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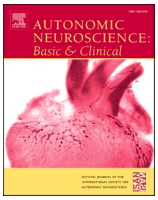

Preferential impairment of parasympathetic autonomic function in type 2 diabetes

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ABSTRACT

Objective: Cardiovascular autonomic neuropathy is a known complication in type 2 diabetes (T2D). However, the extent of sympathetic dysfunction and its relation to blood pressure (BP) dysregulation is insufficiently studied. We therefore assessed the cardiovascular sympathetic function using a standardized autonomic test-battery.
Research design and methods: Forty T2D patients (mean age and duration of diabetes ±SD, 65.5 ± 7.3 and 9.5 ± 4.2 years) and 40 age- and gender-matched controls were examined through autonomic testing, assessing cardiovascular responses to deep breathing, Valsalva maneuver and tilt-table testing. Additionally, 24-hour oscillometric BP and self-reported autonomic symptoms on COMPASS-31 questionnaire was recorded.
Results: Patients with T2D had reduced parasympathetic activity with reduced deep breathing inspiratory: expiratory-ratio (median [IQR] T2D 1.11 [1.08–1.18] vs. controls 1.18 [1.11–1.25] ($p = 0.01$)), and reduced heart rate variability ($p < 0.05$). We found no differences in cardiovascular sympathetic function measured through BP responses during the Valsalva maneuver ($p > 0.05$). 24-hour-BP detected reduced night-time systolic BP drop in T2D (9.8 % ± 8.8 vs. controls 15.8 % ± 7.7 ($p < 0.01$)) with more patients having reverse dipping. Patients with T2D reported more symptoms of orthostatic intolerance on the COMPASS-31 ($p = 0.04$).
Conclusions: Patients with T2D showed reduced parasympathetic activity but preserved short-term cardiovascular sympathetic function, compared to controls, indicating autonomic dysfunction with predominantly parasympathetic impairment. Despite this, T2D patients reported more symptoms of orthostatic intolerance in COMPASS-31 and had reduced nocturnal BP dipping, indicating that these are not a consequence of cardiovascular sympathetic dysfunction.

1. Introduction

Peripheral neuropathy is a common complication of type 2 diabetes (T2D) affecting approximately 50 % during the course of their disease (Feldman et al., 2019; Jensen et al., 2021). Reported prevalence of cardiac autonomic neuropathy in T2D varies from 15 % up to 42 % (Zoppini et al., 2015; Tahrani et al., 2014).

Cardiac autonomic neuropathy affects either the sympathetic or the parasympathetic branch, or both systems simultaneously, although studies rarely distinguish between the two.

Cardiac autonomic dysfunction is particularly important to recognize as it is associated with increased morbidity, cardiovascular disease, and all-cause mortality (Pop-Busui et al., 2010; Chowdhury et al., 2021; Fleg et al., 2016). While parasympathetic dysfunction is used primarily as a prognostic factor for increased mortality (Chowdhury et al., 2021), diagnosing sympathetic dysregulation is clinically essential, as it is manageable through both pharmacological and non-pharmacological conservative interventions (Gibbons et al., 2017).

In practice, the diagnosis of cardiac autonomic neuropathy is usually based on the presence of increased resting heart rate (HR) and reduced heart rate variability (HRV) as tested by cardiac reflex tests, which primarily measure parasympathetic indices of the autonomic nervous

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system (Yun et al., 2018). Few studies have focused on the impact on the sympathetic branch of the autonomic nervous system in T2D. Low et al. found impaired sympathetic cardiovascular function in 48 % of a cohort of T2D patients, and a significantly greater proportion of these patients reporting symptoms of orthostatic intolerance, compared to control subjects (Low et al., 2004).

The sympathetic branch is the main regulator of blood pressure (BP) (Guyenet, 2006) and sympathetic dysfunction is characterized by insufficient HR and BP regulation causing orthostatic hypotension (OH) with an increased risk of syncope, falls and cardiac disease (Goldstein and Sharabi, 2009; Fedorowski et al., 2010). Autonomic nervous system dysfunction is associated with nocturnal BP dysregulation with either reduced dipping or non-dipping/reverse-dipping (Fanciulli et al., 2018). The exact pathophysiological mechanisms involved are not fully understood, although both sympathetic and parasympathetic dysfunction seems to play a role (Milazzo et al., 2018). A better understanding of this process may provide key insights into the pathophysiology and treatment of nocturnal dysregulation.

Methods specifically assessing cardiovascular sympathetic function have been available for years, but require specialized laboratories and trained personnel, which likely have impeded its widespread application in both clinical and research settings. However, simple and clinically accessible methods exist comprising bedside orthostatic BP and 24-hour ambulatory BP monitoring (24-hour ABPM) systems to identify specific characteristic of hemodynamic autonomic dysfunction including nocturnal dysregulation, and impaired BP variability (van Hateren et al., 2012; Yoshinari et al., 2001).

We aimed to assess whether patients with T2D have reduced cardiovascular sympathetic regulatory capabilities, measured as impaired continuous BP reflex responses to standardized autonomic cardiovascular tests, and aimed to assess the association between these measures and 24-hour ABPM indices. Accordingly, we studied the following sympathetic markers: BP responses to the Valsalva maneuver and to tilt table testing and 24-hour ABPM indices - in a group of patients with T2D and a group of sex and age-matched control subjects.

We hypothesized that T2D is a risk factor for the development cardiovascular sympathetic dysfunction. The primary aim of this study was to assess the magnitude of autonomic dysregulation in patients with T2D, with a detailed assessment of established cardiovascular sympathetic markers, in comparison to age- and gender matched control subjects, and to investigate the association between cardiovascular sympathetic function and nocturnal BP dysregulation.

2. Materials and 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 about the study prior to inclusion and gave their written informed consent upon entering the study.

Forty patients with T2D were enrolled in this study, recruited from the Department of Neurology, Aarhus University Hospital, through posters, and from the Diabetes Type 2 Cohort (DD2) described previously (Gylfadottir et al., 2020a). An equal number of age- and gender matched control subjects were recruited through local advertisements. Inclusion criteria for T2D patients were a diagnosis of T2D, and a normal 12-lead ECG. Exclusion criteria were history of ischemic cardiac disease, persistent arrhythmias interfering with the evaluation of autonomic measures, history of hypertensive emergency (previously recorded BP $>$ 220/120 mmHg), neurodegenerative diseases, or intake of medicine with influence on the autonomic nervous system that could not be paused safely. Exclusion criteria for control subjects were any known disease or regular intake of any medication.

2.2. Course of examination

Patients with T2D were seen at two sessions at the Danish Pain Research Center, Aarhus University Hospital, Denmark. At the first session a medical history, demographic and anthropometric data were obtained. A physical examination was performed and a COMPASS 31 questionnaire was completed (Sletten et al., 2012). Symptoms and signs of diabetic neuropathy were assessed using the Utah Early Neuropathy Scale. Patients were instructed in preparations for the second test-day (see below). Control subjects were seen for one session, and were instructed by telephone beforehand. After autonomic testing, all participants were equipped with 24-hour ABPM system. Participants were investigated between May 2019 and February 2021.

