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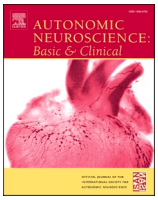

Functional and ^{123}I-MIBG scintigraphy assessment of cardiac adrenergic dysfunction in diabetes

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ABSTRACT

Objectives: To assess the agreement between clinical cardiovascular adrenergic function and cardiac adrenergic innervation in type 2 diabetes patients (T2D).
Methods: Thirty-three patients with T2D were investigated bimodally through (1) a standardized clinical cardiovascular adrenergic assessment, evaluating adequacy of blood pressure responses to the Valsalva maneuver and (2) ^{123}I-meta-iodobenzylguanidine (MIBG) scintigraphy assessing myocardial adrenergic innervation measured as early and delayed heart heart/mediastinum (H/M) ratio, and washout rate (WR).
Results: T2D patients had significantly lower early and delayed H/M-ratios, and lower WR, compared to laboratory specific reference values. Thirteen patients had an abnormal adrenergic composite autonomic severity score (CASS $>$ 0). Patients with abnormal CASS scores had significantly higher early H/M ratios (1.76 [1.66–1.88] vs. 1.57 [1.49–1.63], $p < 0.001$), higher delayed H/M ratios (1.64 [1.51:1.73] vs. 1.51 [1.40:1.61] ($p = 0.02$)), and lower WR (−0.13(0.10) vs −0.05(0.07), $p = 0.01$). Lower Total Recovery and shorter Pressure Recovery Time responses from the Valsalva maneuver was significantly correlated to lower H/M early ($r = 0.55$, $p = 0.001$ and r = 0.5, $p = 0.003$, respectively) and lower WR for Total Recovery ($r = -0.44$, $p = 0.01$).
Conclusion: The present study found impairment of sympathetic innervation in T2D patients based on parameters derived from MIBG cardiac scintigraphy (low early H/M, delayed H/M, and WR). These results confirm prior studies. We found a mechanistically inverted relationship with favourable adrenergic cardiovascular responses being significantly associated unfavourable MIBG indices for H/M early and delayed. This paradoxical relationship needs to be further explored but could indicate adrenergic hypersensitivity in cardiac sympathetic denervated T2D patients.

1. Introduction

Cardiac autonomic neuropathy is a common complication of type 2 diabetes (T2D) affecting up to 36 % of all patients (Kempler, Tesfaye et al., 2002). It is an independent predictor of increased mortality (Maser et al., 2003; Ziegler, 2008; Pop-Busui, Evans et al., 2010). Its diagnosis is made typically through cardiovascular autonomic reflex tests, which assess almost exclusively parasympathetic function (Spallone, Ziegler et al., 2011). As the examination of cardiac adrenergic function requires an extensive laboratory setup, only a limited number of studies have examined the degree of adrenergic dysfunction in T2D (Freccero et al., 2004; Low et al., 2004).

Abbreviations: T2D, type 2 diabetes; MIBG, ^{123}I-metaiodobenzylguanidine; H/M, heart/mediastinum; WR, washout rate; BP, blood pressure; CASS, Composite Autonomic Scoring Scale; HR, heart rate; HC, healthy controls; P2L, Phase 2 late; S3, Sympathetic index 3; P4, Phase 4; PRT, pressure recovery time; TPR, total peripheral resistance; HRV, heart rate variability; IQR, interquartile range.

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^{123}I-metaiodobenzylguanidine (MIBG) scintigraphy is a non-invasive in vivo imaging technique for quantifying postganglionic presynaptic uptake and storage of radiolabeled neurotransmitters in cardiac adrenergic fibers. The MIBG scintigraphy yields several measures of adrenergic innervation: early heart/mediastinum (H/M) ratio reflects the density of presynaptic myocardial adrenergic terminals, and delayed H/M ratio represents a combined parameter reflecting both density and the functional tone of the presynaptic nerve terminals. The difference between delayed and early (delayed - early) H/M-ratio is referred to as washout rate (WR), and is considered to represent vesicular storage capabilities and the adrenergic turnover measuring the degree of sympathetic drive.

Measures of MIBG (Mantysaari et al., 1992; Paolillo et al., 2013) and autonomic cardiovascular reflex responses (Vinik et al., 2003) have both been found to be reduced in T2D patients. Previous studies have assessed the association between cardiovascular parasympathetic function and MIBG in T2D but have only found weak correlations (Scholte et al., 2010; Didangelos et al., 2018; Zobel et al., 2019). It remains unclear, however, if clinical cardiovascular adrenergic function is associated with myocardial MIBG uptake in T2D.

