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ARTICLE

Pulse pressure amplification and cardiac autonomic dysfunction in patients with type 2 diabetes mellitus

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Abstract

The main aim of this cross-sectional study was to investigate the association between pulse pressure amplification (PPA) and cardiac autonomic activity (baroreflex sensitivity (BRS) and heart rate variability (HRV)) in patients with type 2 diabetes mellitus (T2DM). In addition, we examined the association between cardiac autonomic activity and central hemodynamic parameters that may affect PPA such as augmentation index (AIx), aortic stiffness (pulse wave velocity (PWV)), and common carotid artery stiffness distensibility coefficient (DC). A total of 142 patients with T2DM were included in the study. In multivariate linear regression analysis—after controlling for age, diabetes duration, height, waist circumference, aortic PWV, use of β-blockers, and BRS—PPA was associated significantly and independently with male gender (standardized regression coefficient (β) = 0.156, $p = 0.007$), aortic systolic blood pressure ($\beta = -0.221$, $p < 0.001$), heart rate ($\beta = 0.521$, $p < 0.001$), AIx ($\beta = -0.443$, $p < 0.001$), and parameters of HRV, such as total power of HRV ($\beta = -0.157$, $p = 0.005$). No significant associations were found between BRS or parameters of HRV with aortic PWV, AIx, or DC. In patients with T2DM, cardiac autonomic dysfunction was associated with enhanced PPA. This association was independent from the well-described effect of resting heart rate, as well as from traditional cardiovascular risk factors or diabetes-related factors. Moreover, it was not mediated by effects of the autonomic dysfunction on arterial stiffness or on pressure wave reflections. These findings suggest that cardiac autonomic dysfunction affects PPA by mechanisms other than resting tachycardia and arterial properties.

Introduction

Type 2 diabetes mellitus (T2DM) is a common chronic metabolic disease associated with increased cardiovascular (CV) risk and mortality [1]. Hypertension is one of the modifiable CV risks factors, conventionally accessed via peripheral blood pressure (BP) measurements. However, accumulating evidence suggests that central vascular hemodynamics may better correlate with overall CV risk compared to traditional peripheral BP measurements in patients with and without T2DM [2–4]. More specifically, pulse pressure (PP) amplification (PPA) is an emerging biomarker of CV risk stratification that predicts CV mortality over and above peripheral and central BP measurements [5].

PPA reflects the disparity between central and peripheral BP (normally PP is higher in peripheral than in central arteries for a similar mean arterial BP (MBP)) and is mostly dependent on arterial stiffness and wave reflections [6]. Moreover, PPA can be affected by several factors such as age, gender, heart rate, height, and arterial BP [6]. Increased PPA has been reported in the presence of the metabolic syndrome and diabetes and this has been attributed mostly to the increased heart rate observed in these patients [6–9].

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Cardiac autonomic neuropathy (CAN) in diabetes is an increasingly recognized multifaceted syndrome characterized by functional dysregulation of the parasympathetic and sympathetic tone of the heart and blood vessels that increases mortality risk up to 50% [10, 11]. Resting tachycardia and altered beat-to-beat variability are some of the earliest manifestations of CAN and can readily be accessed by the use of baroreflex sensitivity (BRS) and heart rate variability (HRV) [10].

Although increased heart rate has been associated with increased PPA in insulin-resistant states [7, 8, 12], the relationship of cardiac autonomic dysfunction in patients with diabetes and PPA has not been investigated so far. Since cardiac autonomic dysfunction often manifests as increased heart rate and, on the other hand, increased heart rate results in PPA enhancement, we hypothesized that cardiac autonomic dysfunction would be associated with PPA. Thus, the main aim of this cross-sectional study was to investigate the association between PPA and cardiac autonomic activity, as assessed by BRS and HRV, in patients with T2DM. In addition, we examined the association between cardiac autonomic activity and central hemodynamic parameters that may affect PPA such as augmentation index (AIx), aortic stiffness (pulse wave velocity (PWV)), and common carotid artery stiffness (distensibility coefficient (DC)).

Materials and methods

Study population

The study population consisted of 142 patients with T2DM attending the outpatient Diabetes Clinic of our Hospital. Diabetes status was confirmed from the medical records according to the American Diabetes Association criteria [13] and the treatment with antidiabetic medications. Exclusion criteria were arrhythmias, moderate or severe renal disease (estimated glomerular filtration rate (eGFR) < 45 ml/min/1.73 m^2), significant hepatic or cardiorespiratory disease, malignancy, connective tissue disease, hormone replacement therapy, and acute illness.