2.3. Experimental set-up

Autonomic testing was performed on the second day, in a quiet room between the hours 09:00 to 14:00 to minimize circadian hemodynamic variance. Prior to attendance, participants had abstained from strenuous exercise for at least 24 h and from alcohol, caffeine, and nicotine at least 12 h before testing. They fasted for at least 4 h and had no major meal 6 h prior to testing. Medicine potentially influencing the autonomic nervous system, including antihypertensive drugs, was paused for a minimum of five half-lives prior to testing. Participants emptied their bladder immediately before testing and were not allowed to sleep nor talk or move significantly during testing.

The same investigator (TKR) instructed and supervised all tests. Participants were allowed to practice the autonomic testing on the test-day, until it was sufficiently executed, before any measures were recorded. Vital lung capacity was measured to ensure comparable performance between the groups. Usual medication regimens were resumed 24 h after testing, immediately following the dismount of 24-hour AMBP apparatus.

2.4. Cardiovascular autonomic monitoring

A 3-channel electrocardiogram, oscillometric and continuous “beat-to-beat” BP were recorded non-invasively with a Task Force Monitor® (CNSystems Medizintechnik AG, Graz, Austria). 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 photoplethysmographic sensor readings on the hand contralateral to the oscillometric cuff.

2.5. Autonomic testing

Orthostatic BP was measured after 5 min supine rest and every minute for 3 min upon standing (A&D UM201 BP apparatus). Afterwards, subjects rested in the supine position for 20 min before further testing. Subjects performed tests from the Ewing autonomic test battery (Freeman and Chapleau, 2013) in following order: Deep breathing test at least two times. Ten minutes resting HRV-recording followed by passive tilting to 70 degrees for 10 min. Afterwards, 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 test. 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 international guidelines (Thijs et al., 2021; Cheshire et al., 2021).

2.6. 24-hour ABPM

24-hour ABPM was performed non-invasively using an oscillometric BP apparatus (BOSO TM-2430) on their non-dominant arm. Appropriate arm cuff sizes were used. BP was measured every 20 min during daytime (07:00–22:00) and every 30 min during nighttime. Participants were instructed to perform usual daily activities during this period but refrain from strenuous activities, and to stop moving or talking and keep the arm still during cuff inflation. Nighttime intervals were ascertained for each participant from self-reported diaries. Analyzed measures were systolic and diastolic mean, standard deviation (SD) and coefficient of variation (CV) (SD/mean) for 24-hour, daytime, and nocturnal BP (Rothwell et al., 2010). SD and CV indices of systolic BP have previously been found to be associated with sympathetic function (Lodhi et al., 2019).

Nocturnal BP changes were estimated as absolute and relative changes compared to daytime BP. Subjects with a <10 % reduction in mean nocturnal BP compared to mean daytime BP were classified as reduced-dipping, and subjects with no decrease or a rise in nocturnal BP were classified as non-dipping and reverse dipping, respectively (Fanciulli et al., 2018).

2.7. Data analysis

The Valsalva maneuver and deep breathing was analyzed as previously described (Rasmussen et al., 2017; Novak, 2011). The analysis of deep breathing and Valsalva maneuver responses was performed blinded to group status. To avoid analysis of flattop responses, only hemodynamic responses to the Valsalva maneuver performed at 20-degree tilt was used for the analysis ($n = 2$ had flattop responses in all supine Valsalva maneuver tests).

Sympathetic index (SI) 2–5 as described by Novak. P was used as cardiovascular sympathetic markers (Novak, 2011). Phase 2 late (P2L/SI 2), Total recovery (SI 3), Phase 4 overshoot (P4/SI 4), and pressure recovery time (PRT/SI 5) are markers of sympathetic function (Sandroni et al., 1991; Sandroni et al., 2000; Vogel et al., 2005; Salmanpour et al., 2011). The HR response to deep breathing is reported as both absolute (expiratory:inspiratory(E:I)-difference) and relative (I:E-ratio) changes for the five largest HR responses. Changes in BP during tilt table testing was measured both by oscillometry and by continuous BP, compared to resting baseline BP. Oscillometric BP was recorded approximately once per minute during tilting. Continuous BP was calculated as 1 min interval mean averages. During active standing, BP was measured by oscillometry. Definition of OH was a reduction of systolic BP of ≥20 mmHg (≥30 mmHg in patients with supine hypertension) or diastolic BP of ≥10 mmHg within 3 min of active standing or head-up tilt (Freeman et al., 2011). Delayed OH was an equivalent persistent drop in BP after 3 min of standing during tilt table testing. See Fig. 1 for examples of autonomic tests.

2.8. 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).

HRV was analyzed in the time and frequency domain. The time domain measures included HR (bpm), mean of R-R intervals (ms), standard deviation of NN interval time series (SDNN) (ms), and the root mean square of successive differences in the NN intervals (RMSSD) (ms).

The frequency domain spectrum estimates were low frequency (LF (ms^2), 0.04–0.15 Hz), high frequency (HF (ms^2) 0.15–0.4 Hz), coefficient of component variance (CCV)-LF (square root of LF/mean RR) and CCV-HF (square root of HF/mean RR), and LF/HF-ratio.

2.9. Statistical analysis

Data extraction and calculations was performed with Matlab 2021a, and statistical analysis was performed using Stata 15.1 (StataCorp, College Station, Texas).

Data are presented at as mean ± SD and median [IQR]. Continuous variables between T2D patients and the control group were compared using two-tailed unpaired student's *t*-test, and categorical variables were compared using Chi-squared test. Paired data are compared using students paired t-test. Normal distribution was determined using QQ-plots and the Shapiro-Wilk goodness-of-fit test. Non-normally distributed variables were either log-transformed or compared using Wilcoxon rank sum test as appropriate. Spearman's rank correlation was used to assess associations between sympathetic indices and 24-hour ABPM.

3. Results

In the combined cohort, the mean age ± SD was 65.2 (±7.7) years with 45 % males. Diabetes duration was 9.5 years (SD ±4.2). Clinical characteristics for the two groups are summarized in Table 1. Alcohol intake did not differ between the groups. Patients with T2D had significantly higher BMI, resting HR, resting and 24-hour systolic and diastolic BP and scored significantly higher on the Utah Early Neuropathy Scale. The time of day for testing did not have any significant impact on the autonomic outcomes.

Hypertensive patients pausing antihypertensive drugs for five half-lives for the experimental day, showed significantly higher systolic and diastolic BP compared to the day on inclusion (on antihypertensive treatment). Mean systolic/diastolic BP (±SD) on medication vs off medication was 137.7/84.5 mmHg (±12.5/9.7) mmHg vs 147/88 mmHg (±16.7/9.4) mmHg, $p < 0.05$ for both systolic and diastolic BP. Normotensive patients showed no difference in resting BP on the day of inclusion compared to the test-day ($p > 0.05$ for both systolic and diastolic BP). No difference in HR for neither group between days was found. A table of medicine paused prior to testing can be found in Supplementary Table 1.

3.1. Parasympathetic and mixed autonomic measures

The deep breathing test was performed adequately with no significant difference in expiratory volumes between the groups (mean ± SD, T2D 68.9 % ± 15.9 vs controls 75.3 % ± 17.1, $p = 0.09$), measured as proportion of vital lung capacity.

Data of autonomic measures are presented in Table 2. The E:I-ratio was found to be significantly lower in patients with T2D, compared to controls. Both absolute E:I difference and Valsalva ratio were lower in patients with T2D, albeit not significantly different from controls.

