



# Left ventricular remodelling and cardiac chamber sizes in long-term, normoalbuminuric type 1 diabetes patients with and without cardiovascular autonomic neuropathy

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## ABSTRACT

**Aims:** Type 1 diabetes is associated with increased cardiovascular (CV) morbidity and mortality, and cardiovascular autonomic neuropathy (CAN) is an important CV risk factor. The study aimed to explore associations between CAN and altered cardiac chamber sizes in persons with type 1 diabetes.

**Methods:** This was a cross-sectional study of 71 asymptomatic, normoalbuminuric participants with long-term type 1 diabetes (39 with CAN, determined by >1 abnormal autonomic function test) examined with cardiac multi detector computed tomography scans, which allowed measurements of left ventricular mass and all four cardiac chamber volumes. Cardiac chambers were indexed according to body surface area ( $\text{ml}/\text{m}^2$  or  $\text{g}/\text{m}^2$ ).

**Results:** Persons with and without CAN had mean  $\pm$  SD age of  $57 \pm 7$  and  $50 \pm 8$  years ( $p < 0.001$ ) and diabetes duration of  $36 \pm 11$  and  $32 \pm 9$  years ( $p < 0.05$ ), respectively. Increasing autonomic dysfunction, evaluated by decrease in heart rate variability during deep breathing (in beats per minute), was associated with larger right ( $-0.5$ , 95% CI  $-1.0$  to  $-0.0$ ,  $p < 0.05$ ) and trend towards larger left ( $-0.4$ , 95% CI  $-0.8$ – $0.0$ ,  $p < 0.1$ ) ventricular volumes in multivariable linear regression.

**Conclusions:** Our results suggest that impaired autonomic function may be associated with modest enlargement of ventricular volumes; this might be an early sign of progression towards heart failure.

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## 1. Introduction

Type 1 diabetes is associated with increased cardiovascular (CV) morbidity and mortality.<sup>1–3</sup> A common complication in diabetes is cardiovascular autonomic neuropathy (CAN) – with a prevalence of up to 65% of persons with diabetes increasing with age and diabetes duration<sup>4,5</sup> – which further increase mortality in persons with diabetes.<sup>6</sup>

Left ventricular (LV) mass (LVM) and LV remodelling (changes in LV mass:volume ratio) are important CV risk factors, as persons with LV

concentric remodelling, eccentric hypertrophy or concentric hypertrophy have increased CV morbidity and mortality.<sup>7–9</sup> Type 1 diabetes has been associated with increased LV remodelling,<sup>10</sup> LV dysfunction<sup>11–13</sup> and increased LVM,<sup>10</sup> although these results are inconsistent throughout the literature.<sup>12,14</sup> Among other complications in type 1 diabetes, autonomic neuropathy has been associated with increased LV measures to different degrees.<sup>15,16</sup> However, previous studies of persons with type 1 diabetes do either not exclude persons with kidney damage – an important confounder for cardiac morphology<sup>10–12,14,16</sup> – or have few participants.<sup>15</sup>

The aim of this study was to evaluate whether the presence of CAN in persons with long-term type 1 diabetes without albuminuria was associated with changes of cardiac chamber sizes and LV remodelling.

## 2. Materials and methods

This study was a post-hoc analysis of persons with long-term type 1 diabetes without albuminuria included in two previous studies, who

**Abbreviations:** BSA, body surface area; CAN, cardiovascular autonomic neuropathy; CV, cardiovascular; DV, diastolic volume; HRV, heart rate variability; I, Indexed value per BSA; LA, left atrium; LAV, left atrial volume; LV, left ventricle; LVM, left ventricular mass; MDCT, multi detector computed tomography; RA, right atrium; RAV, right atrial volume; RV, right ventricle.

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underwent cardiac multi-detector computed tomography (MDCT)<sup>17,18</sup> scan. The focus of these previous studies was type 1 diabetes complicated by CAN and its impact on the CV system.

Participants with type 1 diabetes were recruited from the Diabetes Unit, Rigshospitalet, Copenhagen University Hospital and Steno Diabetes Center, Gentofte. Inclusion criteria were: type 1 diabetes according to the American Diabetes Association criteria<sup>19</sup> for >10 years; age between 18 and 75 years; and HbA<sub>1c</sub> < 86 mmol/mol (10%). Exclusion criteria were albuminuria (urinary albumin-to-creatinine ratio > 30 mg/g; elevated S-creatinine >120 µmol/l) and electrocardiographic signs or clinical symptoms of heart disease.