The primary aim of this study was to investigate the association between adrenergic function assessed by blood pressure (BP) responses to the Valsalva maneuver and by MIBG scintigraphy in T2D patients with no known ischemic heart disease. Additionally, we aimed to investigate the agreement between MIBG scintigraphy and the adrenergic index of the Composite Autonomic Scoring Scale (CASS) (Low, 1993).

We hypothesized that reduced clinical cardiovascular adrenergic function would be associated with low MIBG scintigraphy measures.

2. Methods

2.1. Participants

The study was carried out according to the Helsinki Declaration and was approved by the local ethics committee (Ref-ID: MJ-1-10-72-256-18), Central Denmark Region, Denmark, and registered at Aarhus University internal notification (no. 2016-051-000001, 1191). All participants received written and oral information prior to inclusion and provided written informed consent upon entering the study. This study is part of another study assessing cardiovascular autonomic function in forty T2D patients (Rasmussen et al., 2022). All T2D patients were invited to undergo MIBG scintigraphy. A total of thirty-three patients completed the MIBG scintigraphy, with the main reason for failure to participate being COVID-19 lockdown and restrictions. Subjects scanned had significantly higher resting HR compared to subjects not scanned (mean ± SD scanned 72.5 ± 13.2 bpm vs subjects not scanned 61.4 ± 7.8 bpm, $p = 0.04$), but did not differ significantly in any other clinical or demographic measures (data not shown).

Inclusion criteria were a diagnosis of T2D and normal 12-lead ECG. Exclusion criteria were history of ischemic cardiac disease or hypertensive emergencies, arrhythmias, BP > 220/120, neurodegenerative diseases, history of drug or alcohol abuse, or regular intake of any medicine with potential influence on the autonomic nervous system or the MIBG scintigraphy that could not be paused. In-house reference MIBG data from healthy controls (HC) of comparable age and sex and imaged with identical methodology are presented in the results section for reference ($n = 14$).

2.2. Course of examination

Participants were seen for three sessions: two sessions on separate days at the Danish Pain Research Center, Aarhus University Hospital, Denmark, and one session at the Department of Nuclear Medicine and PET, Aarhus University Hospital, Denmark. In the first session, a medical history along with demographic and anthropometric data was obtained. A neurological and physical examination was performed. Patients were instructed in preparations regarding the autonomic testing day: no strenuous exercise for at least 24 h and no intake of alcohol, caffeine, or nicotine at least 12 h before testing. They were asked to fast for at least 4 h and to abstain from a major meal for 6 h prior to testing. Medicine potentially influencing the autonomic nervous system was paused for a minimum of five half-lives prior to testing, including all antihypertensive drugs.

Autonomic testing was performed on the second day in a quiet room between the hours 09:00 to 14:00 with a mean (±SD) room temperature of 23.6C (±0.6).

The setup for autonomic testing has previously been described (Rasmussen et al., 2017). Subjects rested in the supine position for at least 20 min before testing. Subjects performed a modified Ewing autonomic battery in the following order (Freeman and Chapleau, 2013): Deep breathing test at least two times and ten minute resting HRV-recording followed by passive tilting to 70° for 10 min. The Valsalva maneuver was performed a minimum of four times, two times in supine, and two times at 20-degree passive tilt, with the order of positioning randomized. They rested for at least 4 min between each test.

Subjects blew into a mouthpiece (Vitalograph 2820 BV filter) connected to a digital pressure transducer during the Valsalva maneuver, and a digital air volume transducer during deep breathing. Real-time respiratory and expiratory measurements were presented on a computer screen, allowing subjects to rapidly adjust respiration volume and expiration pressures. All testing was in compliance with AAN guidelines for autonomic testing (Anon, 1996).

2.3. MIBG

The MIBG scintigraphy was performed using a dual-head gamma camera (Siemens Symbia SPECT/CT, Erlangen, Germany) with a low-energy high-resolution collimator. Images of the thorax were acquired at 15 min (early) and 3.5 h (delayed) after injection of 110 MBq MIBG. Regions of interest were manually defined on the heart and mediastinum by an experienced rater blinded to diagnostic category of the study subjects. Mean heart:mean mediastinum uptake ratios were calculated on early (H/M early) and late (H/M delayed) images, and WR was calculated as $(H/M_{delayed}) - (H/M_{early})$. Thus, a negative WR represents pathologically rapid wash out.

2.4. Cardiovascular autonomic monitoring

A 3-channel electrocardiogram, oscillometric and continuous “beat-to-beat” BP and impedance cardiography was recorded non-invasively with a Task Force Monitor® (CNSystems Medizintechnik AG, Graz, Austria) during autonomic testing. Absolute oscillometric BP was measured with an upper arm cuff on the subject's right arm, initially 3 times during the acclimatizing phase, and subsequently before every test. Continuous “beat-to-beat” BP was measured on the second or third digit by photoplethysmography sensor readings on the fingers contralateral to the oscillometric cuff.

