The systolic-diastolic difference in carotid stiffness is increased in type 2 diabetes

Original Article

The systolic–diastolic difference in carotid stiffness is increased in type 2 diabetes: The Maastricht Study

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Objective: In type 2 diabetes (T2D), increased arterial stiffening results from accelerated arterial wall matrix remodeling. The associated structural alterations modify the pressure dependency of arterial stiffness, which can be quantified by the systolic–diastolic difference in carotid pulse wave velocity (δPWV). We evaluated the association between T2D and δPWV as marker for matrix remodeling and whether δPWV may contain additional information beyond carotid stiffness (cPWV).

Methods: In 746 individuals from The Maastricht Study, 415 with normal glucose metabolism; 126 with prediabetes; and 205 with T2D, carotid pulse wave velocity (cPWV) and δPWV were determined by ultrasonography and tonometry. Multiple linear regression analyses were used to investigate associations of glucose metabolism status (with normal glucose metabolism as reference) with cPWV and δPWV, adjusting for age, sex, mean arterial pressure, prior cardiovascular disease, estimated glomerular filtration rate and smoking, and δPWV or cPWV as appropriate.

Results: After adjustment for age, sex, mean arterial pressure, prior cardiovascular disease, estimated glomerular filtration rate and smoking, T2D was associated with greater cPWV [β (95% confidence interval) 0.376 (0.119; 0.632)] and δPWV [0.301 (0.013; 0.589)]. After additional adjustment for δPWV or cPWV, associations of T2D with cPWV and δPWV were attenuated [0.294 (0.048; 0.539) and 0.173 (−0.103; 0.449), respectively]. Prediabetes was not associated with either cPWV or δPWV.

Conclusion: The systolic-diastolic difference in carotid stiffness is increased in T2D, but not prediabetes. Importantly, the association was not abolished by carotid stiffness, which suggests that systolic–diastolic difference in carotid stiffness carries additional information regarding arterial matrix remodeling.

Keywords: arterial stiffness, collagen crosslinking, elastin degradation, pulse wave velocity, vascular remodeling

Abbreviations: ρ, blood density; δPWV, systolic–diastolic difference in carotid pulse wave velocity; AGE, advanced glycation endproduct; CEL, *N*ε-(carboxyethyl)lysine; cfPWV, carotid-to-femoral pulse wave velocity; CML, *N*ε-(carboxymethyl)lysine; cPWV, carotid pulse wave velocity; CVD, cardiovascular disease; *D*, diameter; DC, distensibility coefficient; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HOMA2-IR, homeostasis model assessment 2 for insulin resistance; HR, heart rate; IMT, intima–media thickness; MAP, mean arterial pressure; NGM, normal glucose metabolism; PP_{car}, local carotid pulse pressure; RAS-inhibitor, renin–angiotensin system inhibitor; SAF, skin autofluorescence; SBP, systolic blood pressure; T2D, type 2 diabetes; Δ*D*, distension

INTRODUCTION

Arterial stiffening underlies increased cardiovascular disease (CVD) and cardiovascular risk [1,2], and is the result of structural alterations in the arterial wall matrix. Arterial matrix remodeling affects the arterial pressure–area relationship [3–5]. The pressure–area relationship defines the change in arterial cross-sectional area in response to changes in transmural pressure. The slope of the pressure–area relationship is directly related to the vessel's stiffness. From the slope, one can derive a corresponding pulse wave velocity, which is the measure used to quantify stiffness. The systolic–diastolic difference in carotid pulse wave velocity (δPWV), as obtained from carotid artery measurements, is a property of the pressure–area relationship, that is, it quantifies the pressure dependency of arterial stiffness (Fig. 1a) [6]. Greater δPWV is associated with left ventricular mass index, independent of existing arterial stiffness measures [6]. It has not been established whether δPWV may add to our understanding of arterial matrix remodeling. Therefore, the goal of the current study

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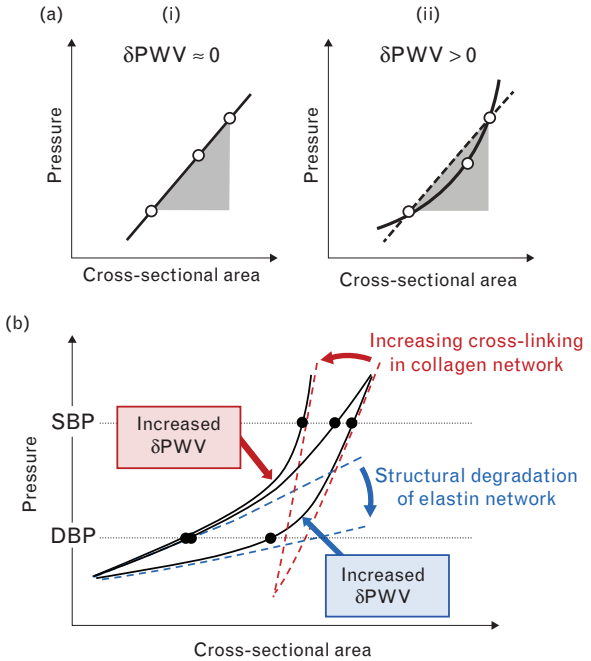