Participants were divided into two groups according to the outcome of four autonomic function tests: heart rate variability (HRV) during deep breathing; Valsalva ratio; lying-to-standing test; and blood pressure (BP) in response to standing up. CAN was defined as two or more abnormal tests.<sup>20–22</sup> Participants were examined with MDCT between November 2009 and July 2014.

The studies were conducted according to the Declaration of Helsinki II and approved by the Danish Scientific Ethical Committee (protocol numbers H-4-2009-091 and H-2-2014-012) and the Danish Data Protection Agency (RH-2017-254). All participants gave oral and written informed consent.

**Demographics:** Interviewing for medical history, blood sampling, measurement of height, weight and blood pressure and CAN-testing was done at inclusion, as previously reported.<sup>17,18</sup> Body surface area (BSA) was calculated according to Du Bois' formula and used for indexed values of the cardiac chambers.

**Laboratory measurements:** Blood for laboratory measurements was collected from the antecubital vein. HbA<sub>1c</sub> was measured using a chromatographic technique (Tosoh, Tokyo, Japan). S-creatinine, total cholesterol, LDL-cholesterol, and HDL-cholesterol were measured with standardised clinical chemistry methods.

### 2.1. Cardiovascular autonomic neuropathy (CAN) tests

The participants were tested with regards to HRV during deep breathing first. If the participants had any contraindication towards Valsalva manoeuvre then the test was not performed. If the participants had two positive tests before all tests were performed, then the remaining tests were not performed. The testing was done throughout the day and participants were not instructed to be fasting, avoiding caffeine or pausing of any medication before CAN testing. As previously described,<sup>17,18</sup> CAN was defined as more than one out of four abnormal autonomic function tests<sup>20,23</sup>: 1) HRV during deep breathing: Participants were asked to breathe deeply at a rate of six breaths per minute while being monitored on a (50 mm/s) 12-lead ECG. The maximum and minimum heart rate during each breathing cycle was measured, and the mean difference of six cycles was calculated. For CAN determination, abnormal values were defined as ≤10 beats per minute and for linear regression analyses HRV was used as continuous variable as measure of increasing autonomic dysfunction.<sup>15,20,24,25</sup> 2) Lying-to-standing heart rate ratio: rest for 5 min in supine position and afterwards the maximal-to-minimal heart rate ratio was calculated from the R-R interval measured after the 30th beat after standing up and the R-R interval measured after the 15th beat after standing up; abnormal values were ≤ 1. 3) Valsalva ratio: forced exhalation into a mouth-piece with a pressure of 40 mm Hg for 15 s, and the ratio of the maximum to the minimum R-R interval during the test was calculated; abnormal ratios were ≤1.10. 4) Orthostatic hypotension: defined as a drop in systolic BP of ≥30 mm Hg when changing from supine to the upright position.<sup>20</sup>

### 2.2. Multi-detector computed tomography (MDCT) scan

Participants were examined with a 320-detector CT (Aquillon One, Toshiba Medical Systems, Japan) using a low dosage of radiation and

images were analysed on dedicated software (Vitrea, Vital Images Inc., USA). Intravenous contrast media (Visipaque 70–110 ml) was infused according to body weight and with a flow rate of 5 ml/s, followed by a saline chaser (30–50 ml) to assess LVM and cardiac chamber volumes. Image acquisition was initiated automatically at a density threshold of 180 Hounsfield Units in the descending aorta. Detector collimation was 320 × 0.5 mm and 100 kV tube voltage. ECG-gated images were acquired during diastolic best-phase and reconstructed with 0.25 increment and 0.5 slice thickness.<sup>26,27</sup>

**Chamber measurements:** LVM was calculated as the LV myocardial volume derived by manual correction of automated delineation of the endocardial and epicardial borders and multiplied with the specific gravity of myocardial tissue (with a tissue density of 1.055 g/ml). Left and right ventricular diastolic volumes (LVDV and RVDV) were derived as manually corrected automated delineation of the endocardial borders. Left and right atrial volumes (LAV and RAV), including appendage, were assessed manually by tracing the endocardial borders on 15–20 tomographic slices. Inter-observer variability using these methods has previously been described.<sup>26,28,29</sup> Chamber measures were then converted to indexed values (I), in order to account for body composition, by dividing the values with BSA (i.e. LVM/BSA = LVMI).