3.2. HRV measures

During a 10-minute rest period, RR interval, SDNN, RMSSD, CCV-LF, LF, HF and total power were significantly lower in patients with T2D compared with controls.

3.3. Sympathetic measures

Patients with T2D and control subjects performed the Valsalva maneuver with similar expiratory straining (median [IQR], T2D 37.6 mmHg [36.2–39.3] vs controls 37.4 mmHg [36.2–38.8], $p = 0.6$), and expiratory duration (median [IQR], T2D 15.6 s [15.5–16.1] vs controls 15.8 s [15.6–16], $p = 0.1$).

No significant difference in sympathetic Valsalva maneuver markers was found between the groups. We found no significant association between duration of T2D and any sympathetic markers.

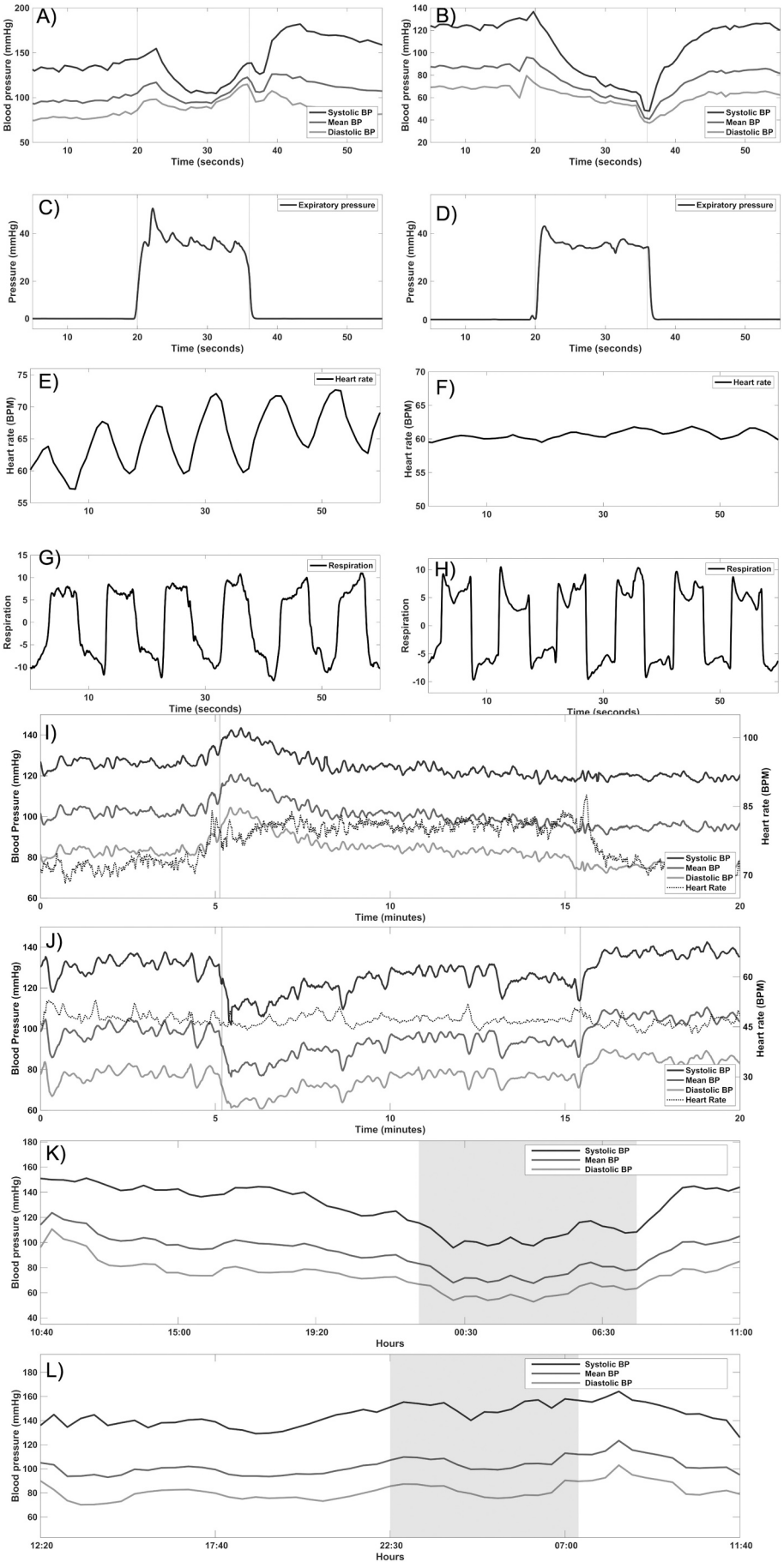

Fig. 1. Illustrative figures of autonomic testing showing (A) a normal BP response to the Valsalva maneuver in all phases, and an (B) abnormal response with a prolonged pressure recovery time of 15 s and absent phase 2 late and phase 4. The test was performed sufficiently in both the normal (C) and abnormal (D) response, blowing 40 mmHg for 15 s. For deep breathing, panel (E) shows a normal HR response to deep in- and expiration, while panel (F) shows an abnormal response, with a reduced inspiratory:expiratory ratio of 1.06. The test was sufficiently performed in both (G + H). A normal BP and HR response to tilt table testing is shown in panel (I), with an initial rise in BP, and relevant HR increase during tilting. Panel (J) shows an insufficient response, with a drop in BP of 30/18 mmHg indicating OH, and no relevant increase in HR suggesting cardiovagal dysfunction. Fig. (K) shows a normal 24-hour BP recording, with a relevant drop in BP during nighttime, whereas fig. (L) shows an abnormal recording, with a nocturnal rise in BP.

Table 1
General characteristics.

	Type 2 diabetes	Controls subjects	*p*-Value
n (%)	40 (50.0)	40 (50.0)	
Age (years), mean (SD)	65.5 (7.3)	64.8 (8.0)	0.67
Gender (male), n (%)	18 (45.0)	18 (45.0)	1.00
BMI (kg/m^2), mean (SD)	31.1 (6.5)	24.2 (3.5)	<0.001
HbA1C (mmol/mol), mean (SD)	51.6 (8.1)		
HbA1C (%), mean (SD)	6.9 (0.7)		
Diabetes duration (years), mean (SD)	9.5 (4.2)		
Receiving antihypertensive treatment, n (%)			
No treatment, n (%)	17 (42.5)	40 (100.0)	
With treatment, n (%)	23 (57.5)	0 (0.0)	<0.001
Tobacco use, n (%)			
Never, n (%)	21 (52.5)	28 (70.0)	
Currently, n (%)	4 (10.0)	2 (5.0)	
Previously, n (%)	15 (37.5)	10 (25.0)	0.26
Office sBP (mmHg), mean (SD)	141.0 (17.5)	129.4 (19.3)	0.01
Office dBP (mmHg), mean (SD)	84.7 (10.2)	78.0 (10.6)	0.01
Office resting HR (bpm), mean (SD)	70.3 (13.1)	56.7 (7.2)	<0.001
24-hour sBP (mmHg), mean (SD)	146.0 (15.0)	132.9 (15.5)	<0.001
24-hour dBP (mmHg), mean (SD)	81.4 (7.0)	77.7 (9.1)	0.04
Daytime sBP (mmHg), mean (SD)	149.9 (13.9)	138.9 (15.7)	0.001
Daytime dBP (mmHg), mean (SD)	83.9 (6.5)	81.7 (9.4)	0.24
Nighttime sBP (mmHg), mean (SD)	135.6 (21.0)	117.0 (16.8)	<0.001
Nighttime dBP (mmHg), mean (SD)	74.9 (11.2)	67.2 (9.4)	0.001
UENS total score, mean (SD)	7.5 (4.3)	3.3 (3.3)	<0.001

Values are the number of subjects (proportion) or means ± SD. sBP systolic blood pressure, dBP diastolic blood pressure, HR heart rate, UENS Utah Early Neuropathy Scale.