FIGURE 1 (a) (i) The systolic–diastolic difference in carotid pulse wave velocity (δPWV) in a linear pressure–area curve is more or less equal to 0, i.e. negligible pressure dependency of arterial stiffness. (ii) δPWV in a curvilinear pressure–area curve is greater than zero, signifying pressure dependency of arterial stiffness (refer to [6]). Note that in both cases, carotid pulse wave velocity is equal, as signified by the shaded areas. (b) δPWV as a marker of arterial matrix remodeling known to occur in type 2 diabetes. Greater δPWV is reflected by progressive degradation of the elastin network, leading to an increase in cross-sectional area, whether or not in combination with stiffening of the collagen network leading to a progressively steeper pressure–area curve. The effect of elastin degradation is indicated with a blue dotted line and the effect of increased collagen crosslinking with a red dotted line.

was to evaluate δPWV as a marker of arterial matrix remodeling.

In type 2 diabetes (T2D), arterial matrix remodeling is increased or 'accelerated' [7]. The elastin and collagen fiber network in T2D may be damaged due to cyclic or metabolic stress, leading to increased arterial stiffness via increased elastin degradation [8–10] and increased collagen cross-linking [10–15]. Given the accelerated elastin and collagen remodeling in T2D, we hypothesized that δPWV is greater in T2D (Fig. 1b).

Therefore, we investigated in a well characterized population-based cohort the association between glucose metabolism status [i.e. normal glucose metabolism (NGM), prediabetes and T2D] and δPWV. In our analysis, we considered whether or not δPWV contains information beyond carotid stiffness, as derived from distensibility and expressed as carotid pulse wave velocity (cPWV).

METHODS

Study population

We used data from The Maastricht Study, an observational prospective population-based cohort study. The rationale and methodology have been described previously [16]. In brief, the study focuses on the etiology, pathophysiology, complications and comorbidities of T2D and is characterized by an extensive phenotyping approach. Eligible for participation were all individuals aged between 40 and 75 years and living in the southern part of The Netherlands. Participants were recruited through mass media campaigns and from the municipal registries and the regional Diabetes Patient Registry via mailings. Recruitment was stratified according to known T2D status for reasons of efficiency. The present report includes cross-sectional data from the first 866 participants who completed the baseline survey between November 2010 and March 2012. The examinations of each participant were performed within a time window of 3 months. The study has been approved by the institutional medical ethical committee (NL31329.068.10) and the Minister of Health, Welfare and Sports of The Netherlands (Permit 131088-105234-PG). All participants gave written informed consent.

Arterial measurements

Carotid and aortic arterial stiffness, and δPWV, were assessed noninvasively by means of vascular ultrasound and applanation tonometry. All measurements (approximately 45 min) were done by trained vascular technicians unaware of the participants' clinical or diabetes status. Measurements took place in a quiet temperature-controlled room (21–23 °C) and were performed in supine position, after 10 min of rest. Participants were asked to refrain from smoking and drinking coffee or tea or alcohol beverages 3 h prior to the study. Participants were allowed to have a light meal (breakfast and/or lunch). Talking or sleeping was not allowed during the examination. A three-lead ECG was recorded continuously during the measurements to facilitate automatic signal processing. In addition, during the vascular assessment, brachial systolic, diastolic and mean arterial pressure (MAP) were determined repeatedly with a 5-min interval, using an oscillometric device (Accutorr Plus; Datascope Inc.; Montvale, New Jersey, USA), and the average of these measurements was calculated and used in the statistical analyses.

Arterial tonometry

Tonometric waveforms were determined at the right common carotid and right common femoral arteries (Sphygmocor; Sydney, Australia). The difference in the time of pulse arrival from the R-wave of the ECG between the two sites (transit time) was determined by an intersecting tangent algorithm. The pulse wave travel distance was calculated as 80% of the direct straight distance (measured with an infantometer) between the two arterial sites. The median of three consecutive carotid-to-femoral pulse wave velocity (cfPWV, defined as traveled distance/transit time) recordings was used in the analyses.

