropathy than those without baseline neuropathy. Specifically, the median annual percentage loss in eGFR\textsubscript{CYSTATIN} C was greater in those with neuropathy (median, −2.3% [IQR, −3.6% to −1.0%] versus −1.0% [IQR, −1.9% to −0.1%] per year; P < 0.001), and early GFR loss was observed in 15 (32%) of the 47 participants with neuropathy compared with 36 (11%) of the 323 participants without neuropathy (P < 0.001). Similarly, incident advanced CKD occurred in 22 (47%) of the 47 participants with baseline neuropathy.
and in 46 (14%) of the 323 participants without baseline neuropathy (P=0.001).

Table 2 shows the results of univariable logistic regression analysis for prevalent early GFR loss and Cox proportional-hazards analysis for incident advanced CKD. As shown in the left columns of Table 2, diastolic BP, HbA1c, triglycerides, AER, and the presence of microalbuminuria were associated with greater odds of early GFR loss (P<0.05 for all comparisons). Impaired values for the HRV parameters mean circular resultant, E/I index, and SD were also associated with early GFR loss. In addition, the presence of cardiac autonomic neuropathy was strongly associated with greater odds of early GFR loss (odds ratio, 2.1%; P=0.001). As shown in the right-hand columns of Table 2, a significant association with risk of incident advanced CKD was observed for the variables sex, age, diabetes duration, total cholesterol, eGFRMDRD, AER, and presence of microalbuminuria (P<0.05 for each comparison). Similarly, association with risk of incident advanced CKD was observed for cardiac autonomic neuropathy and the HRV parameters E/I index and SD (P<0.05 for each comparison).

To investigate the independent effect of baseline cardiac autonomic neuropathy on the development of early GFR loss and advanced CKD, we performed adjusted logistic and Cox proportional-hazards models, respectively (Table 3). Despite adjustment for sex, diabetes duration, systolic BP, diastolic BP, HbA1c, triglycerides, eGFRMDRD, AER, and the presence of microalbuminuria, baseline autonomic neuropathy was independently associated with early GFR loss (odds ratio, 4.09; 95% CI, 1.65 to 10.12; P=0.002). Expressed as a continuous variable, annual percentage change in eGFRCYSTATIN C remained lower in those with cardiac autonomic neuropathy than those without (−2.1%; 95% CI, −2.9% to −1.3%; P<0.001). Similarly, despite adjustment for the above variables, the presence of baseline cardiac autonomic neuropathy was an independent predictor of incident advanced CKD, as shown in Figure 1 (hazard ratio, 2.76; 95% CI, 1.44 to 5.30; P=0.002).

Discussion
In a cohort of 370 patients with type 1 diabetes followed prospectively for 14 years, we found that baseline cardiac autonomic neuropathy, defined by lower HRV, was associated with risk factors (such as older age, longer diabetes duration, higher HbA1c, lower baseline eGFRMDRD) and with the presence of microalbuminuria. Independent of these and other variables, the presence of lower HRV was strongly associated with EPRD, measured by early GFR loss and by incident advanced CKD. The magnitude of the effect was stronger for eGFR loss than for advanced CKD, and the magnitude of the effect varied according to eGFR level.

Table 1. Clinical characteristics of the 370 participants with type 1 diabetes according to presence of cardiac autonomic neuropathy

<table>
<thead>
<tr>
<th>Clinical Characteristic</th>
<th>Presence of Baseline Cardiac Autonomic Neuropathy* (n=47)</th>
<th>Absence of Baseline Cardiac Autonomic Neuropathy* (n=323)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women, n (%)</td>
<td>78 (47)</td>
<td>110 (54)</td>
<td>0.20</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>34 ± 6</td>
<td>30 ± 7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes duration (yr)</td>
<td>21 ± 8</td>
<td>15 ± 8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>123 ± 18</td>
<td>118 ± 14</td>
<td>0.07</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>74 ± 9</td>
<td>72 ± 9</td>
<td>0.10</td>
</tr>
<tr>
<td>Hemoglobin A1c (%)</td>
<td>9.1 ± 1.4</td>
<td>8.4 ± 1.5</td>
<td>0.003</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>205 ± 36</td>
<td>193 ± 43</td>
<td>0.07</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>108 (79–148)</td>
<td>100 (75–137)</td>
<td>0.30</td>
</tr>
<tr>
<td>eGFRMDRD (ml/min per 1.73 m²)</td>
<td>111 ± 29</td>
<td>121 ± 23</td>
<td>0.02</td>
</tr>
<tr>
<td>Albumin excretion rate (mg/min)b</td>
<td>47 (16–102)</td>
<td>19 (12–51)</td>
<td>0.003</td>
</tr>
<tr>
<td>Presence of microalbuminuria, n (%)c</td>
<td>31 (66)</td>
<td>135 (42)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Key:

*Cardiac autonomic neuropathy defined as MCR <20.