24-Hour ambulatory blood pressure was measured non-invasively using an oscillometric apparatus (BOSO TM-2430) on the participant's non-dominant arm. BP was measured every 20 min during daytime (07:00–22:00) and every 30 min during nighttime. Nocturnal time intervals were ascertained for each participant from self-reported diaries.

2.5. Autonomic markers

During tilt table testing the maximum drop in oscillatory systolic BP during tilt was compared to resting baseline systolic BP. Oscillatory BP was recorded approximately once per minute during tilting. The Valsalva maneuver and deep breathing was analyzed as previously described (Rasmussen et al., 2017). Only Valsalva maneuvers performed in the supine position were used for analysis, to allow comparability of

measures according to the CASS classification. No flattop responses were included in the analysis.

Phase 2 late BP rise (P2L), Total Recovery (Sympathetic index 3 (SI 3)), Phase 4 BP overshoot (P4), and pressure recovery time (PRT) were used as short-term cardiovascular sympathetic markers (Sandroni et al., 1991; Denq et al., 1998; Sandroni et al., 2000; Vogel et al., 2005). Total Recovery (SI 3) as described by Novak P. was also used (Novak, 2011). For a list of the calculations used for adrenergic measures, see Online Resource Table 1. Adrenergic function was dichotomized by the adrenergic index of the CASS, a 5-point scale ranging from 0 to 4. Patients were classified as normal (CASS = 0) or impaired (CASS > 0) adrenergic function (Low, 1993). Total peripheral resistance (TPR), stroke volume and cardiac output measured by impedance cardiography by the Task Force Monitor were analyzed during 10-minute supine rest, and during 10-minute passive head up tilt table testing.

2.6. Heart rate variability

Heart rate variability (HRV) was analyzed using Kubios HRV analyzing software (KUBIOS V3.4.1 Biosignal Analysis and Medical Imaging Group, Kuopio, Finland) (Tarvainen et al., 2014).

2.7. Statistical analysis

Statistical analysis was performed, and graphs were generated, using Stata 15.1 (StataCorp, College Station, Texas). Data are expressed as mean (±SD) or median [interquartile range (IQR)]. Continuous variables were compared between groups using two-tailed unpaired Student's *t*-test, Welch's *t*-test, or the Wilcoxon Rank Sum Test, and categorical variables were compared using chi-squared test. Normality of the data was assessed by QQ-plots and the Shapiro-Wilk goodness-of-fit test. All participants were included in all analyses. Spearman's rank order correlation analyses were used to examine the relationship between cardiovascular adrenergic parameters and MIBG early and delayed H/M ratio and WR.

3. Results

A total of 33 T2D patients (19 females) completed the clinical assessment. The mean age was 64.9 ± 6.7 years.

Clinical and demographic data are presented in Table 1. Twenty patients had a CASS score = 0 and 13 patients had a CASS score > 0 (n = 12 CASS = 1, n = 1 CASS = 2). No significant difference in any baseline characteristics was found between the groups, although patients with CASS > 0 tended to have a shorter duration of T2D and were predominantly female.

Table 1
Clinical and demographic data.

Columns by: CASS GROUP	CASS = 0	CASS > 0	*p*-Value
n (%)	20 (60.6)	13 (39.4)	
Age (years), mean (SD)	65.0 (6.9)	64.8 (6.7)	0.97
Gender (Male), n (%)	11 (55.0)	3 (23.1)	0.07
BMI, mean (SD)	32.7 (5.4)	30.2 (9.1)	0.33
HbA1C, mean (SD)	50.9 (7.4)	53.1 (7.2)	0.42
Diabetes duration (years), mean (SD)	10.9 (3.9)	8.0 (5.4)	0.08
eGFR, mean (SD)	86.6 (8.7)	83.3 (12.3)	0.43
Receiving antihypertensive treatment, n (%)			
No treatment, n (%)	10 (50.0)	5 (38.5)	
With treatment, n (%)	10 (50.0)	8 (61.5)	0.52
Tobacco use, n (%)			
Never, n (%)	13 (65.0)	6 (46.2)	
Currently, n (%)	0 (0.0)	3 (23.1)	
Previously, n (%)	7 (35.0)	4 (30.8)	0.08
Office systolic BP, mean (SD)	140.4 (18.5)	137.3 (14.6)	0.61
Office diastolic BP, mean (SD)	85.2 (8.9)	83.4 (9.9)	0.58
Office resting HR, mean (SD)	69.8 (11.3)	76.8 (15.1)	0.13
24-Hour systolic BP, mean (SD)	146.8 (14.6)	144.5 (16.0)	0.67
24-Hour diastolic BP, mean (SD)	81.7 (7.3)	81.3 (6.0)	0.86
Daytime systolic BP, mean (SD)	149.9 (13.4)	148.7 (13.9)	0.80
Daytime diastolic BP, mean (SD)	83.6 (6.8)	83.4 (5.9)	0.94
Nighttime systolic BP, mean (SD)	138.4 (21.7)	133.8 (21.8)	0.55
Nighttime diastolic BP, mean (SD)	76.4 (12.0)	75.9 (12.2)	0.92
UENS total score, mean (SD)	8.1 (5.0)	7.6 (4.1)	0.80