The mean reduction in BP during head-up tilt and active standing was not different. No participants presented with delayed OH during tilt table testing. No significant difference in prevalence of OH was found between the groups (T2D $n = 12$ vs controls $n = 7$, $p = 0.19$), oscillometric passive tilt. Pooling all participants, 75 % of all OH were in subjects aged ≥65 years (<65 years 11.4 % & ≥ 65 years 26.4 %, $p = 0.01$). We found no significant association between duration of T2D and any sympathetic markers or parasympathetic markers.

3.4. 24-hour ABPM

Differences in 24-hour ABPM indices are presented in Table 3. Patients with T2D were found to have increased systolic and diastolic nighttime SD, and decreased systolic 24-hour and daytime CV, compared to controls. They had smaller nocturnal BP decreases, with a higher prevalence of reverse dipping compared to controls.

Mean drop in continuous systolic BP during tilt table testing was significantly correlated to 24-hour and daytime systolic and diastolic SD and CV. No other sympathetic, parasympathetic and 24-hour ABPM measures were significantly correlated. See Supplementary Table 2.

Total COMPASS 31 scores differed significantly between the groups (median [IQR]) T2D 24.6 [20.7–35.3] vs controls 4.0 [1.6–9.9] $p < 0.001$. The orthostatic domain of the COMPASS 31 differed significantly between the groups, when calculated as a continuous variable ($p = 0.03$), although when dichotomizing the orthostatic domain as a categorical variable (orthostatic score 0 vs. >0), no significant difference was found (T2D 40 % vs controls 20,5 % ($p = 0.06$)).

An additional exploratory analysis was performed grouping all participants reporting orthostatic symptoms (COMPASS orthostatic domain >0) comparing autonomic measures to participants without orthostatic symptoms. No difference was found between participants reporting symptoms in the orthostatic domain of COMPASS 31 when comparing autonomic outcomes. Values are presented in Supplementary Table 3.

3.5. Data and resource availability

The data generated and analyzed during this study are available from the corresponding author upon reasonable request.

4. Discussion

The main finding of this study was no significant group differences in cardiovascular neuronal measures of sympathetic function in our cohort of T2D patients compared with age- and gender matched control subjects, despite a significantly higher reporting of orthostatic symptoms on the orthostatic domain on the COMPASS 31 questionnaire. Expectedly, parasympathetic function was reduced in T2D patients, measured as higher resting HR and reduced deep breathing and HRV indices. Patients with T2D had lower night dipping BP with a significantly higher frequency of reverse dipping pattern. We found no significant correlations between sympathetic measures of the Valsalva maneuver and 24-hour ABPM. Thus, sympathetically mediated changes in continuous BP and diurnal changes in 24-hour BP are complementary and not substitutable.

Indices from the Valsalva maneuver have previously been used to assess the integrity of sympathetic neuronal regulation in T2D patients. Low et al. found a sympathetic dysregulation in 48 % of T2D patients in a population-based cohort, when stratifying adrenergic function according to the composite autonomic severity score (Low et al., 2004). Although our findings are measured as continuous variables, we were not able to find any significant impairment in sympathetic function, when comparing directly to age- and gender-matched control subjects. We calculated grouped differences as continuous values. This was chosen to preserve the resolution of our data in spite of the relatively low number of subjects, and although it may hinder the direct clinical transferability and comparability of our findings, this approach was considered most appropriate to assess the magnitude of sympathetic impairment in T2D patients. Comparing the incidence of sympathetic dysregulation on the composite autonomic severity score between T2D patients and controls subjects did not reveal any difference between the groups (data not shown).

Cardiovascular sympathetic function in our cohort of T2D patients was comparable to the sympathetic function of controls subjects, despite unfavorable clinical profiles (higher BMI and higher BP) associated with the development of cardiovascular autonomic neuropathy (Andersen et al., 2018), as well as significant parasympathetic impairment and a higher Utah Early Neuropathy score, indicative of small fiber neuropathy.

We found significant differences in 24-hour BP indices with T2D patients having significantly higher 24-hour systolic and diastolic BP during day- and nighttime and reduced systolic BP night dipping. More T2D patients showed a reverse nocturnal dipping pattern. The differences in absolute 24-hour BP measures are expected, as control subjects were excluded if they took any medication or had any diseases (e.g., hypertension) whereas T2D patients were not excluded upon the presence of comorbidities. A total of 23 T2D patients received treatment for hypertension. Despite these underlying group disparities, we only found minor differences in 24-hour BP between the groups – specifically, an increase in nighttime systolic SD for T2D patients, and a significantly larger 24-hour and daytime CV in the controls, although the absolute differences were small. Nocturnal BP drop was reduced in T2D patients, with a higher prevalence of reverse dipping. Nocturnal dysregulation is a combined product of both humoral and neuronal influences (Tuck et al., 1985), and sympathetic nerve fibers have been found to play a pivotal role in the regulation of nocturnal BP (Sherwood et al., 2002). Studies investigating the applicability of 24-hour ABPM indices as a measure for autonomic dysfunction in T2D have focused mainly on the

Table 2
Clinical autonomic outcomes.

Parasympathetic and mixed autonomic measures			
	Type 2 diabetics	Control subjects	*p*-Value
Inspiratory-expiratory HR difference (bpm)*	7.93 [5.91–12.07]	10.06 [7.70–15.54]	0.11
Inspiratory-expiratory ratio†	1.11 [1.08–1.18]	1.18 [1.12–1.27]	0.01
Valsalva ratio	1.60 (0.33)	1.73 (0.32)	0.08
Heart rate variability measures			
RR interval (ms)	882.9 (158.8)	1082.3 (126.9)	<0.001
SDNN interval (ms)*	20.0 [13.0–29.8]	33.8 [20.7–39.7]	0.002
RMSSD (ms)*	16.7 [12.6–29.7]	29.2 [18.9–40.5]	0.004
LF (ms2)*	192.4 [74.2–292.3]	616.6 [229.6–950.6]	<0.001
CCV-LF (%)*	0.0 [0.0–0.0]	0.0 [0.0–0.0]	0.012
LF (normalized units)†	65.7 [49.8–72.1]	69.9 [61.3–79.1]	0.134
HF (ms2)*	89.1 [44.5–284.1]	214.6 [99.9–511.2]	0.011
CCV-HF (%)*	0.0 [0.0–0.0]	0.0 [0.0–0.0]	0.241
HF (normalized units)*	34.3 [27.9–50.2]	30.0 [20.9–38.6]	0.138
LF/HF (ratio)†	1.9 [1.0–2.6]	2.3 [1.6–3.8]	0.134
Total power (ms2)*	356.9 [153.2–688.7]	1072.0 [404.9–1517.1]	0.001
Sympathetic measures			
Phase 2 late sBP rise (mmHg) (SI 2)†	13.92 (12.58)	15.67 (13.71)	0.55
Total recovery (SI 3)	0.84 [−10.93–9.38]	−9.21 [−16.44–10.24]	0.40
Phase 4 sBP overshoot (mmHg) (SI 4)†	24.59 (15.47)	24.17 (16.42)	0.91
Pressure recovery time (seconds) (SI 5)†	2.86 [1.05–4.10]	1.96 [0.79–4.45]	0.77
Mean continuous sBP drop during tilting (mmHg)	−2.60 [−6.58–0.59]	2.10 [−4.20–6.70]	0.02
Max oscillatory sBP drop during tilting (mmHg)	3.96 (9.30)	4.55 (6.64)	0.74
Bedside orthostatic sBP drop (mmHg)	13.43 (14.31)	10.85 (14.07)	0.42