The purpose of the study was clearly explained to all participants and written informed consent was obtained. The study was conducted according to the principles of the Declaration of Helsinki and was approved by the Ethics Committee of our Hospital.

Clinical and laboratory parameters

This was a cross-sectional study and study visits took place at the Diabetic Laboratory of our Department.

All measurements were performed between 8:00 and 10:00 am after a 10-h fast and in a room with stable temperature (23 ± 1 °C). All medications were withheld and administered to the participants at the end of the examination and after blood was drawn. Blood samples were collected, immediately centrifuged, and deep frozen at −80 °C.

A complete physical examination was performed and a detailed history for current and previous diseases, use of current medications, and smoking habits was obtained. Waist circumference, body weight, and height were measured in light clothing and body mass index (BMI) was calculated.

Coronary artery disease was defined as a history of angina, myocardial infarction, percutaneous transluminal coronary angioplasty, or coronary artery bypass grafting. Peripheral arterial disease was defined as a history of intermittent claudication, revascularization procedure at the aorta or the lower extremities, or as an ankle brachial pressure index < 0.90. Cerebrovascular disease was defined as a history of stroke or revascularization at the carotid arteries.

After a 20-min rest in the Laboratory, BP at the brachial artery was measured using an appropriate cuff three consecutive times at 5-min intervals with the participant in a seated position. The mean value of the last two measurements was used in the analysis [14]. Arterial hypertension was defined according to the current guidelines if systolic BP (SBP) was ≥140 mmHg and/or diastolic BP (DBP) was ≥90 mmHg and/or if patients were on antihypertensive treatment [14].

Plasma glucose, total serum cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, serum urea, and creatinine were measured on an automatic analyzer. Low density lipoprotein (LDL) cholesterol was estimated using Friedewald's equation, while eGFR was calculated according to the equation for Modification of Diet in Renal Disease. HbA1c levels were determined using a DCA analyzer (DCA 2000+, Bayer HealthCare LLC, Elkhart, IN 46514, USA). Albumin to creatinine ratio (ACR) was measured in a first morning void urine sample using the same DCA analyzer.

Assessment of hemodynamic parameters

Assessment of central BP, PPA, and AIx

Central BP, augmentation pressure, and AIx were determined by the technique of pulse wave analysis with a validated noninvasive device (SphygmoCor, AtCor Medical, Sydney, Australia), as previously described [15]. This technique uses the principle of applanation tonometry to noninvasively record peripheral arterial waveforms. After applying a validated integral transfer function, the central

ascending aortic waveform and pressure can be derived and analyzed. In summary, all individuals were studied in the supine position after an acclimatization period of about 20 min. Measurements were performed on the radial artery with the wrist at the heart level by a high fidelity Millar micromanometer (SPC-301; Millar Instruments, Inc., Houston, TX). Radial waveforms calibrated from the measured brachial SBP and DBP were used for the determination of peripheral MBP and central BP components (cSBP, cDBP, cPP, and cMBP). PPA was then calculated as the brachial PP to central PP ratio [6]. After 20 sequential stable radial waveforms were acquired, the SphygmoCor System Software generated an average peripheral and corresponding central ascending aortic pressure waveform, which was subjected to further analysis to determine the augmentation pressure (the difference between the first and the second peak of the central arterial waveform), Aix (the ratio of the augmentation pressure over the PP), and AIx normalized for heart rate of 75 bpm (AIx75) [6].

Assessment of aortic PWV

Aortic PWV was calculated from the measurements of pulse transit time and the distance traveled between the common carotid artery and the common femoral artery with a validated noninvasive device (Sphygmocor, AtCor Medical, Sydney, Australia) [16]. The distance measurements were taken with a measuring tape by subtracting the distance from the suprasternal notch to the carotid from the suprasternal notch to the femoral artery at the sensor location.

Assessment of common carotid DC

Local arterial stiffness was measured in the right and left common carotid artery using a high-resolution B-mode ultrasound device (Vivid 7 Pro, GE Healthcare) with a 14-MHz multi-frequency linear transducer, and the carotid arterial properties were determined according to international guidelines [17]. Common carotid artery DC was calculated according to the following formula:

$$\mathrm{DC} = (2\Delta D \times D + \Delta D^2)/(\mathrm{PP} \times D^2)(10^{-3}/\mathrm{kPa}),$$

where D is the arterial diameter, ΔD is the distension (change in arterial diameter during heart cycle), and PP is the pulse pressure.