Values are the number of subjects (proportion) or mean ± SD. BMI body mass index, BP blood pressure, eGFR estimated glomerular filtration rate, HR heart rate, SD standard deviation, UENS Utah Early Neuropathy Scale.

3.1. MIBG measures and hemodynamic responses during passive tilt table testing

All MIBG parameters were significantly reduced in T2D patients compared to laboratory-specific reference data from HC subjects. T2D vs. HC - Early H/M 1.63 [1.54–1.71] vs. 1.71 [1.66–1.80] $p = 0.03$, for delayed H/M 1.55 [1.46–1.66] vs. 1.76 [1.69–1.85] $p < 0.001$ and WR −0.08 (0.09) vs. 0.04 (0.06) $p < 0.001$. For early H/M, 15.2 % of T2D patients were two SD below HC. For Late H/M and WR, 42.4 % and 54.5 % respectively, of T2D patients were two SD below HC.

3.2. CASS scores

Patients with CASS > 0 showed significantly higher early and delayed H/M-ratio and lower WR, compared to CASS = 0. Mean point estimates of raw cardiac and mediastinum tracer uptake, showed a significantly lower cardiac tracer uptake in both early and delayed scintigraphy for CASS = 0, whereas measures of mediastinum uptake were comparable between the groups (see Table 2). The difference in tracer uptake (early-delayed) was not different between the groups for both the heart and mediastinum (data not shown). For hemodynamic responses during passive tilt table testing, patients with CASS > 0 had an increase in TPR, whereas patients with CASS = 0 had a decrease in TPR. Patients with CASS > 0 had a significantly larger decrease in stroke volume. See Table 2.

3.3. Correlation between adrenergic autonomic measures and MIBG

Early and delayed H/M-ratios were positively correlated with adrenergic measures of the Valsalva maneuver for Total Recovery and Pressure Recovery Time and negatively correlated with Phase 2 late BP rise, Phase 4 Overshoot (see Table 3 and Fig. 1).

No correlations were found between early and delayed H/M ratios, and parasympathetic and HRV indices (see Online Resource Table 2 in Supplementary material). WR was significantly correlated with Total Recovery (SI 3) ($r = -0.44$, $p = 0.01$) and the parasympathetic indices expiratory:inspiratory (E:I)-ratio ($r = 0.35$, $p = 0.048$) and Valsalva ratio ($r = 0.41$, $p = 0.02$). See Online Resource Table 2.

4. Discussion

This study has two major findings. Expectedly, we found significantly reduced cardiac MIBG uptake in our T2D patients compared to in-house reference MIBG data from HC, suggesting lower cardiac adrenergic innervation in T2D patients. Surprisingly, in the present group of cardiac sympathetic denervated T2D patients we found reduced cardiovascular adrenergic function to be significantly correlated with lower washout and increased early and delayed H/M ratios. In short, a paradoxical association between reduced cardiac adrenergic innervation and greater adrenergic cardiovascular responses.

Low measures of early H/M ratio reflect reduced myocardial adrenergic nerve fiber density and norepinephrine transporter activity,

Table 2
^{123}I-MIBG scintigraphy data and changes in hemodynamic parameters during tilt table testing.

MIBG measures			
	CASS = 0	CASS > 0	p-Value
Early H/M*	1.57 [1.49–1.63]	1.76 [1.66–1.88]	0.0001
Delayed H/M*	1.51 [1.40–1.61]	1.64 [1.51–1.73]	0.02
Washout	−0.05 (0.07)	−0.13 (0.10)	0.007

Raw MIBG values			
	CASS = 0	CASS > 0	p-Value
Early heart tracer uptake†	186.35 (29.00)	229.81 (68.48)	0.047
Early mediastinum tracer uptake†	119.70 (16.50)	125.45 (30.01)	0.54
Delayed heart tracer uptake†	122.51 (18.24)	153.68 (44.80)	0.03
Delayed mediastinum tracer uptake†	81.32 (10.42)	90.59 (20.24)	0.15