Carotid artery ultrasound measurements

The left common carotid artery (at least 10 mm proximal to the carotid bulb) was obtained by using a 7.5-MHz linear probe of an ultrasound scanner (MyLab70; Esaote Europe B.V.; Maastricht, The Netherlands). This setup enables the measurement of diameter (D), distension (ΔD) and intima–media thickness (IMT) as described elsewhere [17,18]. During the ultrasound measurements, a B-mode image on the basis of 19 M-lines was depicted on screen, and an online echo-tracking algorithm showed real-time anterior and posterior arterial wall displacements. The

M-mode recordings were composed of 19 simultaneous recordings at a frame rate of 498 Hz. The distance between the M-line recording positions was 0.96 mm; thus, a total segment of 18.24 mm of each artery was covered by the scan plane. For offline processing, the radiofrequency signal was fed into a dedicated PC-based acquisition system (ART.LAB; Esaote Europe B.V.) with a sampling frequency of 50 MHz. Data processing was performed in MATLAB (version 7.5; MathWorks; Natick, Massachusetts, USA). The distension waveforms were obtained from the radiofrequency data with the use of a wall track algorithm [17]. Carotid IMT was defined as the distance of the posterior wall from the leading edge interface between lumen and intima to the leading edge interface between media and adventitia [18]. The median diameter, distension and IMT of three measurements were used in the analyses.

Local carotid pressure calibration

Pulse pressure at the carotid artery (PP_{car}) was calculated by calibrating the systolic–diastolic amplitude of the carotid artery tonometry waveform $(sys\text{-}dias)_{tono}$ to pressure, assuming a constant difference between MAP and diastolic blood pressure (DBP) along the large arteries: $PP_{car} = (sys\text{-}dias)_{tono} \times (MAP - DBP)_{brach}/(mean\text{-}dias)_{tono}$.

This procedure was the same as those described by Kelly and Fitchett [19] and Van Bortel *et al.* [20], except that the MAP and DBP values at the brachial artery were taken as the respective averages over the vascular measurements (i.e. over a 30–45 min period) as obtained with a validated commercial oscillometric device (Accutorr Plus; Datascope Inc.). Cited procedures considered the mean–diastolic difference as obtained from a measured brachial artery pressure or diameter waveform.

Calculation of carotid pulse wave velocity and systolic–diastolic difference in carotid pulse wave velocity

Carotid artery stiffness was expressed in terms of cPWV (in units of m/s) based on the Bramwell–Hill relation [21]:

$$\mathrm{cPWV} = \frac{1}{\mathrm{sqrt}(\rho \mathrm{DC})},$$

with DC, the carotid distensibility coefficient calculated from the ultrasonography and tonometry derived parameters as defined below [22].

$$\mathrm{DC} = \frac{(2\Delta \mathrm{D} \times \mathrm{D} + \Delta \mathrm{D}^2)}{(\mathrm{PP_{car}} \times \mathrm{D}^2)},$$

with PP_{car}, the local carotid PP (calibration described above).

Accordingly, we obtained a systolic and diastolic PWV based on systolic, diastolic and dicrotic notch pressure and diameter values. δPWV was then calculated by subtracting the diastolic from the systolic PWV, as described in further detail by Hermeling *et al.* [6].

Reproducibility

Reproducibility was assessed in 12 individuals (six men; 60.8 ± 6.8 years; six with T2D) who were examined by two observers at two occasions spaced 1 week apart. The intraobserver and interobserver intraclass correlation coefficients were 0.87 and 0.69 for cfPWV and 0.85 and 0.73 for the carotid distensibility coefficient.

Glucose metabolism status and other covariates

Glucose metabolism status was assessed as described previously [16,23]. For the current study, impaired fasting glucose and impaired glucose tolerance were combined into prediabetes. T2D consisted of newly diagnosed and known T2D. Newly diagnosed T2D was defined as having T2D according to the WHO 2006 criteria [23] and no self-reported T2D. Medical history, prior CVD, medication use (glucose-lowering, antihypertensive and lipid-modifying), smoking status (never, former and current), BMI, waist and hip circumference, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, glycosylated hemoglobin (HbA1c) and fasting glucose levels were assessed as described previously [16]. Insulin was quantified on a Meso Scale custom duplex assay (Meso Scale Discovery; Gaithersburg, Maryland, USA). Detection ranges of the assay were 35–25 000 pg/ml, and the interassay coefficient of variation was 10.1%. Insulin was converted from pg/ml to pmol/l using a molar mass of $5808 \times g$. Insulin resistance was estimated from fasting insulin and glucose by the Homeostasis Model Assessment 2 for Insulin Resistance (HOMA2-IR) and calculated using the HOMA2 calculator (version 2.2.3. for Windows; available from https://www.dtu.ox.ac.uk/homacalculator). Prior CVD was defined as a history of myocardial infarction, cerebrovascular infarction or hemorrhage and(or) vascular surgery (including percutaneous angioplasty) of the coronary arteries, abdominal arteries, peripheral arteries or carotid arteries. Waist-to-hip ratio was calculated by dividing waist circumference by hip circumference. Total-to-HDL cholesterol ratio was calculated by dividing total cholesterol by HDL cholesterol.