Values are reported as mean (±SD) or median [IQR], *Log-transformed, †Wilcoxon rank sum test ‡ During deep breathing test, HR Heart rate, SDNN Standard deviation of NN intervals, RMSSD Root Mean Square of the Successive Differences, LFnu Low frequency normalized units, HFnu High frequency normalized units, sBP systolic blood pressure, dBP diastolic blood pressure.

association between 24-hour BP and mixed parasympathetic and sympathetic outcomes, which have yielded low correlations (Cardoso et al., 2008; Spallone et al., 1993). Lohdi et al. reported an association between increased daytime systolic BP SD and CV with presence of sympathetic autonomic dysfunction (Lodhi et al., 2019). We did not find differences between these specific indices, but found significant correlations between mean drop in systolic BP during tilt table testing, and daytime and 24-hour CV and SD. These findings suggest that increased variability measured with 24-hour ABPM might be indicative of orthostatism, although no other sympathetic markers were significantly correlated. Further exploration of these correlations is thus warranted.

We found a marked reduction in the nocturnal BP drop in T2D patients. Reduced nocturnal BP dipping is associated with abnormal parasympathetic function and OH (Costa et al., 2016). A study in multiple system atrophy and Parkinson patients found associations between impaired sympathetic function and nocturnal BP dipping (Fanciulli et al., 2014). Our findings of preserved cardiovascular sympathetic function, measured through the Valsalva maneuver and tilt table testing, and the lack of significant correlations between 24-hour ABPM or any and any other sympathetic markers, suggest that abnormal nocturnal BP regulation could either be an early marker of reduced sympathetic function, or independent of sympathetic dysfunction. Alternatively, different pathophysiological mechanisms for different diseases associated with autonomic dysfunction may explain discrepancy in previous findings, as Parkinson can affect central autonomic regulatory centers (Chen et al., 2020), whereas T2D primarily affects peripheral nerves in a length-dependent manner (Feldman et al., 2019). To address this, patients with a wider spectrum of severity of sympathetic dysfunction need to be assessed.

Orthostatic BP did not differ significantly between the groups despite a reported prevalence of OH of 30 % and 17.5 % for T2D and control subjects respectively. The lack of significance may be due to insufficient power in this study. Although our sample is relatively small, these numbers are in accordance with previous findings in both community dwelling subjects, and patients with T2D (Saedon et al., 2020; Atli and Keven, 2006; Zhou et al., 2017). Generally, OH is considered to present in the later stages of diabetic autonomic neuropathy, and indicate an advanced neuropathic disease state, although no longitudinal studies have investigated this assumption. In the assessment of diabetic autonomic neuropathy, the presence of OH is often interpreted as the sole marker of sympathetic dysfunction (Bernardi et al., 2011). Our findings suggest that despite a tendency of OH being more prevalent in T2D, conclusions regarding presence and/or advancement of autonomic neuropathy based solely on OH, ought to be carefully considered as a notable percentage of non-diabetic subjects may also show OH. Passive tilting is often used for the evaluation of autonomic function, and is considered a purely sympathetic measure (Cheshire and Goldstein, 2019). We found no differences in hemodynamic responses during head up tilting, in line with the above-mentioned findings for active standing BP responses.

Despite no impairment in cardiovascular sympathetic autonomic responses, T2D patients scored significantly higher on the orthostatic domain of COMPASS 31, which measures subjective symptoms of orthostatism. COMPASS 31 is a screening tool for identification of specific autonomic domain insufficiencies; however, previous studies found low correlations to reported symptoms and quantitative testing, including for orthostatic symptoms (Treister et al., 2015). This finding is not novel, as similar low correlations between self-reported symptoms and measures of autonomic function have been reported (Low et al., 2004), however no explanation for this discordance has been put forward. It may be hypothesized that these differences in measures can in part be explained by the temporal aspect of the orthostatic COMPASS questioning, as it covers any symptoms experienced within the last year, whereas the quantitative autonomic testing and hemodynamic recordings are conducted over a span of ~2 h. Under normal circumstances, the hemodynamic balance is intricately regulated to withstand a myriad of both external and internal influences. Factors such as inappropriate HR regulation, fluid depletion, increased prevalence of postprandial hypotension (Hashizume et al., 2019), or other potential conditions associated with T2D, may account for the increased reporting of orthostatic symptoms. Food, fluid and medication intake is standardized during autonomic testing, and augmented influences hereof may therefore go unnoticed during autonomic testing.

In line with previous studies (Subbalakshmi et al., 2014; Sucharita et al., 2011), we found that patients with T2D had affected parasympathetic capabilities, measured specifically as reduced E:I HR-ratio to deep breathing, and reduced HRV indices. Despite the widespread use of HRV in the evaluation of autonomic function, the calculated measures are mainly indicative of either parasympathetic or of mixed sympathetic/parasympathetic function (Reyes del Paso et al., 2013).

4.1. Strengths and limitations

Compliance regarding the performance of autonomic testing is known to significantly influence the hemodynamic outcomes (Rasmussen et al., 2017). In our study, expiratory performance parameters during autonomic testing were recorded and compared to ensure equal exertions between the groups, and all testing was instructed and supervised by the same investigator. Furthermore, external factors known

Table 3
24-hour ambulatory blood pressure.