**LV morphology:** LV hypertrophy was defined as LVMI >80 g/m<sup>2</sup> for men and > 65 g/m<sup>2</sup> for women (>97.5 percentile).<sup>26</sup> Concentric LV was defined as LV mass per diastolic volume-ratio (LVM/DV) >1.44 g/ml for men and > 1.19 g/ml for women (>97.5 percentile).<sup>26,30</sup> Abnormality was characterised as either LV concentric remodelling (normal LVMI, but increased LVM/DV); eccentric LV hypertrophy (high LVMI, but normal LVM/DV); or concentric LV hypertrophy (high LVMI and high LVM/DV).<sup>9,31</sup> Furthermore LV regional asymmetry was assessed according to the American Heart Association 17-segment model, excluding the cardiac apex, in the participants with type 1 diabetes. LV regional asymmetry was defined as a regional myocardial thickness ≥ 13.0 mm and > 1.5 times the opposing segment's thickness.<sup>30–32</sup>

### 2.3. Statistical analysis

Setting power to 80% and a test level of 5%,<sup>33</sup> assuming a standard deviation of 20 g on LVM,<sup>15</sup> a sample size of 70 persons would be able to detect a difference of 9.5 g between the two CAN groups. Sample size was only calculated for LVM as the remaining cardiac chamber analyses were considered exploratory. A two-tailed *p*-value <0.05 was considered significant. Statistical analyses were performed using SPSS 22 (IBM SPSS Statistics, New York, NY). For continuous variables, data are expressed as means ± standard deviation (SD) when data follow normal distribution; otherwise as median (interquartile range (IQR)) when data are skewed. Dichotomous variables are listed as frequency (percentage). Group differences were calculated with Students' *t*-test for continuous, normally distributed variables and Mann-Whitney *U* test for continuous, skewed variables; for dichotomous variables Chi<sup>2</sup> or Fisher exact test was used. Multivariable linear regression analysis was used for determination of factors associated with the chamber measurements in type 1 diabetes. Predefined determinants for cardiac chamber measurements were age, gender, BSA, duration of diabetes, HbA<sub>1c</sub>, HRV and systolic BP.<sup>15,26,34</sup> HRV was used as continuous measure of CAN in the linear regression models.<sup>20</sup> Missing data were handled analysis-by-analysis.

### 2.4. Sensitivity analysis

To evaluate the results from the multivariable analyses, a sensitivity analysis was performed without the demographical differences between those with CAN and those without CAN, namely age, sex and duration of diabetes. This was accomplished by excluding 9 women from the group with CAN and 4 men from the group without CAN and afterwards excluding the youngest man without CAN and the oldest man

**Table 1**

Baseline demographics of participants with type 1 diabetes according to status of cardiovascular autonomic neuropathy.

	–CAN, n = 32	+CAN, n = 39	p-Value
Age (years)	50.5 ± 7.7	57.3 ± 6.9	<0.001 <sup>a</sup>
Gender (men)	23 (71.9)	19 (48.7)	0.06
Diabetes duration (years)	31.6 ± 9.5	36.4 ± 10.5	0.05 <sup>a</sup>
Height (cm)	176.7 ± 9.0	174.4 ± 9.0	0.29
Weight (kg)	75.3 ± 10.2	77.1 ± 16.1	0.59
BMI (kg/m <sup>2</sup> )	24.1 ± 2.5	25.2 ± 4.1	0.17
BSA (m <sup>2</sup> )	1.92 ± 0.17	1.91 ± 0.22	0.93
Statin treatment	12 (37.5)	21 (53.8)	0.23
Antihypertensive medication	13 (40.6)	24 (64.9)	0.06
ACE-inhibitor/angiotensin II antagonist	13 (40.6)	20 (52.6)	0.35
Beta-blocker	0 (0.0)	6 (15.4)	0.03
Calcium antagonist	4 (12.5)	8 (21.1)	0.34
Diuretic	5 (15.6)	14 (35.9)	0.06
Systolic BP (mm Hg)	124 ± 10	126 ± 15	0.46
Diastolic BP (mm Hg)	76 ± 7	74 ± 7	0.23
HbA <sub>1c</sub> (mmol/mol)	61 ± 11	63 ± 9	0.35
(%)	(7.7 ± 1.0)	(7.9 ± 0.8)	
Total cholesterol (mmol/l)	4.6 (4.1–5.3)	4.8 (4.4–5.3)	0.56
HDL-cholesterol (mmol/l)	1.9 (1.4–2.2)	2.0 (1.6–2.4)	0.30
LDL-cholesterol (mmol/l)	2.6 (1.8–3.0)	2.3 (1.8–2.7)	0.47
S-creatinine (µmol/l)	67 ± 9	65 ± 13	0.68