Serum creatinine and serum cystatin C to estimate the glomerular filtration rate (eGFR) according to the Chronic Kidney Disease Epidemiology Collaboration equation [24] were measured as described previously [25]. Twenty-four-hour urinary albumin excretion was assessed as described previously, based on one or preferably two urine collections extrapolated to 24 h [25]. The presence of microalbuminuria or macroalbuminuria (urinary albumin excretion of 30–300 mg or urinary albumin excretion of more than 300 mg per 24 h, respectively [26]) was dichotomized.

MAP determined during vascular assessment was used as a primary covariate in linear regression analyses. Office and ambulatory blood pressures (BPs) were measured as described previously [15,16]. Mean 24-h systolic BP (SBP) and DBP were calculated based on hourly averages [27] and were only calculated if there were more than 14 valid measurements during daytime and more than seven valid measurements at night [28]. Twelve individuals were excluded because of invalid measurements at night. The remaining missing data on 24-h ambulatory BP monitoring were caused by device nonavailability or technical problems. Hypertension was defined as an office, and in a separate definition also 24-h, SBP ≥ 140 mmHg, an office or 24-h DBP ≥ 90 mmHg and(or) the use of antihypertensive medication [16,27].

Advanced glycation endproducts (AGEs) were measured as skin autofluorescence (SAF) using the AGE reader (DiagnOptics Technologies BV, Groningen, The Netherlands) and by means of plasma AGEs using HPLC with fluorescence detection for protein-bound pentosidine and ultra-performance liquid chromatography tandem mass spectrometry for protein-bound *N*ε-(carboxymethyl)lysine (CML) and *N*ε-(carboxyethyl)lysine (CEL), as described previously [12].

Statistical analysis

All statistical analyses were performed using SPSS version 21 (IBM SPSS, IBM Corp; Armonk, New York, USA). A two-sided *P* value of 0.05 was considered statistically significant. Differences in population characteristics between NGM, prediabetes and T2D were tested by ANOVA for continuous variables normally distributed, log transformed if necessary, and with chi-square test for dichotomous or categorical variables.

Associations of glucose metabolism status with cPWV and δPWV were investigated with multivariable linear regression analyses with NGM as reference category. The analyses were executed crude (model 1) and additionally adjusted for sex, age and MAP (model 2); prior CVD, eGFR and smoking (model 3); and the use of renin–angiotensin system inhibitors (RAS-inhibitors) [29] (model 4). Finally, we additionally adjusted the association between glucose metabolism status and δPWV for cPWV and vice versa to investigate whether the associations of glucose metabolism status with δPWV and cPWV were independent of each other (model 5).

RESULTS

Study population

From the initial 866 individuals, we excluded four individuals with type 1 diabetes. In the remaining 862 individuals, pulse wave analysis data were available in 808 participants. The reason for the missing pulse wave analysis data was either for logistical reasons or technical failure. From those 808 individuals, a further 12 were excluded with a δPWV less than 0 m/s due to measurement errors. An additional 50 individuals were excluded because they had missing values on prior CVD, eGFR or smoking (not mutually exclusive).

The current study population therefore consisted of 746 individuals (415 individuals with NGM, 126 individuals with prediabetes and 205 with T2D of whom 32 were newly diagnosed T2D).

The missing or excluded individuals more often had T2D, more often suffered from hypertension, had lower eGFR, greater BMI and cfPWV. In addition, they more often used lipid-modifying medication and lower total and LDL cholesterol levels.

Clinical characteristics of the study population

Table 1 shows the characteristics of the study population according to glucose metabolism status. Individuals with T2D were on average older, were more often men, and had a greater BMI as compared with individuals with NGM and(or) prediabetes. In addition, individuals with T2D suffered more often from hypertension and CVD.

Individuals with T2D had a greater carotid diameter as well as greater IMT. In addition, individuals with T2D had a higher PP_{car} (Table 1).

Both cPWV and δPWV were higher in individuals with T2D as compared with individuals with NGM and(or) prediabetes (Table 1).