Assessment of autonomic cardiac nervous system activity

Assessment of BRS

BRS estimation was performed by the sequence method using the BaroCor System (AtCor Medical, Sydney, Australia) as described before [18]. In brief, all individuals were studied in the supine position after a period of acclimatization of about 20 min. Electrocardiographic signal with a 3-lead electrocardiogram and beat-to-beat BP were continuously and simultaneously recorded for 20 min. Continuous BP measurements were made using a radial tonometer in the dominant arm (CBM 7000; Colins Medical Instruments Corp., San Antonio, TX) that enables the noninvasively derivation of the ascending aortic BP waveform via a generalized transfer function [15]. Time series of inter-beat (RR) intervals and SBP were analyzed by the BaroCor System Software to identify sequences in which SBP and RR interval increased or decreased concurrently over at least three cardiac cycles. A linear correlation was applied for each sequence and the regression slope was calculated. BRS was calculated as the average values of the individual's slopes and expressed in ms/mmHg. Lag 0 value of central BRS was selected for each participant measurement.

Assessment of HRV

Short-term analysis of the HRV was performed using the computer-aided examination and evaluation system VariaCardio TF5 (Medical Research Limited, Leeds, UK) [19]. Frequency domain parameters of HRV were obtained after a 5-min duration recording at each interval on a 256 beat-window basis. Data were analyzed by Fast Fourier Transform modified by the coarse-graining algorithm. Each dataset was filtered automatically by excluding recorded artifacts using a recognition algorithm. Parameters of the frequency-domain were observed within the high frequency (HF) band (0.15 to 0.50 Hz) and within the low frequency (LF) band (0.05 to 0.15 Hz). Power in the HF range (0.15–0.40 Hz) and in the LF range (0.04–0.15 Hz) was recorded. Total power (frequency range: ≤ 0.40 Hz), the sum of all the components, was also obtained. Moreover, LF/HF ratio was calculated. Vagal activity is the major contributor to the HF component, while LF reflects both sympathetic nervous system and vagal activity. Total power represents the sum of all the frequency components, whereas LF/HF ratio is considered by some investigators as a surrogate marker of sympathovagal balance [10, 19].

Statistical analysis

Statistical analysis was performed using the SPSS 21.0 statistical package (SPSS, Inc., Chicago, IL, USA). All data were assessed for normal distribution of their values. Normally distributed continuous variables were presented as means ± standard deviation (SD). Independent samples Student's *t*-test was used for the comparison of normally distributed continuous variables between males and

Table 1 Demographic, clinical characteristics and laboratory parameters of the study participants, stratified by gender

	$n = 142$	Males ($n = 70$)	Females ($n = 72$)	p
Age (years)	61.4 ± 8.2	60.4 ± 8.3	62.4 ± 8.0	0.138*
Duration of diabetes (years)	10.0 [5.3–18.8]	10.0 [5.0–19.0]	12.0 [6.0–19.0]	0.767**
Height (m)	1.65 ± 0.10	1.73 ± 0.07	1.58 ± 0.06	<0.001*
Body mass index (kg/m^2)	31.7 ± 5.1	31.0 ± 4.6	32.3 ± 5.6	0.145*
Waist circumference (cm)	106.0 ± 12.1	108.9 ± 11.4	103.2 ± 12.2	0.006*
Hypertension *n* (%)	102 (71.8)	48 (68.6)	54 (75.0)	0.395†
Glucose (mg/dl)	149.7 ± 50.2	155.6 ± 51.8	143.9 ± 48.2	0.185*
HbA1c (%)	7.5 ± 1.2	7.5 ± 1.2	7.5 ± 1.2	0.811*
Total cholesterol (mg/dl)	186.8 ± 37.1	182.6 ± 32.3	191.1 ± 41.2	0.194*
HDL-cholesterol (mg/dl)	46.7 ± 12.4	42.2 ± 9.1	51.4 ± 13.6	<0.001*
LDL-cholesterol (mg/dl)	108.8 ± 32.8	108.9 ± 29.5	108.6 ± 36.3	0.963*
Triglycerides (mg/dl)	136.0 [94.5–195.8]	144.0 [99.0–191.5]	129.0 [87.5–196.5]	0.272**
eGFR (ml/min/1.73 m^2)	76.3 ± 23.1	76.1 ± 23.5	76.5 ± 22.9	0.908*

Data are means ± SD (standard deviation) or median value (25, 75 percentile)

HDL high-density lipoprotein, *LDL* low-density lipoprotein, *eGFR* estimated glomerular filtration rate

**p* Values for the comparison between males and females with Independent Samples Student's *t*-test

***p* Values for the comparison between males and females with Mann–Whitney *U* test

†*p* Values for the comparison between males and females with the Chi-squared test.

females. Non-normally distributed continuous variables, presented as median (25th–75th percentile), were compared using a Mann–Whitney *U* test. Categorical variables were compared with a Chi-squared test and/or Fisher's exact test, as appropriate. Univariate and multivariate linear regression analyses (stepwise backward method) were performed to look for associations between the studied parameters and the hemodynamic parameters (PPA, PWV, AIx75, left and right common DC). Parameters that were significantly associated with the hemodynamic parameters in univariate analysis were entered in multivariate analysis models.To avoid multi-collinearity, several models of multivariate analysis were created for each one of the HRV parameters. All tests were two-sided and a p value < 0.05 was considered statistically significant for all analyses.