Data presented as mean±SD, median (interquartile range), or n (%). P values for continuous normally distributed variables were calculated using the t test; P values for continuous non-normally distributed variables were calculated using the Wilcoxon rank-sum test (indicated in the table by the presence of median [interquartile range] values), and P values for categorical variables were calculated using the chi-square test. eGFRMDRD, eGFR assessed by four-variable Modification of Diet in Renal Disease (MDRD) equation; MCR, mean circular resultant.

Data converted from logarithmic means and SDs.

Presence of microalbuminuria, defined as new-onset microalbuminuria. Defined as decline in eGFRCYSTATIN C >3.3%/yr.
Table 2. Univariable analysis of variables associated with the two measures of early progressive renal decline in the 370 participants with type 1 diabetes

<table>
<thead>
<tr>
<th>Baseline Clinical Characteristic</th>
<th>Present (n=51)</th>
<th>Absent (n=319)</th>
<th>Odds Ratio (95% CI)</th>
<th>P Value</th>
<th>Present (n=68)</th>
<th>Absent (n=302)</th>
<th>Hazard Ratio (95% CI)</th>
<th>P Value</th>
</tr>
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<tbody>
<tr>
<td><strong>Women, n (%)</strong></td>
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<td></td>
<td>31 (61)</td>
<td>157 (49)</td>
<td>1.60 (0.88 to 2.92)</td>
<td>0.13</td>
<td>48 (71)</td>
<td>140 (46)</td>
<td>2.32 (1.38 to 3.91)</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Age (yr)</strong></td>
<td>31±7</td>
<td>30±7</td>
<td>1.01 (0.97 to 1.05)</td>
<td>0.65</td>
<td>32±7</td>
<td>30±7</td>
<td>1.05 (1.02 to 1.09)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>Diabetes duration (yr)</strong></td>
<td>15±8</td>
<td>16±9</td>
<td>0.99 (0.95 to 1.03)</td>
<td>0.54</td>
<td>19±8</td>
<td>15±8</td>
<td>1.05 (1.02 to 1.08)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Systolic BP (mmHg)</strong></td>
<td>120±15</td>
<td>119±14</td>
<td>1.00 (0.99 to 1.03)</td>
<td>0.45</td>
<td>119±16</td>
<td>119±14</td>
<td>1.00 (0.98 to 1.02)</td>
<td>0.94</td>
</tr>
<tr>
<td><strong>Diastolic BP (mmHg)</strong></td>
<td>75±9</td>
<td>72±9</td>
<td>1.04 (1.01 to 1.08)</td>
<td>0.02</td>
<td>73±9</td>
<td>72±9</td>
<td>1.00 (0.98 to 1.03)</td>
<td>0.81</td>
</tr>
<tr>
<td><strong>Hemoglobin A1c (%)</strong></td>
<td>9.4±1.5</td>
<td>8.3±1.4</td>
<td>1.57 (1.28 to 1.91)</td>
<td>&lt;0.001</td>
<td>8.7±1.7</td>
<td>8.4±1.4</td>
<td>1.08 (0.93 to 1.27)</td>
<td>0.31</td>
</tr>
<tr>
<td><strong>Total cholesterol (mg/dl)</strong></td>
<td>205±44</td>
<td>193±42</td>
<td>1.01 (0.99 to 1.01)</td>
<td>0.09</td>
<td>209±48</td>
<td>192±41</td>
<td>1.01 (1.01 to 1.02)</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>eGFRMDRD (ml/min per 1.73 m²)</strong></td>
<td>116±35</td>
<td>120±22</td>
<td>0.99 (0.98 to 1.00)</td>
<td>0.21</td>
<td>121±100</td>
<td>121±98</td>
<td>1.00 (1.00 to 1.00)</td>
<td>0.65</td>
</tr>
<tr>
<td><strong>Albumin excretion ratio (mg/min)</strong></td>
<td>51 (26–102)</td>
<td>18 (12–49)</td>
<td>1.01 (1.00 to 1.00)</td>
<td>0.002</td>
<td>33 (14–78)</td>
<td>19 (12–52)</td>
<td>1.00 (1.00 to 1.01)</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Presence of microalbuminuria, n (%)</strong></td>
<td>37 (73)</td>
<td>129 (40)</td>
<td>3.89 (2.02 to 7.49)</td>
<td>&lt;0.001</td>
<td>38 (56)</td>
<td>128 (42)</td>
<td>1.78 (1.10 to 2.88)</td>
<td>0.02</td>
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<tr>
<td><strong>Heart rate variability parameters</strong></td>
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<td><strong>Mean circular resultant</strong></td>
<td>43.7±31.3</td>
<td>66.0±55.9</td>
<td>0.98 (0.97 to 0.99)</td>
<td>0.001</td>
<td>54.2±74.3</td>
<td>64.9±47.9</td>
<td>0.99 (0.99 to 1.00)</td>
<td>0.05</td>
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<tr>
<td><strong>Expiration/inspiration index</strong></td>
<td>1.28±0.23</td>
<td>1.38±0.24</td>
<td>0.81 (0.69 to 0.95)</td>
<td>0.01</td>
<td>1.31±0.27</td>
<td>1.38±0.23</td>
<td>0.86 (0.76 to 0.97)</td>
<td>0.02</td>
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<tr>
<td><strong>SD (msec)</strong></td>
<td>71.1±45.7</td>
<td>91.9±48.1</td>
<td>0.99 (0.98 to 0.99)</td>
<td>0.005</td>
<td>74.4±51.8</td>
<td>92.3±46.9</td>
<td>0.99 (0.9 to 1.00)</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>Posture index</strong></td>
<td>1.18±0.42</td>
<td>1.26±0.44</td>
<td>0.96 (0.90 to 1.03)</td>
<td>0.22</td>
<td>1.25±0.50</td>
<td>1.25±0.42</td>
<td>0.99 (0.94 to 1.05)</td>
<td>0.74</td>
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<tr>
<td><strong>Cardiac autonomic neuropathy</strong></td>
<td></td>
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<tr>
<td><strong>MCR&lt;20, n (%)</strong></td>
<td>15 (29)</td>
<td>32 (10)</td>
<td>3.74 (1.85 to 7.56)</td>
<td>&lt;0.001</td>
<td>22 (32)</td>
<td>25 (8)</td>
<td>3.99 (2.40 to 6.65)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data presented as mean±SD, median (interquartile rang), or n (%). Odds ratios and hazard ratios are expressed per unit for each baseline clinical characteristic, except for the heart rate variability parameters expiration/inspiration index and posture index, which are expressed per 0.1 units. 95% CI, 95% confidence interval; eGFRMDRD, eGFR assessed by four-variable Modification of Diet in Renal Disease (MDRD) equation; MCR, mean circular resultant.