	Type 2 diabetics	Control subjects	*p*-Value
24 h SD sBP	20.55 (4.04)	22.53 (6.42)	0.1
Daytime SD sBP*	17.92 [15.65–24.19]	20.75 [16.75–27.81]	0.13
Nighttime SD sBP*	14.14 [12.38–18.23]	12.11 [8.87–16.36]	0.01
24 h SD dBP	15.59 (4.23)	16.52 (5.32)	0.39
Daytime SD dBP	15.19 (5.05)	16.57 (6.27)	0.28
Nighttime SD dBP*	10.54 [7.94–14.56]	8.06 [5.76–11.35]	0.01
24 h sCV*	0.13 [0.11–0.17]	0.16 [0.14–0.20]	0.004
Daytime sCV*	0.12 [0.10–0.17]	0.15 [0.12–0.19]	0.01
Nighttime sCV	0.11 (0.03)	0.11 (0.03)	0.42
24 h dCV	0.19 (0.05)	0.21 (0.07)	0.15
Daytime dCV	0.18 (0.06)	0.20 (0.08)	0.19
Nighttime dCV*	0.16 [0.11–0.20]	0.13 [0.09–0.16]	0.08
Night sBP drop (mean % of daytime sBP)	9.77 (8.84)	15.75 (7.67)	0.002
Night dBP drop (mean % of daytime dBP)	10.80 (10.09)	17.64 (7.72)	0.001
Dipping, n (%)			
Normal dipping, n (%)	23 (57.5)	31 (77.5)	
No dipping, n (%)	8 (20.0)	8 (20.0)	
Reverse dipping, n (%)	9 (22.5)	1 (2.5)	0.02

Values are reported as mean (±SD), median [IQR] or number (%), *Log-transformed. sBP systolic blood pressure, dBP diastolic blood pressure, sCV systolic coefficient of variation, dCV diastolic coefficient of variation.

to influence autonomic testing (medication, time since last meal, caffeine, etc.), was controlled for in our study (Low, 2003).

It is worth noting that despite our finding of no significant impairment in neuronally regulated cardiovascular measures of sympathetic function in T2D patients, we cannot rule out the possibility of a type II error either due to the low number of subjects, insufficient sensitivity of the applied methodology, or the combination of both. Although no averaged group difference was found, we cannot rule out the possibility of specific individuals being susceptible to sympathetic neuropathy, which would be drowned out by analyzing mean point estimates. An analysis revealed no significant differences between the groups when comparing upper and lower centiles and quartiles of sympathetic markers (data not presented). Control subjects were not formally screened for T2D when entering the study, and inclusion of subjects with unrecognized T2D in the control group can therefore not be ruled out. The control subjects were normotensive with a BMI of only 24.2 and thus less likely to have metabolic syndrome or T2D. Additionally, based on improved diagnostic activity over the last decade in Denmark the prevalence of non-diagnosed diabetes is low with estimated 0.6–0.8 % undiagnosed T2D patients in the aged 20–85 years (Jørgensen et al., 2020; Bruun-Rasmussen et al., 2020). Therefore, although not tested, cases of undiagnosed T2D in our control cohort is unlikely. Control subjects had an UENS score of 3.3. Although significantly lower than in patients T2D, we cannot rule out that this could represent a minor state of neuropathy in our control subjects. However, previous studies utilizing the UENS in subjects without neuropathy, have presented scores comparable to our findings (Boger et al., 2012; Gylfadottir et al., 2020b).

Patients with history of hypertensive emergency were excluded from this study. Although this criterion was considered reasonable for safety-concerns, we cannot rule out it may have disproportionately excluded patients with marked autonomic failure.

5. Conclusion

In conclusion, our population of T2D patients presented with reduced parasympathetic function measured as higher resting HR, impaired HR responses to deep breathing, and reduced HRV. We found preserved cardiovascular sympathetic function in our cohort, despite T2D patients reporting significantly more orthostatic symptoms on the orthostatic domain of COMPASS 31 and despite altered 24-hour BP regulation with reduced nocturnal dipping. We found no significant correlation between 24-hour ABPM and sympathetic markers based on continuous BP. Thus, careful consideration should be taken when making inferences regarding sympathetic dysfunction based solely on 24-hour ABPM indices, in T2D patients. Our findings are indicative of reduced parasympathetic function, but preserved sympathetic function in our T2D cohort.

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Prior presentation

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Data availability

Data will be made available on request.

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T.K.R. designed the study, collected the data, performed the statistical analyses, wrote the first draft, and contributed to the discussion. A. J.T. designed the study, contributed to the discussion, and reviewed and edited the manuscript. N.B.F., T.S.J., J.H., and W. S. contributed to the discussion, and reviewed and edited the manuscript. All authors approved the final manuscript. T.K.R. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.autneu.2022.103026.

References

Andersen, S.T., Witte, D.R., Fleischer, J., Andersen, H., Lauritzen, T., Jorgensen, M.E., Jensen, T.S., Pop-Busui, R., Charles, M., 2018. Risk factors for the presence and progression of cardiovascular autonomic neuropathy in type 2 diabetes: ADDITION-Denmark. Diabetes Care 41, 2586–2594.

Atli, T., Keven, K., 2006. Orthostatic hypotension in the healthy elderly. Arch. Gerontol. Geriatr. 43, 313–317.

Bernardi, L., Spallone, V., Stevens, M., Hilsted, J., Frontoni, S., Pop-Busui, R., Ziegler, D., Kempler, P., Freeman, R., Low, P., Tesfaye, S., Valensi, P., Toronto Consensus Panel on Diabetic, N., 2011. Methods of investigation for cardiac autonomic dysfunction in human research studies. Diabetes Metab. Res. Rev. 27, 654–664.

Boger, M.S., Hulgan, T., Haas, D.W., Mitchell, V., Smith, A.G., Singleton, J.R., Peltier, A. C., 2012. Measures of small-fiber neuropathy in HIV infection. Auton. Neurosci. 169, 56–61.

Bruun-Rasmussen, N.E., Napolitano, G., Kofoed-Enevoldsen, A., Bojesen, S.E., Ellervik, C., Rasmussen, K., Jepsen, R., Lynge, E., 2020. Burden of prediabetes, undiagnosed, and poorly or potentially sub-controlled diabetes: Lolland-Falster health study. BMC Public Health 20.

Cardoso, C.R., Leite, N.C., Freitas, L., Dias, S.B., Muxfeld, E.S., Salles, G.F., 2008. Pattern of 24-hour ambulatory blood pressure monitoring in type 2 diabetic patients with cardiovascular dysautonomy. Hypertens. Res. 31, 865–872.