ACE, angiotensin converting enzyme; BSA, body surface area; BMI, body mass index; BP, blood pressure; CAN, cardiovascular autonomic neuropathy; +CAN, presence of CAN; –CAN, no presence of CAN.

<sup>a</sup>  $p < 0.05$ .

with CAN creating a sensitivity analysis with 27 persons without CAN compared to 27 persons with CAN as shown in Table 3.

### 3. Results

Of the 75 previously included persons with type 1 diabetes, 4 persons did not have a valid MDCT scan (two without contrasts and two not in diastolic phase) for measurements of cardiac chambers and they were therefore excluded from further analysis.

#### 3.1. Baseline demographics

As shown in Table 1, type 1 diabetes participants with CAN, compared to those without CAN, were older and had longer duration of diabetes. There was a trend towards a higher proportion of women and higher frequency of persons treated with BP medication with CAN ( $p = 0.06$ ). HRV was median (range) 9 (3–32) beats per minute in the included population.

#### 3.2. Cardiac morphology

Eight (11%) participants with type 1 diabetes had abnormal LV morphology (>97.5 percentile)<sup>26</sup>: 2 women (with CAN) had eccentric hypertrophy; 3 women (with CAN) had concentric remodelling; and 3 men had concentric remodelling (1 with CAN, 2 without CAN). Even though more participants with CAN had remodelling, CAN was not statistically associated with abnormal LV morphology (data not shown). There were no differences according to CAN-status in indexed chamber sizes, LVM/DV or LV asymmetry according the 17-segment model when examining the entire study population (data not shown).

In univariable analyses, BSA was strongly associated ( $p < 0.001$ ) with all cardiac chamber sizes (except for LVM/DV). Fig. 1 illustrates indexed ventricular volumes plotted against HRV. The figures show a trend towards increasing volumes with increasing autonomic dysfunction, expressed as decrease in HRV. Univariable predictors ( $p < 0.05$ ) for LVMI were gender and diabetes duration; for LVDVI it was gender; for RVDVI it was gender; for LAVI it was age and HRV; none for RAVI. For LVM/DV, only HbA<sub>1c</sub> approached significance ( $p = 0.05$ ) in univariable analyses.

Results from the multivariable analyses of the cardiac chambers are shown in Table 2. In summary, cardiovascular autonomic dysfunction