**Early GFR loss defined by eGFR_CYSTATIN C slope <−3.3%/yr;** associations examined by logistic regression.

**Incident advanced CKD defined by onset of CKD stage ≥3;** associations examined by Cox proportional-hazards.

**Data converted from logarithmic means and SDs.**

**Compared with prevalent long-standing normoalbuminuria. Presence of microalbuminuria defined as prevalent or new-onset microalbuminuria.**

**Odds ratios and hazard ratios are expressed as per 0.1-unit change.**
of risk was represented by a 4-fold higher adjusted odds of early GFR loss and a nearly 3-fold adjusted hazard of incident advanced CKD. Given the temporal association over a long follow-up period between baseline cardiac autonomic neuropathy and these outcomes, these data suggest that early abnormalities in the autonomic nervous system may contribute to the pathogenesis of DN and the subsequent development of CKD.

The association between cardiac autonomic neuropathy and renal dysfunction has been explored in patients with type 2 diabetes (17,19). In smaller studies of type 1 diabetes, autonomic dysfunction was associated with increased albuminuria and higher rates of eGFR loss, although cases of abnormal eGFR were rare (16,34). The long-term temporal relationship between the presence of autonomic dysfunction and the development of complications observed in these studies and the current analysis supports the view that the relationship between autonomic neuropathy and future CKD is causal in type 1 diabetes.