Hemodynamic response to tilt table testing			
	CASS = 0	CASS > 0	p-Value
Change in TPR (dyne*s/cm^5)	138.59 (313.75)	−128.16 (299.88)	0.02
Change in CO (l/min)	−0.48 (1.04)	0.15 (0.73)	0.07
Change in SV (ml)	2.29 (11.94)	12.08 (11.19)	0.02
Change in systolic BP (mmHg)	−0.88 (8.00)	−2.29 (5.00)	0.57
Change in HR (bpm)	−9.62 (3.89)	−11.20 (5.76)	0.35

Data are presented as mean (±SD), or median [IQR]. *Wilcoxon rank sum test, †Welch's *t*-test, H/M Heart/mediastinum ratio, Washout measured as: delayed H/M – early H/M, BP Blood pressure, CO Cardiac output, SV Stroke volume, TPR Total peripheral resistance. Changes in hemodynamic parameters – difference between mean during 10-minute rest and mean during 10-minute tilt table testing ((mean during 10-minute rest) – (mean during 10-minute tilt table testing)).

Table 3
Correlation coefficients between adrenergic measures.

Valsalva maneuver indices	H/M Early		H/M Delayed		Washout	
	r	p-Value	*r*	p-Value	*r*	p-Value
Phase 2 late mean BP rise	−0.28	0.112	−0.27	0.122	0.03	0.883
Total recovery (SI 3)	**0.55**	**0.001**	0.27	0.125	**−0.44**	**0.010**
Phase 4 systolic BP overshoot	−0.28	0.112	−0.18	0.311	0.26	0.144
Pressure recovery time	**0.50**	**0.003**	**0.41**	**0.018**	−0.13	0.463

Calculated as Spearman's rank correlations. Statistically significant values are written in bold font. H/M Heart: mediastinum ratio, BP Blood pressure, SI Sympathetic Index.

whereas delayed H/M is considered a combined measure of receptor density, norepinephrine vesicular storage capacity and sympathetic activity. Washout reflects adrenergic turnover, measuring the sympathetic drive – a negative WR is indicative of sympathetic dysfunction, either due to low vesicular storage capacity with reduced retention of MIBG tracer, or increased sympathetic drive. Our total cohort of T2D patients presented with both lower early and delayed H/M ratios, as well as lower (negative) WR, in comparison to HC subjects.

The absolute values of MIBG measures are typically dependent on the specific technical laboratory setup, which prevents a direct comparison of absolute MIBG outcomes across studies and research laboratories. Despite this limitation, our findings are in line with previous studies in documenting impaired myocardial adrenergic innervation in T2D patients, with mean MIBG scores significantly lower than HC, and with a substantial percentage of T2D patients scoring measures below ≤2 SD of HC (Mantysaari et al., 1992; Kreiner et al., 1995). Decreased delayed H/M and lower washout have been found to be associated with adverse cardiac and cerebrovascular events (Nagamachi et al., 2006, Yufu et al., 2012).

Looking at cardiovascular adrenergic function in our cohort of T2D patients with MIBG-quantified cardiac adrenergic denervation, we found significant correlations between adrenergic cardiovascular function measured by the Valsalva maneuver and MIBG scintigraphy measures of adrenergic integrity. This association persisted both when the patients were dichotomized according to CASS, and when adrenergic Valsalva maneuver indices were analyzed as continuous variables. Contrary to our hypothesis, the associations between Valsalva maneuver and H/M early and delayed were mechanistically inverted, with decreased cardiac adrenergic nerve density (lower values of H/M early and H/M delayed MIBG) being associated with signs of increased adrenergic function during the Valsalva maneuver (smaller Total Recovery, greater Phase 2 late BP rise and Phase 4 Overshoot BP rise, and reduced Pressure Recovery Time). These correlations were significant for Total Recovery and H/M early ($r = 0.55$, $p = 0.001$) and for Pressure Recovery Time to H/M early and delayed (r = 0.5, $p = 0.003$, and 0.41, $p = 0.02$ respectively).

When stratifying adrenergic dysfunction according to CASS, we saw the same trend with an association between abnormal adrenergic CASS (signs of sympathetic deficit during the Valsalva maneuver) and increased H/M early and delayed measures. Thus, the density of the presynaptic cardiac adrenergic nerves appeared to be greatest in patients with clinical signs of sympathetic adrenergic dysfunction. Heightened WR, which may indicate decreased adrenergic storage capacity or sympathetic overactivity, was on the other hand, correlated with decreased Valsalva maneuver function in all adrenergic indices, albeit only significant for Total recovery. An abnormal CASS was significantly associated with a greater WR. All these associations are novel findings that have, to our knowledge, not previously been reported in T2D patients.