Results

Baseline characteristics

A total of 142 participants with T2DM were included in the study. Baseline characteristics of the study participants are presented in Table 1 and Table 1S, while hemodynamic parameters are shown in Table 2.

Parameters associated with PPA

Univariate linear regression analysis showed that PPA was associated positively with male gender, height, waist circumference, and heart rate, and negatively with age, brachial and central SBPs, PPs and MBP, aortic PWV, AIx75, use of β-blockers, BRS, and several parameters of HRV, such as total power and power in LF range (Table 3). Multivariate linear regression analysis, after controlling for age, diabetes duration, height, waist circumference, aortic PWV, use of β-blockers, and BRS, demonstrated that PPA was associated significantly and independently with male gender (standardized regression coefficient (β) = 0.156, $p = 0.007$, aortic SBP ($\beta = -0.221$, $p < 0.001$), heart rate ($\beta = 0.521$, $p < 0.001$), AIx75 ($\beta = -0.443$, $p < 0.001$), and total power ($\beta = -0.157$, $p = 0.005$)) (Table 4).

Parameters associated with aortic PWV

Univariate linear regression analysis showed that PWV was positively associated with age, diabetes duration, brachial and central BPs, AIx75, and presence of CV disease and negatively associated with PPA, left DC, total and LDL cholesterol, and eGFR (Table 3). Multivariate linear regression analysis, after controlling for age, diabetes duration, AIx75, and left DC, demonstrated that PWV was associated significantly and independently with aortic SBP ($\beta = 0.422$, $p < 0.001$), eGFR ($\beta = -0.278$, $p = 0.015$), and presence of CV disease ($\beta = 0.301$, $p = 0.007$) (Table 4).

Parameters associated with AIx

Univariate linear regression analysis showed that AIx75 was positively associated with brachial and central SBPs, PPs and MBP, PWV, and use of statins and negatively

Table 2 Hemodynamic parameters of the study participants, stratified by gender

	$n = 142$	Males ($n = 70$)	Females ($n = 72$)	p
Brachial SBP (mmHg)	143.0 ± 21.3	143.5 ± 20.2	142.5 ± 22.5	0.870*
Brachial DBP (mmHg)	78.3 ± 10.9	80.7 ± 11.4	76.0 ± 10.0	0.016*
Brachial PP (mmHg)	64.6 ± 17.4	62.8 ± 15.7	66.5 ± 18.8	0.091*
Aortic SBP (mmHg)	131.7 ± 20.7	130.5 ± 19.8	132.9 ± 21.6	0.496*
Aortic DBP (mmHg)	79.0 ± 11.2	81.4 ± 11.7	76.8 ± 10.3	0.014*
Aortic PP (mmHg)	52.7 ± 16.8	49.2 ± 15.0	56.2 ± 17.8	0.012*
MBP (mmHg)	96.6 ± 12.8	97.8 ± 13.1	95.5 ± 12.5	0.294*
Heart rate (bpm)	68.6 ± 9.0	69.0 ± 9.8	68.1 ± 8.2	0.556*
PPA	1.22 [1.15–1.31]	1.26 [1.20–1.39]	1.18 [1.13–1.27]	<0.001**
PWV (m/s)	9.2 [8.0–11.3]	9.3 [8.2–11.2]	9.1 [7.8–11.4]	0.524**
AIx (%)	29.8 ± 8.9	26.6 ± 9.4	32.9 ± 7.2	<0.001*
AIx 75 (%)	26.4 ± 7.9	23.5 ± 8.1	29.1 ± 6.6	<0.001*
Right DC (10^{-3}/kPa)	3.54 ± 1.66	3.43 ± 2.02	3.62 ± 1.34	0.662*
Left DC (10^{-3}/kPa)	3.48 ± 1.67	3.95 ± 1.71	3.11 ± 1.56	0.046*

Data are means ± SD (standard deviation) or median value (25, 75 percentile)

SBP systolic blood pressure, *DBP* diastolic blood pressure, *PP* pulse pressure, *MBP* mean blood pressure, *PPA* pulse pressure amplification, *PWV* pulse wave velocity, *AIx* augmentation index, *AIx 75* augmentation index normalized for heart rate of 75 bpm, *DC* common carotid distensibility coefficient

*p Values for the comparison between males and females with Independent Samples Student's t-test

**p Values for the comparison between males and females with Mann–Whitney U test

associated with male gender, height, PPA, and left DC (Table 3). Multivariate linear regression analysis, after controlling for height, gender, aortic SBP, PWV, and use of statins, demonstrated that AIx75 was associated significantly and independently with PPA ($\beta = -0.642$, $p < 0.001$) (Table 4).