Association of glucose metabolism status with carotid pulse wave velocity and systolic–diastolic difference in carotid pulse wave velocity

Table 2 shows the associations of T2D with cPWV and δPWV as compared with NGM. After adjustment for sex, age and MAP (covariates of model 2), T2D was associated with greater cPWV and δPWV: regression coefficient β (95% confidence interval): 0.377 (0.124; 0.629) and 0.311 (0.027; 0.595), respectively (model 2; Table 2). Further adjustment for prior CVD, eGFR and smoking (model 3; Table 2) did not materially change the results: 0.376 (0.119; 0.632) and 0.301 (0.013; 0.589), respectively. If we then further adjusted these associations for the use of RAS-inhibitor, the results did not materially alter: 0.322 (0.051; 0.594) and 0.331 (0.026; 0.636), respectively (model 4; Table 2).

Prediabetes was not associated with cPWV or δPWV after adjustment for any of the variables of the models 2, 3 and 4 (Supplemental Table S2, http://links.lww.com/HJH/A741).

Mutual adjustments for carotid pulse wave velocity and systolic–diastolic difference in carotid pulse wave velocity

If we additionally adjusted the association between T2D and cPWV for δPWV, the results showed that the association between T2D and cPWV attenuated from 0.376 (0.119; 0.632) to 0.294 (0.048; 0.539) (model 5; Table 2).

If we additionally adjusted the association between glucose metabolism status and δPWV for cPWV; the results showed that the association between T2D and δPWV attenuated from 0.301 (0.013; 0.589) to 0.173 (−0.103; 0.449) (model 5; Table 2).

Additional analyses

The results were not materially altered if we additionally adjusted the association of T2D with cPWV and δPWV for cfPWV (Supplemental Table S1, http://links.lww.com/HJH/A741). In addition, the results were not materially altered if we additionally adjusted the association for SAF, plasma AGEs, insulin or HOMA2-IR (Supplemental Table S3b, http://links.lww.com/HJH/A741). The results did however attenuate if we additionally adjusted the association of T2D with cPWV and δPWV for HbA1c (Supplemental Table S3b, http://links.lww.com/HJH/A741).

The results between T2D and cPWV were not materially altered if we additionally adjusted model 3 for either hypertension based upon office or ambulatory BPs and(or) the use of antihypertensive medication or specifically the use of beta-blockers. In a series of exploratory analyses, the results were also not materially altered if we additionally adjusted model 3 for heart rate (HR) at time of vascular

TABLE 1. General characteristics of the study population according to glucose metabolism status

	Total study population	Normal glucose metabolism ($n = 415$)	Prediabetes (n = 126)	Type 2 diabetes (n = 205)	*P*-value
Demographics					
Men, *n* (%)	416 (56)	194 (47)	75 (60)	147 (72)	<0.001
Age (years)	60 ± 8	57 ± 8	62 ± 7	63 ± 7	<0.001
Blood pressure					
24-h systolic blood pressure (mmHg)[b]	119 ± 12	117 ± 11	122 ± 13	124 ± 13	<0.001
24-h diastolic blood pressure (mmHg)[b]	74 ± 7	74 ± 7	75 ± 8	74 ± 7	0.870
Hypertension (%)[c]	57	40	66	86	<0.001
Hypertension 24 h (%)[d]	44	25	55	74	<0.001
Anti-hypertensive medication (%)	38	21	43	71	<0.001
RAS-inhibitors (%)	29	14	33	57	<0.001
ACE-inhibitors (%)	12	6	10	25	<0.001
Angiotensin II inhibitors (%)	17	9	22	31	<0.001
Renin inhibitors (%)	<1	0	0	1	0.267
Beta-blockers (%)	17	8	21	35	<0.001
Metabolic variables					
BMI (kg/m^2)	27.2 ± 4.4	25.7 ± 3.7	28.1 ± 4.0	29.6 ± 4.6	<0.001
Waist-to-hip-ratio[a]	0.95 ± 0.09	0.91 ± 0.08	0.96 ± 0.08	1.01 ± 0.08	<0.001
Total cholesterol (mmol/l)[e]	5.25 ± 1.20	5.53 ± 1.09	5.56 ± 1.17	4.48 ± 1.09	<0.001
High-density lipoprotein cholesterol (mmol/l)[f]	1.32 ± 0.43	1.43 ± 0.41	1.31 ± 0.37	1.12 ± 0.42	<0.001
Low-density lipoprotein cholesterol (mmol/l)[g]	3.31 ± 1.04	3.59 ± 0.92	3.54 ± 1.01	2.60 ± 0.96	<0.001
Triglycerides (mmol/l)[f]	1.20 (0.86; 1.75)	1.02 (0.74; 1.46)	1.41 (1.01; 2.07)	1.63 (1.15; 2.29)	<0.001
Lipid-modifying medication (%)	35	15	36	75	<0.001
HbA1c (%)[h]	6.0 ± 0.8	5.6 ± 0.3	5.9 ± 0.4	6.9 ± 0.9	<0.001
FPG (mmol/l)[i]	6.1 ± 1.5	5.2 ± 0.4	6.0 ± 0.6	7.8 ± 1.8	<0.001
Glucose-lowering medication					
None (%)	–	100	100	22*	
Oral glucose-lowering medication (%)					
Insulin use (%)	–	–	–	5	
Oral glucose-lowering medication and insulin use (%)	–	–	–	14	
Other cardiovascular risk factors					
Prior cardiovascular disease (%)	18	12	18	29	<0.001
Smoking status: never/former/current (%)	31.1/52.5/16.4	36.9/45.8/17.3	27.8/57.1/15.1	21.5/63.4/15.1	<0.001
eGFR (ml/min per 1.73m^2)	88.3 ± 14.6	90.9 ± 13.7	85.3 ± 14.2	85.0 ± 15.6	<0.001
Albuminuria (%)[j]	7	3	6	16	<0.001
Vascular measurements					
Carotid diameter (mm)	7.83 ± 0.85	7.64 ± 0.82	8.00 ± 0.88	8.11 ± 0.79	<0.001
Intima–media thickness (mm)	0.85 ± 0.15	0.82 ± 0.13	0.88 ± 0.18	0.89 ± 0.15	<0.001
cPWV (m/s)	8.5 ± 1.8	8.1 ± 1.7	8.8 ± 1.8	9.1 ± 1.9	<0.001
δPWV (m/s)	3.9 ± 1.9	3.6 ± 1.8	4.0 ± 1.7	4.4 ± 2.0	<0.001
cfPWV (m/s)[k]	8.9 ± 2.1	8.3 ± 1.7	9.3 ± 2.0	9.8 ± 2.3	<0.001
Mean arterial pressure (mmHg)	98 ± 10	96 ± 10	100 ± 10	99 ± 9	0.002
Systolic blood pressure(mmHg)[l]	129 ± 14	126 ± 14	132 ± 14	132 ± 14	<0.001
Diastolic blood pressure (mmHg)[m]	77 ± 8	76 ± 8	78 ± 7	77 ± 7	0.742
Heart rate (beats/min)[m]	63 ± 9	62 ± 8	63 ± 9	66 ± 10	<0.001
Brachial pulse pressure (mmHg)[n]	52 ± 10	49 ± 9	54 ± 10	55 ± 11	<0.001
Carotid pulse pressure (mmHg)	51 ± 14	48 ± 13	54 ± 14	56 ± 15	<0.001

