Non-invasive Carotid Pressure-Diameter Loops to Identify Viscoelastic Properties in Ageing, Hypertension and Type 2 Diabetes

Short title: **Carotid Viscosity in Hypertension**

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ABSTRACT

Objectives

Arterial stiffness as pulse wave velocity (PWV) predicts cardiovascular events independently of blood pressure (BP). PWV does not distinguish between stiffness in systole and diastole. This cross-sectional study aimed to test the hypothesis that viscous and elastic carotid wall properties differ between systole and diastole, distinguishing effects of ageing, hypertension and T2 diabetes (T2DM).

Methods

We examined carotid visco-elasticity in 307 people (180 men), with hypertension alone (n=69), combined hypertension /T2DM (H-T2DM, n=99), normotensive (N-)T2DM (n=25) and healthy controls (n=114). Diameter (*D*) /Pressure (*P*) waveforms were measured at right /left common carotid arteries, respectively. Local carotid PWV and distensibility in systole and diastole were evaluated by the D^2P-loop method, and wall viscosity from hysteresis, the area (H_A) within the *P-D* loop, as a dynamic measure of systolic loading and diastolic unloading.

Results

Controls' hysteresis fell quadratically with age (R^2=0.23, p<0.001). Yet mean H_A in hypertensives (0.95, 95%CI 0.65−1.23) was >6-fold higher than in age-matched controls (0.14, -0.20−0.49, p<0.001) with a 2.5x difference between diastolic ($_dDs$) to systolic ($_sDs$) distensibility (p<0.05) in hypertensives. H_A was higher in hypertensives and H-T2DMs (0.80, 0.58−1.04) than N-T2DMs (0.20, -0.17−0.54, p<0.05), but similar between controls and N-T2DMs. BP-adjusted carotid diameters in all T2DM were significantly greater compared with controls and hypertensives.

Conclusions

Higher BP increased wall viscosity, hysteresis and relative difference between systolic and diastolic distensibility across groups. Carotid diameters were increased in all T2DMs, more in H-T2DM, probably altering BP-flow dynamics in T2DM.

SHORT ABSTRACT

Arterial stiffness as measured by pulse wave velocity is a predictor of cardiovascular events. Whilst distensibility in early systole indicates arterial wall visco-elastic properties, in late diastole it indicates the elastic properties. Here, we introduce a technique to isolate the viscous properties of the carotid wall and show that systolic is lower than diastolic distensibility. Carotid artery diameters are increased in people with Type 2 diabetes independently of pressure. While higher blood pressure contributes to increasing wall viscosity and decreased distensibility in all patient groups, in healthy middle-aged and older adults, wall viscosity falls while arterial stiffness still increases with age.

Key Words: systolic-diastolic viscoelastic properties, hypertension, diabetes, ageing, distensibility, local pulse wave velocity.

INTRODUCTION

Arterial stiffness, estimated by aortic pulse wave velocity (PWV), is a powerful predictor of mortality independent of known risk factors including blood pressure (BP) [1,2]. Patients with hypertension or diabetes generally have increased PWV compared with healthy people independently of BP differences [3–6]. Further, carotid function predicts stroke events and cognitive decline independently of aortic PWV and BP [7,8]. However, the complex mechanical behaviour of the arterial wall and consequent arterial stiffening over time result from interaction of viscous and elastic elements. Viscous forces, depending on smooth muscle cell (SMC) content [9,10], act in response to abrupt changes in blood pressure (BP) so are high during the systolic upstroke and negligible during arterial wall recoil when luminal pressure decreases in diastole. Therefore, PWVs measured in systole with the 'foot-to-foot' techniques (eg: carotid-femoral PWV, brachial-ankle PWV) reflect a combination of both viscous and elastic wall properties, while wall elasticity would be better detectable in diastole. Additionally, such PWVs over long arterial lengths describe average mechanical properties which may correspond poorly with local changes in arterial stiffness. Techniques, such as the *PU*-loop [11] and the ln*DU*-loop [12], help to define these local mechanical arterial properties *in-vivo*, allowing the evaluation of how ageing, various risk factors, and vascular disease affect arterial stiffness at specific arterial locations [12]. These methods estimate local PWV from arterial waveforms in early systole therefore assessing wall viscosity and elasticity combined. Conversely, the D^2P-loop method, introduced by Alastruey [13], estimates local PWV from the pressure (P) and diameter (D) relationship in late diastole when viscous forces are at their minimum with relatively slow elastic recoil of the arterial wall, allowing elastic features to be assessed more independently of complex viscous properties.