Associations between cardiovascular autonomic functions and indices of MIBG scintigraphy have previously been investigated in T2D patients (Nagamachi et al., 1998; Scholte et al., 2010), although all previous studies have utilized HR derived autonomic measures, such as HR changes to deep breathing and HRV, which mainly represent parasympathetic function (Araujo et al., 1992; Billman, 2013). We found significant correlations between WR and Valsalva ratio ($r = 0.36$, $p = 0.04$) and E:I-ratio ($r = 0.41$, $p = 0.02$), but not to early or delayed H/M ratios. Previous studies investigating the association between autonomic function and MIBG scintigraphy have reported mainly negative correlations (Langer et al., 1995; Murata et al., 1996; Nagamachi et al., 1998; Uehara et al., 1999; Schnell et al., 2002; Scholte et al., 2010; Asghar et al., 2017) between parasympathetic (HRV and HR indices) and MIBG, with impaired autonomic function being associated with lower H/M. Contrary to these previous findings, our results suggest that favourable clinical adrenergic function is associated with reduced cardiac presynaptic nerve density. Additionally, we found patients with abnormal CASS (CASS > 0) had greater H/M ratios but an increase in TPR and reduction in cardiac output - contrary to patients with normal CASS – during tilt table testing. Patients with abnormal CASS also had a significantly greater decrease in stroke volume during tilt table testing compared to the group with normal CASS. I summation, these findings could suggest a somehow generalized adrenergic hyperactivity, both in the myocardium and in the smooth muscles of the peripheral vasculature, potentially driven by an increased adrenergic expression and/or sensitivity (Cohen et al., 1990). Notably, patients with normal CASS tended to have a longer disease duration, suggestive of a possible initial adrenergic dysfunction which at later stages revert back to a clinically