Parameters associated with right and left carotid DC

Univariate linear regression analysis showed that right DC was negatively associated with age, waist circumference, and brachial and central BPs and positively associated with HbA1c (Table 3). Multivariate linear regression analysis, after controlling for eGFR, demonstrated that right DC was associated significantly and independently with age ($\beta = -0.200$, $p = 0.001$), waist circumference ($\beta = -0.376$, $p = 0.003$), and HbA1c ($\beta = 0.445$, $p < 0.001$) (Table 4).

Univariate linear regression analysis showed that left DC was negatively associated with age, brachial and central BPs, PWV, AIx75, HDL cholesterol, use of statins, and angiotensin converting enzyme inhibitors or angiotensin II receptor antagonists and positively associated with male gender, height, LDL cholesterol, and eGFR (Table 3). Multivariate linear regression analysis, after controlling for age, gender, height, AIx75, and PWV, demonstrated that left DC was associated significantly and independently with aortic SBP ($\beta = -0.356$, $p = 0.003$), eGFR ($\beta = 0.235$, $p = 0.041$), and use of statins ($\beta = -0.263$, $p = 0.020$) (Table 4).

Discussion

The main finding of our study was that cardiac autonomic dysfunction, as measured by HRV, was associated with enhanced PPA in patients with T2DM, while no significant associations were found between cardiac autonomic dysfunction and PWV, AIx, and DC. The association between PPA and HRV was independent of traditional CV risk factors and suggests that in the early asymptomatic phase of autonomic dysfunction in diabetes, altered sympathetic–vagal interactions may exert a paradoxical compensatory effect on overall vascular homeostasis. However, no independent associations were observed between PPA and BRS.

It should be also noted that although in univariate analysis both BRS and HRV parameter were significantly associated with PPA, in multivariate analysis no significant association was found between PPA and BRS. Nevertheless, both HRV and BRS are used for the assessment of cardiac autonomic dysfunction. However, it should be noted that diagnosis of CAN, according to current guidelines, can be based on the assessment of HRV, but not on the assessment of BRS [10].

PPA represents the widening of the PP as it travels from the aorta to the periphery of the arterial tree and depends mostly on the large artery stiffness, the peripheral arterial resistance, and the timing and amplitude of the forward and reflected BP waves [6]. In our study population, male gender and heart rate were found to be positively and independently

Table 3 Associations between studied parameters and pulse pressure amplification, pulse wave velocity, augmentation index normalized for heart rate of 75 bpm, right and left common carotid distensibility coefficient by univariate linear regression analysis