$N = 746$. Data are presented as mean ± SD, median (interquartile-range) or frequencies (in %) as appropriate. Linear trend across glucose metabolism status was tested by ANOVA for continuous variables normally distributed, log transformed if necessary. For dichotome variables a chi-square test was used.
ACE, angiotensin-converting-enzyme; BMI, body mass index; cPWV, local carotid pulse wave velocity; cfPWV, carotid-to-femoral pulse wave velocity; δPWV, systolic-diastolic difference in carotid pulse wave velocity; eGFR, estimated glomerular filtration rate; HbA1c, glycosylated hemoglobin.
Numbers for specific variables for the total study population, and according to glucose metabolism status are [a]waist-to-hip-ratio 742, 412/126/204; [b]24-h blood pressure measurements 668, 374/112/182; [c]hypertension 745, 414/126/205; [d]hypertension 24 h 704, 381/120/203; [e]total cholesterol 743, 414/125/204; [f]high-density lipoprotein cholesterol, triglycerides 743, 413/125/205; [g]low-density lipoprotein cholesterol 739, 411/125/203; [h]Hb1Ac 742, 412/125/205; [i]FPG 744, 414/126/204; [j]presence of albuminuria 736, 411/125/200; [k]cfPWV 735, 411/124/200; [l]mean SBP 744, 414/126/205; [m]mean DBP and mean HR 745, 415/126/204; [n]brachial PP 744, 414/126/204.
*None in type 2 diabetes consisted of 32 patients with newly diagnosed type 2 diabetes and 13 on which no further information was available.

measurement, BMI, waist circumference or waist–hip ratio, lipid profile, the use of lipid-modifying medication or the presence of albuminuria (Supplemental Table S1, http://links.lww.com/HJH/A741).

The results between T2D and δPWV were not materially altered if we additionally adjusted model 3 for hypertension based upon ambulatory BPs and(or) the use of antihypertensive medication or the presence of albuminuria. In a series of exploratory analyses, the results were materially altered if we additionally adjusted model 3 for hypertension based upon office BPs and the use of antihypertensive medication, BMI or waist circumference or waist-to-hip ratio, lipid profile and the use of lipid-modifying medication, or HR at time of vascular measurement. Overall, the associations attenuated, except for HR in which the association between T2D and δPWV became stronger (Supplemental Table S1, http://links.lww.com/HJH/A741).