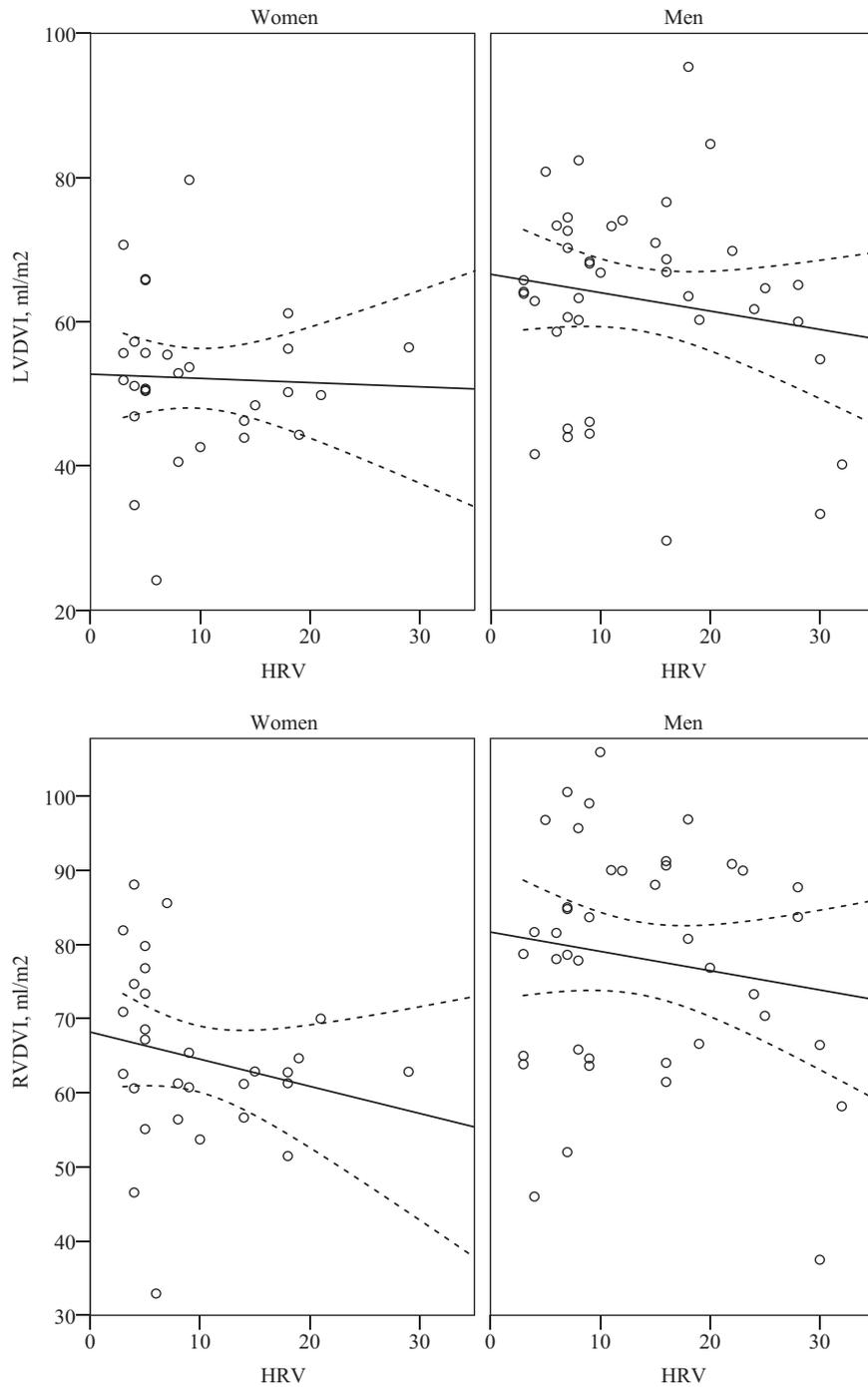
(expressed as HRV in the analyses) was negatively associated with LVDVI and RVDVI (LVDVI approached significance and RVDVI significantly), but not with LVMI or LVM/DV. HbA<sub>1c</sub> was positively associated with LVM/DV. Male gender was associated with increased LVMI, LVDVI, and RVDVI and there was a trend towards increased atrial volumes. HbA<sub>1c</sub> was positively associated and duration of diabetes was negatively associated with LVMI. Performance indicators for the multivariable models were: for LVM/DV  $R = 0.367$ ,  $R^2 = 0.135$ ; for LVMI  $R = 0.641$ ,  $R^2 = 0.410$ ; for LVDVI  $R = 0.488$ ,  $R^2 = 0.238$ ; for RVDVI  $R = 0.534$ ,  $R^2 = 0.285$ ; for LAVI  $R = 0.471$ ,  $R^2 = 0.222$ ; and for RAVI  $R = 0.287$ ,  $R^2 = 0.083$ .

Table 3 shows results from the sensitivity analysis of 27 gender-matched persons with and without CAN furthermore without significant difference in age and diabetes duration. Table 3 shows non-significant differences in cardiac chamber values, but with numerically larger LVDVI and RVDVI in persons with CAN compared to those without CAN.