	PPA		PWV		AIx 75		Right DC		Left DC	
	β	*p*	*β*	*p*	*β*	*p*	*β*	*p*	*β*	*p*
Age (years)	−0.356	**<0.001**	0.333	**<0.001**	0.076	0.366	−0.312	**0.014**	−0.449	**<0.001**
Male gender	0.217	**0.010**	0.030	0.724	−0.357	**<0.001**	−0.057	0.662	0.253	**0.046**
Diabetes duration (years)	−0.158	0.062	0.184	**0.030**	−0.013	0.878	−0.086	0.512	−0.143	0.264
Current smoking	0.054	0.526	−0.086	0.312	−0.015	0.862	0.065	0.618	0.226	0.074
Height (cm)	0.277	**0.001**	0.006	0.943	−0.354	**<0.001**	0.100	0.444	0.313	**0.013**
Body mass index (kg/m^2)	0.163	0.053	−0.098	0.247	−0.022	0.797	−0.191	0.141	−0.150	0.241
Waist (cm)	0.191	**0.025**	0.006	0.941	−0.141	0.098	−0.264	**0.040**	−0.200	0.115
Brachial SBP (mmHg)	−0.183	**0.029**	0.562	**<0.001**	0.209	**0.012**	−0.482	**0.001**	−0.393	**0.001**
Brachial DBP (mmHg)	−0.009	0.919	0.300	**<0.001**	0.130	0.124	−0.314	**0.014**	−0.272	**0.031**
Brachial PP (mmHg)	−0.219	**0.009**	0.494	**<0.001**	0.175	**0.037**	−0.410	**0.001**	−0.310	**0.013**
Aortic SBP (mmHg)	−0.390	**<0.001**	0.586	**<0.001**	0.360	**<0.001**	−0.425	**0.001**	−0.458	**<0.001**
Aortic DBP (mmHg)	−0.041	0.625	0.315	**<0.001**	0.159	0.060	−0.291	**0.023**	−0.282	**0.025**
Aortic PP (mmHg)	−0.453	**<0.001**	0.504	**<0.001**	0.287	**0.001**	−0.328	**0.010**	−0.374	**0.003**
MBP (mmHg)	−0.234	**0.005**	0.506	**<0.001**	0.340	**<0.001**	−0.394	**0.002**	−0.406	**0.001**
Heart rate (bpm)	0.451	**<0.001**	0.036	0.678	0.048	0.576	−0.022	0.866	−0.073	0.574
PPA	–	–	−0.232	**0.006**	−0.469	**<0.001**	0.203	0.117	0.110	0.391
PWV (m/s)	−0.232	**0.006**	–	–	0.267	**<0.001**	−0.239	0.069	−0.383	**0.002**
AIx (%)	−0.624	**<0.001**	0.230	**<0.006**	–	–	−0.100	0.442	−0.244	0.054
AIx 75 (%)	−0.469	**<0.001**	0.267	**<0.001**	–	–	−0.131	0.316	−0.288	**0.022**
Right DC (10^{-3}/kPa)	0.203	0.117	−0.127	0.334	−0.131	0.316	–	–	–	–
Left DC (10^{-3}/kPa)	0.236	0.063	−0.383	**0.002**	−0.288	**0.022**	–	–	–	–
Total cholesterol (mg/dl)	0.033	0.710	−0.214	**0.015**	0.051	0.564	0.110	0.419	0.115	0.392
HDL cholesterol (mg/dl)	−0.124	0.168	−0.013	0.883	0.137	0.126	−0.137	0.324	−0.339	**0.011**
LDL cholesterol (mg/dl)	0.010	0.910	−0.187	**0.039**	0.048	0.598	0.232	0.092	0.306	**0.022**
Triglycerides (mg/dl)	0.053	0.551	−0.029	0.745	−0.017	0.850	−0.076	0.578	0.045	0.735
HbA_{1c} (%)	0.031	0.726	−0.013	0.879	0.110	0.206	0.404	**0.002**	0.234	0.077
Glucose (mg/dl)	0.115	0.191	−0.030	0.734	−0.088	0.317	0.156	0.264	0.247	0.069
eGFR (ml/min/1.73 m^2)	0.124	0.156	−0.333	**<0.001**	−0.151	0.084	0.265	0.052	0.367	**0.005**
Cardiovascular disease	−0.128	0.130	0.195	**0.021**	0.062	0.463	−0.076	0.562	−0.001	0.991
BRS (ms/mmHg)	−0.236	**0.010**	−0.031	0.741	−0.058	0.532	−0.189	0.215	−0.022	0.881
Total power (ms^2)	−0.231	**0.012**	−0.026	0.784	−0.044	0.636	0.055	0.721	−0.058	0.703
LF power (ms^2)	−0.238	**0.009**	0.006	0.948	−0.003	0.973	0.002	0.991	−0.050	0.742
HF power (ms^2)	−0.179	0.052	−0.057	0.544	−0.071	0.443	0.081	0.603	−0.030	0.842
LF/HF ratio	0.177	0.055	0.036	0.700	0.004	0.963	−0.091	0.557	−0.066	0.664
Use of statins	−0.038	0.650	0.047	0.581	0.176	**0.036**	−0.142	0.276	−0.303	**0.016**
Use of ACEi or ARBs	−0.068	0.419	0.120	0.157	0.025	0.764	−0.154	0.237	−0.294	**0.019**
Use of CCBs	−0.045	0.597	0.158	0.063	−0.053	0.528	−0.075	0.565	−0.097	0.448
Use of β-blockers	−0.173	**0.039**	0.084	0.326	0.045	0.592	−0.090	0.489	−0.097	0.451
Use of diuretics	−0.085	0.315	0.112	0.188	0.024	0.781	−0.025	0.850	−0.087	0.497