Chen, Z., Li, G., Liu, J., 2020. Autonomic dysfunction in Parkinson's disease: implications for pathophysiology, diagnosis, and treatment. Neurobiol. Dis. 134, 104700.
Cheshire, W.P., Goldstein, D.S., 2019. Autonomic uprising: the tilt table test in autonomic medicine. Clin. Auton. Res. 29, 215–230.
Cheshire, W.P., Freeman, R., Gibbons, C.H., Cortelli, P., Wenning, G.K., Hilz, M.J., Spies, J.M., Lipp, A., Sandroni, P., Wada, N., Mano, A., Ah Kim, H., Kimpinski, K., Iodice, V., Idiáquez, J., Thaisetthawatkul, P., Coon, E.A., Low, P.A., Singer, W., 2021. Electrodiagnostic assessment of the autonomic nervous system: a consensus statement endorsed by the american autonomic society, American Academy of Neurology, and the International Federation of Clinical Neurophysiology. Clin. Neurophysiol. 132, 666–682.
Chowdhury, M., Nevitt, S., Eleftheriadou, A., Kanagala, P., Esa, H., Cuthbertson, D.J., Tahrani, A., Alam, U., 2021. Cardiac autonomic neuropathy and risk of cardiovascular disease and mortality in type 1 and type 2 diabetes: a meta-analysis. BMJ Open Diabetes Res. Care 9.
Costa, A., Bosone, D., Ramusino, M.C., Ghiotto, N., Guaschino, E., Zoppi, A., D'angelo, A., Fogari, R., 2016. Twenty-four-hour blood pressure profile, orthostatic hypotension, and cardiac dysautonomia in elderly type 2 diabetic hypertensive patients. Clin. Auton. Res. 26, 433–439.
Fanciulli, A., Strano, S., Ndayisaba, J.P., Goebel, G., Gioffrè, L., Rizzo, M., Colosimo, C., Caltagirone, C., Poewe, W., Wenning, G.K., Pontieri, F.E., 2014. Detecting nocturnal hypertension in Parkinson's disease and multiple system atrophy: proposal of a decision-support algorithm. J. Neurol. 261, 1291–1299.
Fanciulli, A., Jordan, J., Biaggioni, I., Calandra-Buonaura, G., Cheshire, W.P., Cortelli, P., Eschlboeck, S., Grassi, G., Hilz, M.J., Kaufmann, H., Lahrmann, H., Mancia, G., Mayer, G., Norcliffe-Kaufmann, L., Pavy-Le Traon, A., Raj, S.R., Robertson, D., Rocha, I., Struhal, W., Thijs, R., Tsioufis, K.P., Van Dijk, J.G., Wenning, G.K., 2018. Consensus statement on the definition of neurogenic supine hypertension in cardiovascular autonomic failure by the American Autonomic Society (AAS) and the European Federation of Autonomic Societies (EFAS). Clin. Auton. Res. 28, 355–362.
Fedorowski, A., Stavenow, L., Hedblad, B., Berglund, G., Nilsson, P.M., Melander, O., 2010. Orthostatic hypotension predicts all-cause mortality and coronary events in middle-aged individuals (The malmo preventive Project). Eur. Heart J. 31, 85–91.
Feldman, E.L., Callaghan, B.C., Pop-Busui, R., Zochodne, D.W., Wright, D.E., Bennett, D. L., Bril, V., Russell, J.W., Viswanathan, V., 2019. Diabetic neuropathy. Nat. Rev. Dis. Primers. 5, 41.
Fleg, J.L., Evans, G.W., Margolis, K.L., Barzilay, J., Basile, J.N., Bigger, J.T., Cutler, J.A., Grimm, R., Pedley, C., Peterson, K., Pop-Busui, R., Sperl-Hillen, J., Cushman, W.C., 2016. Orthostatic hypotension in the ACCORD (Action to control cardiovascular risk in Diabetes) blood pressure trial. Hypertension 68, 888–895.
Freeman, R., Chapleau, M.W., 2013. Testing the autonomic nervous system. Handb. Clin. Neurol. 115, 115–136.
Freeman, R., Wieling, W., Axelrod, F.B., Benditt, D.G., Benarroch, E., Biaggioni, I., Cheshire, W.P., Chelimsky, T., Cortelli, P., Gibbons, C.H., Goldstein, D.S., Hainsworth, R., Hilz, M.J., Jacob, G., Kaufmann, H., Jordan, J., Lipsitz, L.A., Levine, B.D., Low, P.A., Mathias, C., Raj, S.R., Robertson, D., Sandroni, P., Schatz, I., Schondorff, R., Stewart, J.M., Van Dijk, J.G., 2011. Consensus statement on the definition of orthostatic hypotension, neurally mediated syncope and the postural tachycardia syndrome. Clin. Auton. Res. 21, 69–72.
Gibbons, C.H., Schmidt, P., Biaggioni, I., Frazier-Mills, C., Freeman, R., Isaacson, S., Karabin, B., Kuritzky, L., Lew, M., Low, P., Mehdirad, A., Raj, S.R., Vernino, S., Kaufmann, H., 2017. The recommendations of a consensus panel for the screening, diagnosis, and treatment of neurogenic orthostatic hypotension and associated supine hypertension. J. Neurol. 264, 1567–1582.
Goldstein, D.S., Sharabi, Y., 2009. Neurogenic orthostatic hypotension. Circulation 119, 139–146.
Guyenet, P.G., 2006. The sympathetic control of blood pressure. Nat. Rev. Neurosci. 7, 335–346.
Gylfadottir, S.S., Christensen, D.H., Nicolaisen, S.K., Andersen, H., Callaghan, B.C., Itani, M., Khan, K.S., Kristensen, A.G., Nielsen, J.S., Sindrup, S.H., Andersen, N.T., Jensen, T.S., Thomsen, R.W., Finnerup, N.B., 2020a. Diabetic polyneuropathy and pain, prevalence, and patient characteristics: a cross-sectional questionnaire study of 5,514 patients with recently diagnosed type 2 diabetes. Pain 161, 574–583.
Gylfadottir, S.S., Itani, M., Krøigård, T., Kristensen, A.G., Christensen, D.H., Nicolaisen, S. K., Karlsson, P., Callaghan, B.C., Bennett, D.L., Andersen, H., Tankisi, H., Nielsen, J. S., Andersen, N.T., Jensen, T.S., Thomsen, R.W., Sindrup, S.H., Finnerup, N.B., 2020b. Diagnosis and prevalence of diabetic polyneuropathy: a cross-sectional study of danish patients with type 2 diabetes. Eur. J. Neurol. 27, 2575–2585.
Hashizume, M., Kinami, S., Sakurai, K., Izawa, K.P., Shiotani, H., 2019. Postprandial blood pressure decrease in patients with type 2 diabetes and mild or severe cardiac autonomic dysfunction. Int. J. Environ. Res. Public Health 16.
Jensen, T.S., Karlsson, P., Gylfadottir, S.S., Andersen, S.T., Bennett, D.L., Tankisi, H., Finnerup, N.B., Terkelsen, A.J., Khan, K., Themistocleous, A.C., Kristensen, A.G., Itani, M., Sindrup, S.H., Andersen, H., Charles, M., Feldman, E.L., Callaghan, B.C., 2021. Painful and non-painful diabetic neuropathy, diagnostic challenges and implications for future management. Brain 144, 1632–1645.
Jørgensen, M.E., Ellervik, C., Ekholm, O., Johansen, N.B., Carstensen, B., 2020. Estimates of prediabetes and undiagnosed type 2 diabetes in Denmark: the end of an epidemic or a diagnostic artefact? Scand. J. Public Health 48, 106–112.
Lodhi, H.A., Peri-Okonny, P.A., Schesing, K., Phelps, K., Ngo, C., Evans, H., Arbique, D., Price, A.L., Vernino, S., Phillips, L., Mitchell, J.H., Smith, S.A., Yano, Y., Das, S.R., Wang, T., Vongpatanasin, W., 2019. Usefulness of blood pressure variability indices derived from 24-hour ambulatory blood pressure monitoring in detecting autonomic failure. J. Am. Heart Assoc. 8, e010161.