### 4. Discussion

In this cohort of long-term, normoalbuminuric persons with type 1 diabetes, we found cardiovascular autonomic dysfunction, expressed by decrease in HRV, to be associated with larger biventricular volumes, after adjustment for important confounders of cardiac chambers (age, gender, BSA, HbA<sub>1c</sub>, duration of diabetes and systolic BP). Fig. 1 illustrate the basis for this link between HRV and ventricular volumes (although weak univariable association) and the results in Table 2 show that a decrease of 1 beat per minute in HRV during deep breathing was associated with an increase in ventricular volumes of  $\approx 0.5$  ml/m<sup>2</sup> in the participants in this study. This was also the trend in the sensitivity analysis shown in Table 3, which demonstrates larger numerical values of LVDVI and RVDVI in persons with CAN compared to those without. We can only speculate about this finding; perhaps these increases in volumes with worse HRV indicate a decompensatory mechanism as a result of increased denervation – and perhaps development towards heart failure.<sup>35,36</sup> Interestingly, HRV was not associated with LVM or LVM/DV in this study. It is also noteworthy that HRV not only influence LV, but also RV – to our knowledge, this has not been described before.

Two other studies have examined CAN and cardiac chambers sizes in type 1 diabetes.<sup>15,16</sup> Taskiran et al.<sup>15</sup> examined 10 type 1 diabetes persons with CAN, 10 type 1 diabetes persons without CAN and 10 controls



**Fig. 1.** Scatter plots of left and right indexed ventricular volumes against heart rate variability during deep breathing in men and women with type 1 diabetes. Lines are linear fit of mean and dashed lines are 95% CI of the mean. HRV, heart rate variability during deep breathing; LVDVI, indexed left ventricular diastolic volume; RVDVI, indexed right ventricular diastolic volume. Indexed values are per body surface area.

and used echocardiographic measurements for assessment of systolic and diastolic function and MRI for LVM and LVDV. Pop-Busui et al.<sup>16</sup> investigated CAN in 966 persons with type 1 diabetes included in the DCCT/EDIC (Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications) cohorts through MRI. Similar cardiac chamber values are reported in MDCT and MRI studies.<sup>26,37</sup>

Our findings are not consistent with the previous results; Taskiran et al.<sup>15</sup> found smaller LVDVI in persons with CAN compared to those without. Surprisingly, we find the opposite trend – that decrease in cardiovascular autonomic function is associated with larger LVDVI. Their participants were younger, had shorter duration of diabetes and higher

HbA<sub>1c</sub> – thus it could be that the differences between our cohorts correspond to different time domains in the progression towards dilated cardiomyopathy and heart failure in persons with type 1 diabetes complicated by autonomic neuropathy. Taskiran et al.<sup>15</sup> did not find difference between LVMI in participants with CAN compared to those without; this was in line with our results, but opposite the results by Pop-Busui et al.<sup>16</sup> who did find CAN associated with increased LVM. However, Pop-Busui et al.<sup>16</sup> did not adjust for BSA or kidney damage, very important confounders.<sup>12,26,38</sup> Interestingly, both studies found HRV related to LVM in multiple regression analyses; however, only the study by Taskiran et al.<sup>15</sup> adjusted for BSA and kidney function.

**Table 2**

Multivariable model of factors associated with cardiac chamber measurements in normoalbuminuric persons with type 1 diabetes.

	Age (years)	Gender (men)	Duration diabetes (years)	HbA <sub>1c</sub> (%)	HRV (bpm)
LVM/DV (g/ml)	0.0 (−0.0–0.0) <sup>a</sup>	–	–	0.1 (0.0–0.2) <sup>b</sup>	–
LVMI (g/m <sup>2</sup> )	–	11.4 (7.2–15.7) <sup>c</sup>	−0.2 (−0.5 to −0.0) <sup>b</sup>	2.5 (0.3–4.7) <sup>b</sup>	–
LVDVI (ml/m <sup>2</sup> )	−0.4 (−0.8–0.0) <sup>a</sup>	12.4 (6.1–18.7) <sup>c</sup>	–	–	−0.4 (−0.8–0.0) <sup>a</sup>
RVDVI (ml/m <sup>2</sup> )	–	14.9 (7.9–21.8) <sup>c</sup>	–	–	−0.5 (−1.0 to −0.0) <sup>b</sup>
LAVI (ml/m <sup>2</sup> )	0.3 (0.0–0.6) <sup>b</sup>	3.4 (−0.6–7.3) <sup>a</sup>	–	–	–
RAVI (ml/m <sup>2</sup> )	–	4.9 (−0.9–10.6) <sup>a</sup>	–	–	–

Linear regression results expressed as coefficients (95% CI). HbA<sub>1c</sub> is in %. Systolic blood pressure was also in the model, but non-significant in all analysis and therefore not shown.

bpm, beats per minute; HRV, heart rate variability during deep breathing; LAVI, indexed left atrial volume; LVDVI, indexed left ventricular diastolic volume; LVMI, indexed left ventricular mass; RAVI, indexed right atrial volume; RVDVI, indexed right ventricular diastolic volume. Indexed values are measurements per body surface area.