PPA pulse pressure amplification, *PWV* pulse wave velocity, *AIx 75* augmentation index normalized for heart rate of 75 bpm, *DC* common carotid distensibility coefficient, *β* standardized regression coefficient, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *PP* pulse pressure, *MBP* mean blood pressure, *HR* heart rate, *AIx* augmentation index, *HDL* high density lipoprotein, *LDL* low density lipoprotein, *eGFR* estimated glomerular filtration rate, *BRS* baroreflex sensitivity, *LF* low frequency, *HF* high frequency, *ACEi* angiotensin converting enzyme inhibitors, *ARBs* angiotensin II receptor antagonists, *CCBs* calcium channel blockers

Table 4 Associations between studied parameters and pulse pressure amplification, pulse wave velocity, augmentation index normalized for heart rate of 75 bpm, right and left common carotid distensibility coefficient by multivariate linear regression analysis

	PPA[a]		PWV[b]		AIx 75[c]		Right DC[d]		Left DC[e]	
	β	*p*	*β*	*p*	*β*	*p*	*β*	*p*	*β*	*p*
Age (years)	–	–	–	–	–	–	−0.200	**0.001**	–	–
Male gender	0.156	**0.007**	–	–	–	–	–	–	–	–
Aortic SBP (mmHg)	−0.221	**<0.001**	0.422	**<0.001**	–	–	−0.208	0.098	−0.356	**0.003**
HR (bpm)	0.521	**<0.001**	–	–	–	–	–	–	–	–
Total power ($msec^2$)[f]	−0.157	**0.005**	–	–	–	–	–	–	–	–
PPA	n/a	n/a	–	–	−0.642	**<0.001**	–	–	–	–
PWV (m/sec)	–	–	n/a	n/a	–	–	–	–	–	–
AI 75 (%)	−0.443	**<0.001**	–	–	n/a	n/a	–	–	–	–
Left DC (10^{-3}/kPa)	–	–	–	–	−0.168	0.086	–	–	n/a	n/a
eGFR (ml/min/1.73 m^2)	–	–	−0.278	**0.015**	–	–	–	–	0.235	**0.041**
Cardiovascular disease	–	–	0.301	**0.007**	–	–	–	–	–	–
Waist (cm)	–	–	–	–	–	–	−0.376	**0.003**	–	–
HbA1c (%)	–	–	–	–	–	–	0.445	**<0.001**	–	–
Use of statins	-	-	-	-	-	-	-	-	−0.263	**0.020**
Use of ACEi or ARBs	-	-	-	-	-	-	-	-	−0.194	0.087

PPA pulse pressure amplification, *PWV* pulse wave velocity, *AIx 75* augmentation index normalized for heart rate of 75 bpm, *DC* common carotid distensibility coefficient, *β* standardized regression coefficient, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *PP* pulse pressure, *MBP* mean blood pressure, *HR* heart rate, *AIx* augmentation index, *HDL* high density lipoprotein, *LDL* low density lipoprotein, *eGFR* estimated glomerular filtration rate, *BRS* baroreflex sensitivity, *LF* low frequency, *HF* high frequency, *ACEi* angiotensin converting enzyme inhibitors, *ARBs* angiotensin II receptor antagonists, *CCBs* calcium channel blockers

[a]Multivariate analysis after adjustment in addition for age, diabetes duration, height, waist circumference, pulse wave velocity, baroreflex sensitivity, and use of β-blockers

[b]Multivariate analysis after adjustment in addition for age, diabetes duration, augmentation index normalized for heart rate of 75 bpm, and left common carotid artery distensibility coefficient

[c]Multivariate analysis after adjustment in addition for height, gender, aortic systolic blood pressure, pulse wave velocity, and statin use

[d]Multivariate analysis after adjustment in addition for estimated glomerular filtration rate

[e]Multivariate analysis after adjustment in addition for age, gender, height, augmentation index normalized for heart rate of 75 bpm, and pulse wave velocity

[f]Total power was used in the multivariate model; when any parameter of heart rate variability that was significantly associated with pulse pressure amplification in the univariate analysis was used instead (low frequency power or high frequency power), the results of the multivariate model did not change

associated with PPA, while aortic SBP, AIx, and parameters of HRV reflecting both individual and reciprocal components of the sympathetic and parasympathetic activity were found to be negatively and independently associated with PPA. Regarding male gender, heart rate, aortic SBP, and AIx, these results are consistent with literature findings [6, 7, 20]. No association between PPA and smoking was observed in the univariate analysis, although other investigators have reported that PPA is significantly lower in smokers when compared with non-smokers [21]. Nevertheless, the relatively small number of our participants who were current smokers (46 out of 142) may not have allowed a possible association to emerge.