The mechanisms by which cardiac autonomic neuropathy may contribute to renal dysfunction in humans are unknown. For cardiovascular regulation, experimental models have demonstrated that the development of cardiac autonomic neuropathy relies on complex interactions between the degree of glycemic control, age-related neuronal attrition, diabetes duration, and BP. Abnormalities in these clinical factors promote progressive cardiac autonomic dysfunction in a length-dependent fashion akin to injury of peripheral somatic nerves in diabetic sensorimotor polyneuropathy (35). Early in the progression of cardiac autonomic neuropathy, there is a compensatory increase in cardiac sympathetic nervous system (SNS) tone in response to subclinical peripheral denervation of parasympathetic nerves (35–37). With time, sympathetic denervation progresses from the apex of the ventricles toward the base (35,36). In addition to these effects in the heart, several studies have demonstrated that cardiac autonomic neuropathy is associated with increased SNS activation, which may play a critical role in the pathogenesis of renal dysfunction. In young patients with type 1 diabetes, SNS activity causes early, preclinical BP changes that are in turn associated with increased glomerular basement membrane thickness and mesangial matrix expansion (38). Autonomic dysfunction also correlates with higher-range normoalbuminuria in patients with type 1 diabetes, suggesting a possible role for SNS activation in the transition from normo- to microalbuminuria (39) and activation of intrarenal RAAS, intraglomerular hypertension, and hyperfiltration in animals and in humans with type 1 diabetes (40–42). Therapeutic interventions using pharmacologic SNS blockade in type 1 diabetes resulted in lower BP and improved HRV (43). Meanwhile, in streptozotocin-induced diabetic rats, renal hyperfiltration can be prevented with sympathetic renal denervation (40). Similar strategies for renal sympathetic denervation have been targeted in humans with treatment-resistant hypertension (44), although their long-term utility in preventing CKD is unknown.

Our results highlight the importance of the SNS as a potential initiating factor in the development of CKD. The SNS is associated with other novel biomarkers of renal dysfunction, such as changes in albumin excretion ratio and evidence of increased glomerular basement membrane thickness, which may be markers of early renal injury. Further studies are needed to determine the role of SNS activation in the pathogenesis of CKD and to explore potential therapeutic strategies to prevent or delay its development.
injury and GFR loss, including neurohormonal activation, markers of cell apoptosis, inflammation, and immune responses (45). For instance, among rats with induced aortic regurgitation, increased intrarenal production of angiotensin II, reactive oxygen species, and nicotinamide adenine dinucleotide phosphate oxidase components resulted in podocyte injury and subsequent albuminuria—effects that are attenuated with renal SNS denervation (46). Similarly, the SNS activates proinflammatory pathways, including cytokines/chemokines (47). Importantly, longitudinal studies in humans have also demonstrated high predictive validity of the circulating inflammatory factors, such as TNF receptors 1 and 2, for early GFR loss and advanced CKD (48). The interaction among these key injurious pathways with classic markers of neurohormonal activation, including the SNS, therefore warrants further investigation to determine their mechanistic relationship in the cause of early and late renal function loss in diabetes.

Although unique as a prospective cohort study, this investigation has potential limitations. First, we cannot confirm—despite conditioning on confounding variables—the independent causal role of cardiac autonomic neuropathy. Specifically, subclinical renal disease may instead lead to autonomic dysfunction (39). Ultimately, pharmacologic approaches or blockade of the SNS will be required to elucidate the role of cardiac autonomic neuropathy in the initiation and progression of DN. Second, cardiac autonomic neuropathy was assessed only at baseline, with no assessment of its progress during the 14-year follow-up period. Similarly, cardiac autonomic neuropathy was defined on the basis of a single CART, whereas a formal clinical diagnosis generally requires two or more abnormal CARTs from a broader panel of tests (11). We also acknowledge that HRV has varies considerably day to day, particularly among individuals without neuropathy (49). This phenomenon has been previously reported among patients with cardiac conditions, such as congestive heart failure (49). Finally, the relevance of these data in patients with type 2 diabetes will require further study.

In conclusion, cardiac autonomic neuropathy is an independent risk factor for early progressive renal decline in nonmacroalbuminuric patients with type 1 diabetes. Further studies are required to determine the effects of cardiac autonomic neuropathy on DN, as well as the role of the SNS in initiating renal function loss.

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