Low, P.A., 2003. Testing the autonomic nervous system. Semin. Neurol. 23, 407–421.
Low, P.A., Benrud-Larson, L.M., Sletten, D.M., Opfer-Gehrking, T.L., Weigand, S.D., O'brien, P.C., Suarez, G.A., Dyck, P.J., 2004. Autonomic symptoms and diabetic neuropathy: a population-based study. Diabetes Care 27, 2942–2947.
Milazzo, V., Di Stefano, C., Vallelonga, F., Sobrero, G., Zibetti, M., Romagnolo, A., Merola, A., Milan, A., Espay, A.J., Lopiano, L., Veglio, F., Maule, S., 2018. Reverse blood pressure dipping as marker of dysautonomia in parkinson disease. Parkinsonism Relat. Disord. 56, 82–87.
Novak, P., 2011. Assessment of sympathetic index from the valsalva maneuver. Neurology 76, 2010–2016.
Pop-Busui, R., Evans, G.W., Gerstein, H.C., Fonseca, V., Fleg, J.L., Hoogwerf, B.J., Genuth, S., Grimm, R.H., Corson, M.A., Prineas, R., 2010. Effects of cardiac autonomic dysfunction on mortality risk in the action to control cardiovascular risk in diabetes (ACCORD) trial. Diabetes Care 33, 1578–1584.
Rasmussen, T.K., Hansen, J., Low, P.A., Sandroni, P., Singer, W., Jensen, T.S., Terkelsen, A.J., 2017. Autonomic function testing: compliance and consequences. Auton. Neurosci. 208, 150–155.
Reyes Del Paso, G.A., Langewitz, W., Mulder, L.J., Van Roon, A., Duschek, S., 2013. The utility of low frequency heart rate variability as an index of sympathetic cardiac tone: a review with emphasis on a reanalysis of previous studies. Psychophysiology 50, 477–487.
Rothwell, P.M., Howard, S.C., Dolan, E., O'brien, E., Dobson, J.E., Dahlöf, B., Sever, P.S., Poulter, N.R., 2010. Prognostic significance of visit-to-visit variability, maximum systolic blood pressure, and episodic hypertension. Lancet 375, 895–905.
Saedon, N.I.Z., Pin Tan, M., Frith, J., 2020. The prevalence of orthostatic hypotension: a systematic review and meta-analysis. J. Gerontol. A Biol. Sci. Med. Sci. 75, 117–122.
Salmanpour, A., Frances, M.F., Goswami, R., Shoemaker, J.K., 2011. Sympathetic neural recruitment patterns during the valsalva maneuver. Annu. Int. Conf. IEEE Eng. Med. Biol. Soc. 2011, 6951–6954.
Sandroni, P., Benarroch, E.E., Low, P.A., 1991. Pharmacological dissection of components of the valsalva maneuver in adrenergic failure. J. Appl. Physiol. 1985 (71), 1563–1567.
Sandroni, P., Novak, V., Opfer-Gehrking, T.L., Huck, C.A., Low, P.A., 2000. Mechanisms of blood pressure alterations in response to the valsalva maneuver in postural tachycardia syndrome. Clin. Auton. Res. 10, 1–5.
Sherwood, A., Steffen, P.R., Blumenthal, J.A., Kuhn, C., Hinderliter, A.L., 2002. Nighttime blood pressure dipping: the role of the sympathetic nervous system. Am. J. Hypertens. 15, 111–118.
Sletten, D.M., Suarez, G.A., Low, P.A., Mandrekar, J., Singer, W., 2012. COMPASS 31: a refined and abbreviated composite autonomic symptom score. Mayo Clin. Proc. 87, 1196–1201.
Spallone, V., Bernardi, L., Ricordi, L., Solda, P., Maiello, M.R., Calciati, A., Gambardella, S., Fratino, P., Menzinger, G., 1993. Relationship between the circadian-rhythms of blood-pressure and sympathovagal balance in diabetic autonomic neuropathy. Diabetes 42, 1745–1752.
Subbalakshmi, N.K., Adhikari, P., Shanmugavel Jeganathan, P., 2014. Comparative study on cardiac autonomic modulation during deep breathing test and diaphragmatic breathing in type 2 diabetes and healthy subjects. J. Diabetes Investig. 5, 456–463.
Sucharita, S., Bantwal, G., Idiculla, J., Ayyar, V., Vaz, M., 2011. Autonomic nervous system function in type 2 diabetes using conventional clinical autonomic tests, heart rate and blood pressure variability measures. Indian J. Endocrinol. Metab. 15, 198–203.
Tahrani, A.A., Dubb, K., Raymond, N.T., Begum, S., Altaf, Q.A., Sadiqi, H., Piya, M.K., Stevens, M.J., 2014. Cardiac autonomic neuropathy predicts renal function decline in patients with type 2 diabetes: a cohort study. Diabetologia 57, 1249–1256.
Tarvainen, M.P., Niskanen, J.P., Lipponen, J.A., Ranta-Aho, P.O., Karjalainen, P.A., 2014. Kubios HRV–heart rate variability analysis software. Comput. Methods Prog. Biomed. 113, 210–220.
Thijs, R.D., Brignole, M., Falup-Pecurariu, C., Fanciulli, A., Freeman, R., Guaraldi, P., Jordan, J., Habek, M., Hilz, M., Traon, A.P.-L., Stankovic, I., Struhal, W., Sutton, R., Wenning, G., Van Dijk, J.G., 2021. Recommendations for tilt table testing and other provocative cardiovascular autonomic tests in conditions that may cause transient loss of consciousness. Clin. Auton. Res. 31, 369–384.
Treister, R., O'neil, K., Downs, H.M., Oaklander, A.L., 2015. Validation of the composite autonomic symptom scale 31 (COMPASS-31) in patients with and without small fiber polyneuropathy. Eur. J. Neurol. 22, 1124–1130.
Tuck, M.L., Stern, N., Sowers, J.R., 1985. Enhanced 24-hour norepinephrine and renin secretion in young patients with essential hypertension: relation with the circadian pattern of arterial blood pressure. Am. J. Cardiol. 55, 112–115.
Van Hateren, K.J.J., Kleefstra, N., Blanker, M.H., Ubink-Veltmaat, L.J., Groenier, K.H., Houweling, S.T., Kamper, A.M., Van Der Meer, K., Bilo, H.J.G., 2012. Orthostatic hypotension, diabetes, and falling in older patients: a cross-sectional study. Br. J. Gen. Pract. 62, e696.
Vogel, E.R., Sandroni, P., Low, P.A., 2005. Blood pressure recovery from valsalva maneuver in patients with autonomic failure. Neurology 65, 1533–1537.
Yoshinari, M., Wakisaka, M., Nakamura, U., Yoshioka, M., Uchizono, Y., Iwase, M., 2001. Orthostatic hypertension in patients with type 2 diabetes. Diabetes Care 24, 1783–1786.

Yun, J.S., Park, Y.M., Cha, S.A., Ahn, Y.B., Ko, S.H., 2018. Progression of cardiovascular autonomic neuropathy and cardiovascular disease in type 2 diabetes. Cardiovasc. Diabetol. 17, 109.

Zhou, Y., Ke, S.-J., Qiu, X.-P., Liu, L.-B., 2017. Prevalence, risk factors, and prognosis of orthostatic hypotension in diabetic patients: a systematic review and meta-analysis. Medicine 96.

Zoppini, G., Cacciatori, V., Raimondo, D., Gemma, M., Trombetta, M., Dauriz, M., Brangani, C., Pichiri, I., Negri, C., Stoico, V., Bergamini, C., Targher, G., Santi, L., Thomaseth, K., Bellavere, F., Bonadonna, R.C., Bonora, E., 2015. Prevalence of cardiovascular autonomic neuropathy in a cohort of patients with newly diagnosed type 2 diabetes: the Verona newly diagnosed type 2 diabetes study (VNDS). Diabetes Care 38, 1487–1493.