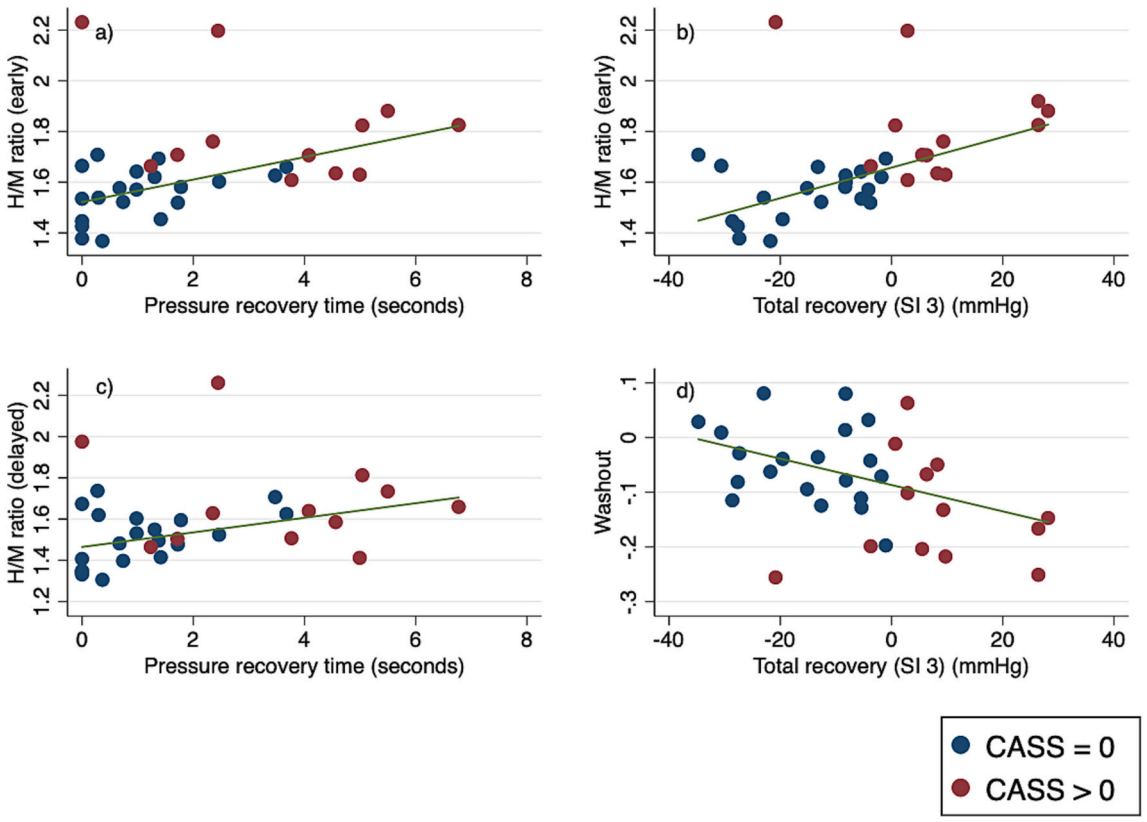

Fig. 1. Scatterplots for MIBG and adrenergic Valsalva maneuver indices.
Correlations between early H/M ratio, and (a) Pressure Recovery Time and (b) Total recovery (SI 3), delayed H/M ratio and (c) Pressure Recovery Time, and Washout and (d) Total recovery (SI 3). For illustrative purposes regression lines are fitted using ordinary least squares regression with removal of highly influential cases defined as Cooks Distance > 4/(total number cases). Extremely high pressure recovery times are not illustrated.

normal CASS, potentially due to a adrenergic hypersensitivity.

These findings could indicate altered adrenergic receptor dynamics, with either a marked upregulation of adrenoceptors, increased transmitter availability, or an adrenergic hyperreactive response state in different patient subgroups; although the exact biological mechanisms explaining these findings need to be further explored. Increased adrenergic sensitivity in denervated cardiac tissue is a known phenomenon, and has been documented in multiple animal models, although primarily in models of surgical denervation (Tapa et al., 2018; Tapa et al., 2020). Animal diabetic models with cardiac denervation have yielded inconclusive results with both down- and upregulation of adrenergic receptor reactivity, adrenergic receptor protein concentrations and mRNA expression both in insulin dependent diabetes mellitus and non-insulin dependent diabetes mellitus (Erdogan et al., 2020).

Research into adrenergic receptor hyperreactivity in humans is sparse. Increased sensitivity to adrenergic stimulation in insulin dependent diabetes mellitus is known (Scobie et al., 1987; Bernjak et al., 2020). A limited number of studies have assessed adrenergic receptor reactivity in T2D. Bruce et al. documented increased pressor responses to exogenous noradrenaline in T2D patients (Bruce et al., 1992). Wilson et al. found T2D patients to have increased sensitivity to dobutamine infusions during stress echocardiography, compared to HC, and had increased resting HR in response to lower plasma norepinephrine levels (Wilson, Wilson et al., 2017). Diabetes is in itself associated with an increased risk of a hypertensive reaction during dobutamine stress echocardiography (Abram et al., 2017).

Hilsted et al. have made substantial contributions investigating this phenomenon of adrenergic denervation hypersensitivity in insulin dependent diabetes. They convincingly demonstrated increased hemodynamic reactivity to both adrenaline (Hilsted et al., 1987) and noradrenaline (Dejgaard et al., 1996) in diabetic patients with clinical signs of autonomic neuropathy, although further work done to clarify the potential physiological mechanisms responsible for this phenomenon failed to identify an increase in adrenergic receptors (Dejgaard et al., 1991), or any changes in noradrenaline kinetics (Dejgaard et al., 1986; Hilsted, 1995).

Similar presentations of denervation hypersensitivity, seen as increased physiological responses to exogenous adrenergic agonists, have been documented in other conditions with associated autonomic dysfunction such as Shy-Drager syndrome (Davies et al., 1982) and Parkinson's disease (Nakamura et al., 2007; Shirai et al., 2018). This condition could thus be suspected to be a consequence of autonomic neuropathy, rather than an isolated phenomenon associated with diabetes, although associations between diabetes and increased adrenergic receptor expression have been established (Rosengren et al., 2010; Tang et al., 2014; Schlereth et al., 2021).

Despite the solid evidence in the literature for the presence of adrenergic hypersensitivity in T2D, the interest has waned significantly in recent decades, and limited attention has been paid to the potential implications of this relationship between diabetes and adrenergic reactivity.

In contrast to previous research, our study differs in mainly two ways. First, whereas previous research has investigated the reactivity to exogenous catecholamines, our findings suggest this increased adrenergic activity is present for endogenous adrenergic transmitters. Secondly, as previous studies have dichotomized patients as either with or without autonomic neuropathy, our results indicate that this reactivity varies on a continuum and therefore not only presents as an either-or phenomenon.

Although the presence of adrenergic hyperreactivity as discussed above is proposed as an explanation for the link between reduced H/M ratios and normal adrenergic function seen here, it is first and foremost

speculative, and fails to address the potential underlying mechanisms responsible hereof. Possible hypotheses could be an upregulation of myocardial postsynaptic adrenergic receptors, greater synaptic noradrenaline availability due to reduced presynaptic reuptake, alterations in intracellular receptor signal coupling, or ultimately centrally mediated changes.