The relationship between changes in P and D^2 (which, under the assumption of a circular cross-sectional area, is proportional to changes in the luminal area) defines arterial

distensibility ($Ds = (dD^2 \,/\, D^2 dP)$ [14]. *Ds* describes the ability of the arterial wall to distend and store blood and elastic energy in systole then releasing that energy during diastole, pushing blood downstream in the systemic and coronary circulations. While a single value of *Ds* is commonly assumed throughout the cardiac cycle, *Ds* is not necessarily equal in systole and diastole [15]. Unlike Hermeling *et al.'s* work [14] which indicated different arterial wall systolic and diastolic stiffness at respective pressures, the present work adopts a different approach; we focus on differences between distensibility (*Ds*) during the systolic upstroke (so ${}_{s}Ds$) and during the elastic recoil that characterises the entire diastolic phase (so ${}_{d}Ds$) of the cardiac cycle, as done previously [15].

Arterial wall viscosity was typically characterised *ex-vivo* when wall tissue was subjected to dynamic loading to quantify the variation of arterial stiffness with load frequency [16,17]. Due to wall viscosity, stress-strain or *P-D* graphs display different dynamic paths with systolic loading and diastolic unloading curves, creating an area, hysteresis (H_A), enclosed between the two curves [17]. Alteration of *in-vitro* arterial viscosity with age and pathology has long been known [10]. However, the impact of wall viscosity on arterial distensibility *in-vivo* is still uncertain, as it was so far investigated by acquiring arterial waveforms in only a few studies [14,18,19].

Here, we aimed to refine age and pathology-related changes in viscous and elastic properties of the human carotid artery wall *in-vivo*. We hypothesised that carotid viscosity, derived from differences between systolic and diastolic distensibility and local wave speed, would illustrate pathological changes to discriminate between controls, patients with hypertension without diabetes, with type 2 diabetes mellitus without defined hypertension (N-T2DM), and with both T2DM and hypertension (H-T2DM).

MATERIAL AND METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Population

Clinical data were recorded in Pisa and Massa (Tuscany, Italy) hospitals from 2015 to 2020. The study sample came from individuals undergoing standard out-patient cardiovascular risk assessment, omitting anyone with histories of major cardiovascular events, atrial fibrillation, malignancy or chronic inflammatory disease. All patients were free of intimal thickening >2.0mm, and all measurements were performed at 1.5 cm from the carotid bulb or a few mm apart when focal intimal thickening (small plaque) was detected. Hypertension was defined as BP ≥ 140 and/or 90 mmHg or active antihypertensive therapy. Controls were those free from therapy, not hypertensive and normoglycaemic. Only those over 50 years were compared to patients with hypertension or diabetes. The protocol of the study followed principles of the Declaration of Helsinki and was approved by the institutional ethics committee "Comitato Etico di Area Vasta Nord Ovest" (reference number: 3146/2010). All subjects gave their informed consent to participate. The final study population included 55 healthy controls, 69 hypertensives, 25 N-T2DMs, and 99 H-T2DMs.

Study Protocol

Vascular examination was performed in the morning, at least 2 hours after a light breakfast, in a quiet room with a stable temperature of 22°, after resting comfortably for at least 15 min in the supine position. All subjects were asked to abstain from cigarette smoking, caffeine, alcohol consumption and vigorous physical activity for 24 hours.