<sup>a</sup> p < 0.1.<sup>b</sup> p < 0.05.<sup>c</sup> p < 0.001.

We did not find HRV independently associated with LVM after adjustment for BSA, gender and other risk factors – we solely found HbA<sub>1c</sub> and diabetes duration influencing LVMI in this cohort. Interestingly, they influenced LVMI in opposite directions: HbA<sub>1c</sub> was associated with an increase in LVMI, whereas duration of diabetes was associated with decrease in LVMI.

Pop-Busui et al.<sup>16</sup> found increased LVM/DV in participants with CAN; we did not replicate this finding. It could be due to our reduced number of participants compared to their study; we do find more persons with CAN with abnormal LV morphology (8 (11%) persons with type 1 diabetes had a measure >97.5 percentile, 6 of 8 had CAN) – although not statistically significant. Another possibility – besides the above-mentioned inclusion of persons with kidney damage in the DCCT/EDIC cohort as an important confounder – is that we use a more strict definition of CAN: we use HRV ≤10 + one more abnormal CAN test, whereas they defined CAN as HRV <20 + one more abnormal test. Lastly, it could also mean that autonomic neuropathy is not related to LV remodelling after adjustment for other factors, as their association disappeared after adjustment for other factors<sup>16</sup> and HRV was not significant in our multivariable analysis (Table 2).

As discussed, the results regarding LV in type 1 diabetes complicated by CAN are not in agreement. From other studies regarding type 1 diabetes and LVM, the diversity in influential factors are evident: normoalbuminuric persons have been described as having both smaller<sup>12</sup> and unaltered LVMI<sup>39</sup> compared to controls. Macroalbuminuria, however, is consistently reported as risk factor of increased LVMI.<sup>10,38,40–42</sup> We found HbA<sub>1c</sub> and diabetes duration independently associated with LVMI; previous studies found that reduction in HbA<sub>1c</sub> resulted in reduction of LVM<sup>43,44</sup> – however only in persons with preserved kidney function<sup>43</sup> – which is in line with our results.

**Table 3**

Sensitivity analysis of cardiac chamber sizes of persons with and without CAN of comparable age, gender and diabetes duration.

	−CAN, n = 27	+CAN, n = 27	p-Value
Age (years)	52.1 ± 7.2	55.5 ± 7.3	0.09
Gender (men)	18 (67%)	18 (67%)	1.00
Diabetes duration (years)	33.0 ± 9.3	35.6 ± 11.1	0.36
LVM/DV (g/ml)	0.88 (0.76–1.08)	0.93 (0.82–1.04)	0.36
LVMI (g/m <sup>2</sup> )	53.7 (44.6–59.2)	55.8 (48.6–63.2)	0.38
LVDVI (ml/m <sup>2</sup> )	57.9 ± 14.5	59.2 ± 11.8	0.72
RVDVI (ml/m <sup>2</sup> )	71.7 ± 15.6	74.0 ± 15.3	0.58
LAVI (ml/m <sup>2</sup> )	39.4 ± 6.9	40.8 ± 9.3	0.52
RAVI (ml/m <sup>2</sup> )	51.6 ± 12.3	50.8 ± 11.2	0.81

CAN, cardiovascular autonomic neuropathy; +CAN, presence of CAN; −CAN, no presence of CAN; LAVI, indexed left atrial volume; LVDVI, indexed left ventricular diastolic volume; LVMI, indexed left ventricular mass; RAVI, indexed right atrial volume; RVDVI, indexed right ventricular diastolic volume. Indexed values are measurements per body surface area.

Data are mean ± SD, median (IQR) or number of observations (percentage).