Previous studies have highlighted the association between enhanced PPA and increased heart rate [6, 7]. Unlike increased arterial stiffness, which results in earlier central arrival of pressure wave reflections, increased heart rate and thus a shorter systolic phase of the cardiac cycle are associated with a delayed (during the diastolic phase of the cardiac cycle) central arrival of pressure wave reflections, a lower augmentation of the peak cSBP, and higher PPA [6].

Data regarding the complex and incompletely understood association of PPA, tachycardia, and T2DM are scarce. Yannoutsos et al. investigated the association of arterial hypertension and diabetes on arterial stiffness and PPA and reported that patients with diabetes and arterial hypertension had higher PPA when compared with individuals without diabetes and hypertension [8]. This difference, however, disappeared after adjustment for heart rate. In another study, Agnoletti et al. reported slightly higher PPA values in patients with diabetes than in non-diabetic participants, but the difference became again non-significant after adjustment

for age, gender, MBP, and heart rate [9]. In our study as well, PPA values were positively and independently associated with heart rate, providing further confirmation of previous findings of this association in insulin-resistant states such as T2DM [7, 12].

The novel finding of our study was that PPA was significantly and independently associated with cardiac autonomic dysfunction in patients with T2DM. Heart rate-induced PPA enhancement could represent a putative mechanism linking cardiac autonomic dysfunction and PPA in diabetes. Parasympathetic impairment through vagal denervation represents an early perturbation process in CAN that leads to unopposed sympathetic tone which, in turn, can lead to both resting tachycardia and increased arterial stiffness [10, 22, 23]. We found that several HRV parameters were negatively associated with PPA. Low levels of the LF component were associated with a higher PPA, while HF power values showed a trend toward negative association with PPA ($p = 0.052$). Lower total power values were also associated with higher PPA. These findings suggest that autonomic imbalance in T2DM patients, driven mostly by vagal tone impairment and aberrant sympathetic activity, can have a profound impact on CV hemodynamics by simultaneously influencing vascular tone and cardiac chronotropy. CAN follows a natural history of parasympathetic-to-sympathetic "gradient" of progressive pathological damage with vagal nerve myelinated fibers being affected early during the disease process [10, 24]. This fact corroborates with the detection of an early alteration in HRV, which predominantly represents an assessment tool for vagal activity, and its functional association with an augmentation effect on PPA. Nevertheless, our finding that cardiac autonomic dysfunction is associated with higher PPA values independently of the positive association of the latter with heart rate could suggest that this association may be related to underlying mechanisms other than resting tachycardia.

The pathogenic mechanisms underlying the relationship between heart rate, CAN, and PPA in T2DM remain to be explored. Although it seems intriguing to suggest that heart rate-induced PPA enhancement could exert a compensatory vascular effect, given the deleterious consequences of CAN on CV prognosis, in the present state of knowledge such observations should be interpreted with great caution. Prospective longitudinal studies that examine the relative influence of enhanced PPA in association with increased heart rate on CV morbidity and mortality in T2DM are needed in order to better clarify the importance, if any, of this pathophysiological "paradox" and its impact on treatment and prognostication issues. Nevertheless, until then, since presence of cardiac autonomic dysfunction seems to modulate PPA, PPA should be interpreted with caution in patients with T2DM.

Clinical studies have reported associations between arterial stiffness and cardiac autonomic dysfunction in patients with type 1 and T2DM [23, 25]. Abnormal aortic PWV has been associated with low values of the HRV parameters in patients with T2DM, but not with BRS [23]. In our study, aortic PWV was not associated with cardiac autonomic indices such as HRV parameters and BRS. One possible explanation is that the smaller sample included in our study may not have allowed the possible association between PWV and HRV parameters to emerge.

AIx and DC were not found to be associated with autonomic function. A potential explanation is that longer disease duration may be necessary in order to observe any significant alteration in similar markers of central vascular function.

A strength of our study is a multimodality approach by the use of most currently available methods to study cardiac autonomic dysfunction in a well characterized cohort of subjects with T2DM. The main limitation of our study is its cross-sectional design that does not allow the investigation of a causal association between impaired cardiac autonomic function and increased PPA. Our study also did not include a control group to allow comparisons of cardiac autonomic function and PPA between individuals with and without T2DM. However, cardiac autonomic dysfunction is extremely uncommon in persons without diabetes.

In summary, our study showed that several indices of cardiac autonomic dysfunction are associated with enhanced PPA. To our knowledge, this is the first study to examine the association between PPA and other central hemodynamic parameters and impaired cardiac autonomic function in T2DM. Further prospective studies are needed in order to elucidate the possible causal mechanisms and the reciprocal associations between PPA and autonomous nervous system impairment, as well as to provide important information on long-term prognostic issues.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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