TABLE 2. Associations of type 2 diabetes with carotid pulse wave velocity and systolic–diastolic difference in carotid pulse wave velocity (as compared with normal glucose metabolism)

Models	T2D and cPWV		T2D and δPWV	
	β	95% CI	β	95% CI
1. Crude	0.955	(0.654; 1.256)	0.821	(0.516; 1.126)
2. Model 1 + sex, age and MAP	0.377	(0.124; 0.629)	0.311	(0.027; 0.595)
3. Model 2 + prior CVD, eGFR and smoking	0.376	(0.119; 0.632)	0.301	(0.013; 0.589)
4. Model 3 + use of RAS-inhibitor	0.322	(0.051; 0.594)	0.331	(0.026; 0.636)
5. Model 3 + δPWV/cPWV[a]	0.294	(0.048; 0.539)	0.173	(−0.103; 0.449)

$N = 746$. β, regression coefficient; δPWV, systolic–diastolic difference in carotid pulse wave velocity in m/s; CI, confidence interval; cPWV, local carotid pulse wave velocity in m/s; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; MAP, mean arterial pressure; RAS-inhibitor, renin–angiotensin system inhibitor; T2D, type 2 diabetes.
[a]As appropriate. Data of prediabetes as compared with normal glucose metabolism not shown (Table S2, http://links.lww.com/HJH/A741).

DISCUSSION

To the best of our knowledge, the present population-based study is the first to investigate the association between glucose metabolism status and δPWV. We found that T2D, but not prediabetes, was associated with greater δPWV, independent of age, sex, MAP, prior CVD, eGFR and smoking. The association of T2D with δPWV was not abolished by carotid stiffness, which suggests that δPWV contains additional information regarding arterial matrix remodeling, that is beyond carotid stiffness.

Biomechanically, δPWV reflects the degree to which stiffness at lower arterial pressure or distension increases toward greater stiffness at higher pressure or distension. This intrinsic pressure-dependent behavior is caused by the properties and configuration of the elastin collagen matrix (Fig. 1b). In the current study, we utilized T2D as a setting with known increased arterial matrix remodeling to assess the potential of δPWV therein. This increased matrix remodeling in T2D may be the result of increased cyclic and/or metabolic stress due to increased elastin degradation [8–10] and increased collagen crosslinking [10–15,30]. The evidence for the contribution of elastin degradation to increased δPWV in T2D, as supported by studies in the context of vascular aging [8,9,31], appears more indirect. It is found that in the arterial media of stiffer arteries, focal calcifications are colocalized with fragmented elastin [10,32]. However, it remains to be established whether the calcium crystals are responsible for increased stiffness or that the coincident elastin degradation causes the increased stiffness. Nonetheless, the higher prevalence of calcified peripheral arteries in T2D [33] may reflect increased elastin degradation in T2D. With regard to increased collagen crosslinking, it has been hypothesized that this is particularly driven by the formation of AGEs in T2D [10,12,13,15,34,35]. In our study however, further adjustment for SAF or the plasma AGEs – pentosidine, CML and CEL – did not materially alter our results. This could potentially be explained by the fact that SAF and plasma AGEs might not adequately represent AGEs at tissue level of the arterial wall itself [36] and thus may not adequately represent any underlying remodeling processes. Furthermore, it has been shown that AGEs do not necessarily behave similar with deteriorating glucose metabolism status [37] to such an extent that it has been shown that plasma AGEs were associated with left ventricular dysfunction in NGM, but not in T2D. Finally, AGEs do not solely determine matrix remodeling on their own, and other mechanisms (e.g. altered matrix metalloproteinase function) might be operative [38–40]. Interestingly, HbA1c levels did attenuate the association between T2D and, cPWV and δPWV, which may be explained by the fact that HbA1c may also represent disease severity and duration.

Assuming that both elastin-related and collagen-related processes contribute to increased matrix remodeling in T2D, δPWV acts in the current study as a marker of matrix remodeling, describing changes above and beyond carotid stiffness. The latter is supported by our finding that adjustment for cPWV does not abolish the association of T2D with δPWV. Interestingly, the fact that δPWV is also independent of aortic stiffness (cfPWV) further supports the notion that δPWV reflects arterial matrix remodeling above and beyond existing measures of arterial stiffness.