We find HbA<sub>1c</sub> associated with an increase in LVM/DV – this has been found before in a study from the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC), but that study included persons with albuminuria.<sup>10</sup> However, our results and theirs suggest that an increase in HbA<sub>1c</sub> results in increase in LVM/DV in persons with type 1 diabetes regardless of albuminuria status.

Table 2 shows that gender is independently associated with increased LVM and increased chamber volumes; this is in accordance with a healthy background population.<sup>26</sup>

#### 4.1. Limitations

As this was a post-hoc analysis of previous trials, the results should be interpreted with caution, although the aims of the previous trials also were CAN and CV risk factors. There was a weak univariable in Fig. 1 due to the large variation, however the trend is similar in all chambers and corresponds to the findings in the multivariable analyses and assumptions for the multivariable models were met; thus, we interpret the results as valid. The demographic differences in the two participant groups shown in Table 1 complicate meaningful univariable analyses of the influence of CAN on cardiac chambers. Ideally the groups would have matched on determinants of cardiac chamber sizes, but it was not possible due to the post-hoc nature of this study; instead we adjusted for the differences in multivariable analyses and also performed a sensitivity analysis in some of the participants of similar age, gender and diabetes duration. A future study should include more participants or better matching on confounders; some of our analyses showed tendency towards significance, which may be a power-issue (i.e. LVDVI approached statistical significance in Table 2 (p < 0.1), more persons with CAN had abnormal LV remodelling and the numerical difference in the sensitivity analysis was non-significant). Classes of antihypertensive drugs could theoretically influence CAN testing, but were not paused prior to CAN testing, which could confound the test results; however, only six persons were prescribed beta-blockers. To our knowledge there are no validated methods for correction of CAN test results obtained during antihypertensive treatment. We have few participants without much variance in CV risk factors, which complicate multifactorial analyses identifying risk factors for the different cardiac measurements – however, our focus was on CAN and not type 1 diabetes in general. The relatively small number of participants also limited inclusion of more potential confounders in the multivariable models, e.g. antihypertensive medication. The cross-sectional design impairs determination of a causal relationship between autonomic dysfunction and increased volumes; a longitudinal study is required to confirm this finding. Some possible confounders for cardiac chambers were not registered e.g. data on physical exercise, coronary angiography or other microvascular complications as retinopathy or peripheral neuropathy. Furthermore, we do not have functional, e.g. echocardiographic, measures; however, CAN was previously reported associated with

decreased left ventricular function in some of the included patients.<sup>13,17</sup> Since the patients with type 1 diabetes have long diabetes duration, we cannot exclude a slight microalbuminuria at some point during their disease; however, all participants have persistently been classified as normoalbuminuric before and at the time of the studies, which is why we exclude albuminuria as a confounder. Strengths in this study include use of a robust method for measurement of cardiac chambers and the largest quantity of participants with CAN and without nephropathy compared to other studies.

#### 4.2. Conclusions

Our results suggest that cardiovascular autonomic dysfunction in persons with long-term type 1 diabetes, in particular heart rate variability, may be associated with enlargement of right and left ventricular volumes, which could indicate progression towards heart failure. To our knowledge, this is the first description that autonomic neuropathy not only influence the left, but also the right ventricle, indicating global myocardial influence of cardiovascular autonomic neuropathy – however, the clinical implication of this remains to be determined. We also found that HbA<sub>1c</sub> and duration of diabetes are important factors regarding left ventricular mass in normoalbuminuric persons with long-term type 1 diabetes.

As this study demonstrates, we do not know much about the impact of CAN on cardiac dimensions and the few existing studies are ambiguous; larger studies are needed in order to confirm our findings. As there is no effective treatment for CAN, it may only be possible to reduce CV morbidity and mortality in persons with CAN by treatment of the physiological CV complications – but first we need to extend our knowledge of these complications. Thus, there is a need for more studies examining how CAN influence the cardiovascular system.

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#### Contribution statement

HØH, TJ, JH, UMM, PR, LK and KFK made substantial contributions to conception and design. HØH, TJ, UMM and HC made substantial contributions to acquisition or analysis of data. HØH, TJ, JH and KFK made substantial contribution to interpretation of the data and drafting the article. UMM, PR, HC and LK revised it critically for important intellectual content. All authors have approved the final version to be published. HØH is the guarantor of the study.

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