Brachial Systolic (SBP_b) and Diastolic Blood Pressure (DBP) were measured by a digital Omron device (model 705cp, Kyoto, Japan). Pressure waveforms were recorded on the left

common carotid artery by tonometry (PulsePen, DiaTecne, Milan, Italy) with a sampling frequency of 500-1000 samples/s according to the PulsePen model used. PulsePen recordings were calibrated assuming constant DBP and mean BP (MBP) along the arterial tree, with brachial DBP and SBP as reference values.[20] MBP was estimated by the widely used equation [21,22], $\mathrm{MBP} = \mathrm{DBP} + (\mathrm{SBP_b} - \mathrm{DBP})/3$.

Diameter distension waveforms were acquired using ultrasound echotracking systems (Aloka Prosound 10, Hitachi Ltd., Japan, 1000 samples/s, or MyLabOne, Esaote SpA, Italy, 660 samples/s, with RF-data output) on the right common carotid artery. When acquisition quality was high, the diameter (ultrasound) and pressure (PulsePen) waveforms were recorded simultaneously on the right and left carotid arteries, respectively, for correspondence in heartbeats. When accurate simultaneous acquisition was not possible, the two signals were acquired sequentially, minimising delay between the two acquisitions.

Intima-media thickness (IMT) was estimated on the far wall of the right common carotid artery using a 10-MHz linear probe implemented with automatic, radiofrequency-based tracking of arterial wall with high spatial resolution (QIMT®, Esaote MyLab70 and MyLabOne, Esaote SpA, Genova, Italy) [23].

Data analysis

A custom Matlab (The MathWorks, Inc., MA) code was used for analysing *P* and *D* waveforms, incorporating the Savitzky-Golay smoothing filter to increase the signal-to-noise ratio. When *P* and *D* had been acquired simultaneously, the analysis was performed only on simultaneously recorded 7-10 cardiac cycles.

Different ultrasound systems might apply different filters on the acquired arterial waveforms and ECG signals, increasing signal-to-noise ratio, thus introducing unknown delays between the two. To avoid a possible misalignment between *P* and *D*, the second derivative of the two

signals was calculated; using its first and second major peaks, we identified the foot of the wave and the dicrotic notch, respectively, and considered these as a reference points to correct for any delay between *P* and *D* [14].

Characterisation of arterial stiffness

Carotid PWV (cPWV) was estimated using the D^2P-loop method [13], which assumes that in late diastole the viscous forces tend to a constant close to 0 allowing estimation of a wave speed describing the elastic properties of the wall. Therefore, we identified late diastole as the diastolic decay portion of the D^2P-loop delimited by DBP and the pressure at the dicrotic notch (**Fig.1A-1B**). Linear regression was then performed on the D^2P-loop in late diastole (**Fig.1B**), and cPWV was calculated as:

$$\mathrm{cPWV} = {}_{\mathrm{d}}D\sqrt{\frac{\mathrm{d}P}{\rho\ \mathrm{d}(D^2)}} \tag{1}$$

where ${}_{\mathrm{d}}D$ is the diastolic diameter, dP the change in pressure, d(D^2) is the change in squared diameter and ρ is the blood density assumed equal to 1060 kg/m^3. Distensibility in diastole (${}_{\mathrm{d}}Ds$) was calculated as

$${}_{\mathrm{d}}Ds = \frac{1}{\rho\ \mathrm{cPWV}^2} \tag{2}$$

To verify the impact of using sequentially acquired *P* and *D* signals on cPWV, we carried out a preliminary study on N=25 individuals, randomly chosen between those whose *P* and *D* had been acquired simultaneously, where we calculated cPWV by sequential and simultaneous acquisition analysis as above. There, differences between the subjects' cPWV with the two analysis protocols was much smaller than the intra-subject variability of cPWV across different heartbeats (**Table S3**); we concluded that the acquisition modality was unlikely to have affected results accuracy.

Additionally, Equation 1 was also applied to the early systolic part of the D^2P-loop to evaluate the distensibility in systole ($_sDs$) (**Fig.1B**), as viscous forces are then at their maximum due to the abrupt increase in BP and luminal diameter. Therefore, we expect that $_sDs$ describes both elastic and viscous properties. Early systole was defined using the same criteria adopted for late diastole but applied to the systolic part of the D^2P-loop, i.e.: the portion of the systolic upstroke delimited by DBP and the pressure at the dicrotic notch (**Fig.1A-1B**). This choice ensured that both $_sDs$ and $_dDs$ were calculated over the same pressure range, eliminating the issue of the pressure-dependency of arterial stiffness in the comparison.