In our additional analyses, we noted that the association of T2D with δPWV attenuated after further adjustment for LDL cholesterol and use of lipid-modifying medication, or BMI. This negative confounding of LDL cholesterol and the use of lipid-modifying medication could be explained by known positive remodeling effects of lipid-modifying medication, which are more often used in individuals with T2D. These positive remodeling effects, including elastin and collagen alterations, are thought to be caused via improving endothelial function and preventing angiotensin II–induced vascular remodeling [41–44]. Therefore, we cannot exclude a mediating role of LDL cholesterol and lipid-modifying medication in the process of arterial matrix remodeling, which may have led to overadjustment in the investigated association with T2D [45]. The negative confounding of BMI in the association between T2D and δPWV could be due to the fact that BMI can be considered as either a confounder or ascending proxy in this association. It has been suggested that weight loss could reduce arterial stiffness and specifically arterial matrix remodeling [46,47], but weight loss could also improve T2D and therefore arterial stiffness and arterial matrix remodeling [48].

Recently, it has been shown that higher HR is associated with a small increase in aortic stiffness [49]. Although T2D is associated with a higher HR due to sympathetic overactivity as part of autonomic neuropathy [50], the association between T2D and carotid stiffness was not affected by HR, whereas the association of T2D with δPWV increased. The first could be explained by the fact that the previously found differences were only small and may be even less prominent in the carotid artery [40]. The latter mechanism is

not exactly known, but possible vasovagal imbalance in T2D and its effect on HR [50] might complicate the interpretation of positive confounding of HR on the association between T2D and δPWV.

Previously, T2D has been associated with structural alterations in the arterial wall in relation to wall stress [51], as reflected by carotid artery geometry. As such, the current study supports the disturbed arterial structure–function relationship in T2D, by considering the dynamic aspect of arterial distension, rather than geometry. Furthermore, although crude analyses of δPWV show a trend among glucose metabolism status, further adjustments for other CVD risk factors showed that prediabetes was not associated with δPWV. Also this finding is in line with Henry *et al.* [51] who found that prediabetes was not associated with carotid arterial remodeling. It might be that in prediabetes, maladaptation is not clearly present yet.

Over the last decade, T2D has been associated with increased carotid stiffness independent of other cardiovascular risk factors [2,52,53]. The current study supports these previous studies by using cPWV as a measure derived from distensibility. Moreover, it has been shown that even before the onset of T2D, signs of abnormal arterial stiffness are evident in individuals with prediabetes, but this is not so clear for specifically carotid stiffness [54]. Although crude analyses of carotid stiffness show a trend among glucose metabolism status, our study supports previous studies [52,54–56] by showing that prediabetes was not associated with carotid stiffness independent of other CVD risk factors.

A major strength of the current study is its use of deep phenotyping that allowed us to adjust for an extensive series of CVD risk factors in a population-based setting, including 24-h ambulatory BP and the use of RAS-inhibitors. Apparently, the association of T2D with cPWV and δPWV is independent of possible known arterial remodeling effects by these medications [29,42,57–59]. However, our study also had some limitations. First, the cross-sectional design of the study does not allow us to draw strong causal inferences. Second, the derivation of δPWV involves combining two measurements (tonometry and ultrasound), in which the aggregate uncertainty of the estimate is of consideration. Nevertheless, the measured mean δPWV 3.9 ± 1.9 m/s ranging from 1.2 to 7.2 m/s in our 746 participants compares well with previous found values of δPWV [6], given that our study population is older. Third, it should be noted that increased δPWV, as applied in the current study, does not allow differentiation between elastin degradation and collagen crosslinking, whereas it reflects both. Finally, our study population consisted of a relatively healthy population, and possible selection bias could have led to underestimation of our results.

Future studies utilizing δPWV should further explore the relative contributions of elastin degradation and collagen crosslinking, such as including plasma biomarkers for oxidative stress, chronic low-grade inflammation and endothelial dysfunction in the analyses.

In conclusion, the systolic–diastolic difference in carotid stiffness is increased in T2D, but not prediabetes. Importantly, the association was not abolished by carotid stiffness, which suggests that systolic–diastolic difference in carotid stiffness carries additional information. As such, the systolic–diastolic difference in carotid stiffness might be considered as a marker of arterial matrix remodeling.

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Conflicts of interest

There are no conflicts of interest.

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Reviewers' Summary Evaluations

Reviewer 1

This is an excellent study in which the authors performed a thorough phenotypic analysis of arterial stiffness in type 2 Diabetes, finding that some arterial matrix remodelling has causative effects on it. The strength of this paper is in its methodology. On the other hand, the small sample and the lack of biomarkers are aspects that require further studies in this area.

Reviewer 2

Authors investigated in a well characterized population-based cohort the association between glucose metabolism status and arterial properties. Individuals with T2 diabetes had a greater carotid diameter, greater intima–media thickness, a higher carotid pulse pressure and higher cPWV and δPWV compared to individuals with normal glucose metabolism and prediabetes, suggesting that functional and structural properties of the arterial tree are seriously damaged in T2 diabetes.