4.1. Limitations

A major limitation of our study is the limited assessment of cardiac integrity of our T2D cohort. T2D is a known risk factor for the development of cardiovascular disease (Abdul-Ghani et al., 2017), and the risk of structural myocardial damage influencing MIBG findings is high. Nevertheless, myocardial infarction would mainly present as lowered early and delayed H/M ratio, with a preserved washout, as the functional capacity would be intact. Most of our patients presented with negative washouts, indicating damaged vesicular storage capabilities. In addition, all patients had normal resting ECG upon inclusion in the study and had no history of cardiac disease. There might be a disproportionate denervation of the myocardium and vessels, which our study fails to sufficiently elucidate.

Overall, these results showed lower cardiac sympathetic innervation (low H/M, early and delayed) and higher WR in the CASS 0 subgroup. As these findings diverged significantly from our initial hypothesis, additional focused studies are needed to replicate and confirm these findings. Most of our T2D patients had limited adrenergic impairment with a PRT < 6 s. It would be highly relevant to look at a cohort of T2D patients covering a wider spectrum of adrenergic neuropathy, from minimal to severe adrenergic impairment. A full dataset in HC is needed, to verify the correlational trajectory in a non-disease-state. HC were not recruited specifically for this study, due to ethical concerns regarding radiation in HC.

An additional assessment of denervation hypersensitivity through the traditional investigation of hemodynamic responses to exogenous adrenergic agonists is imperative in a future study iteration.

Assessment of cardiovascular adrenergic function is complex, as it alters cardiovascular responses both through myocardial and peripheral vascular innervation. MIBG primarily assess myocardial adrenergic innervation, whereas the Valsalva maneuver and tilt table test responses represent both myocardial and peripheral adrenergic innervation (Sandroni et al., 1991, 2000; Cheshire Jr. and Goldstein, 2019). Autonomic tests such as the hand grip or the cold pressor test are considered to primarily represent peripheral adrenergic innervation, and the inclusion of these tests in this study could potentially have yielded greater insight into the correlations found in this study (Yamamoto et al., 1992; Quispe and Novak, 2021).

Notably, two patients presented with very high early and delayed H/M values (>2 SD above mean). These patients did not differ in any anthropometric measures or take any extraordinary medicine (data not shown). As no clinical or methodological confounding parameters of these patients could explain their large H/M values, these were included in all calculations. A post hoc analysis excluding these two patients only further augmented the already significant correlations and made early H/M significantly correlated with all four adrenergic domains and led to delayed H/M being significantly correlated with Phase 4 overshoot. See Online Resource Table 3 for data.

5. Conclusion

We found significantly reduced measures of MIBG in our cohort of T2D patients compared to HC subjects supporting previous findings of reduced cardiac sympathetic innervation in patients with T2D. In these cardiac sympathetically denervated patients, a higher cardiovascular adrenergic function correlated to lower innervation. Our findings shine new light on the potential role of adrenergic hypersensitivity in T2D. Although these findings may prompt more questions than they answer, they nonetheless offer a novel insight to the cardiac neuronal conditions of T2D patients. Further investigation into the reactivity of cardiac and vascular adrenergic receptors is imperative, as this knowledge may influence future adrenergic treatment regimens for T2D patients and could fundamentally question the applicability of functionally derived measures of cardiovascular autonomic neuropathy.

Ethics approval and consent to participate

The study was carried out according to the Helsinki Declaration and was approved by the local ethics committee (Ref-ID: MJ-1-10-72-256-18), Central Denmark Region, Denmark, and registered at Aarhus University internal notification (no. 2016-051-000001, 1191). All participants received written and oral information prior to inclusion and provided written informed consent upon entering the study.

Prior presentation

Results of this study have been presented as an abstract and poster at Neuroscience Day 2021, NeuroCampus Aarhus, May 6, 2021. Demographic and values of MIBG data in this cohort has been presented by T. D. Fedorova et al. in IBRO Neuroscience Reports (2023), https://doi.org/10.1016/j.ibneur.2023.03.006.

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CRediT authorship contribution statement

Thorsten K. Rasmussen: Conceptualization, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **Per Borghammer:** Investigation, Methodology, Writing – review & editing. **Nanna B. Finnerup:** Writing – review & editing, Funding acquisition. **Troels S. Jensen:** Funding acquisition, Writing – review & editing. **John Hansen:** Software, Writing – review & editing. **Karoline Knudsen:** Methodology, Writing – review & editing. **Wolfgang Singer:** Writing – review & editing. **Guillaume Lamotte:** Writing – review & editing. **Astrid J. Terkelsen:** Conceptualization, Formal analysis, Supervision, Writing – review & editing.

Declaration of competing interest

We report no potential conflicts of interest in relation to this manuscript.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.autneu.2024.103155.

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