Characterisation of arterial viscosity

Two indices were used to measure carotid wall viscosity. The first was the *PD*-loop H_A, as 'hysteresis', the area enclosed in the loop quantifying the difference between systolic and diastolic paths (**Fig.1C**). H_A was calculated both in absolute terms and as the hysteresis index (H_I) by normalising H_A with respect to Pulse Pressure (PP) (i.e. SBP-DBP) and Pulse Diameter (ΔD) (i.e. $_sD$-$_dD$) (**Fig.1C**). H_I is therefore unaffected by inter-subject variability of size and pressure/diameter variation and quantifies the distance between the systolic and diastolic portion of the *PD*-loop. The second parameter is the relative difference between $_sDs$ and $_dDs$ (ΔDs). We hypothesised that, as the hysteresis increases, the relative difference between $_sDs$ and $_dDs$ would also increase and therefore provide additional insight into arterial viscosity (**Fig.1B**).

Arterial viscosity was evaluated only on the subgroup of participants who had simultaneous recordings of *P* and *D* (in 94 controls, 22 N-T2DM, 91 H-T2DM and 41 hypertensive patients). H_A depends on the correct alignment of the *P* and *D* waveforms over the entire duration of the cardiac cycle, which can be achieved only when the two signals are acquired simultaneously.

Statistical analysis

Simple analysis was performed for each outcome variable by and across clinical groups, using SPSS 23 (SPSS, Chicago, IL) and taking $p<0.05$ for statistical significance.

Analysis of ageing effects examined our whole sample of controls (n=114) with an age range 18-80 years, using correlation and regression analysis.

Then, the control-pathology comparisons were carried out in two ways: first, between hypertensives, N-T2DMs, H-T2DMs and controls >50 years (55 out of the 114 controls) (**Tables 1** and **2**). This analysis progressed first with age and sex adjustments, then with other potential confounders, as shown in the Tables, using covariance ('ANCOVA'). These confounders included the different ultrasound devices in the two hospitals (Hitachi-Aloka in Pisa; Esaote in Massa), as well as antihypertensives, oral antidiabetic drugs, insulin and statin treatments. BP adjustments of arterial stiffness metrics were carried out with respect to DBP as Eq.1 depends chiefly on DBP.[24] Note also that ${}_{s}Ds$ and ${}_{d}Ds$ were estimated over the same BP range and, hence, both adjusted for DBP (**Fig.1**). For each parameter, when the permissive ANCOVA reached statistical significance, we proceeded with the post-hoc pairwise comparison between groups (Bonferroni test).

In the second analysis, we removed potentially artificial BP categories (hypertensive or not), the data were used continuously in the regression analysis, no longer for each category but for the outcomes of interest, with carotid diameters, H_A, ${}_{s}Ds$ and ${}_{d}Ds$, as the dependent variable, and mean pressure, treatments and T2DM or not as exposures (**Table 3**).

RESULTS

Haemodynamic data for our study sample of 55 healthy controls over 50 years, 69 patients with hypertension without T2 diabetes, 25 N-T2DMs and 99 H-T2DM are shown in **Table 1** with details of drug treatment in **Table S1**.

The groups were reasonably comparable, with a higher proportion of women and slightly younger mean ages in controls; age was an adjustment for all comparisons (**Table 1**). Dyslipidemia persisted in 49% of hypertensive, 28% N-T2DM and 77% of H-T2DM patients, despite statin treatment in 25%, 16% and 73% respectively. Of the H-T2DMs, 93% were on antihypertensive treatment; their BP did not differ from controls (Table 2). Conversely, only 59% of the plain hypertensive patients, without T2DM, were on antihypertensives, so SBP and DBP were significantly higher in these hypertensives than in either controls or T2DM patients.

Effect of ageing in healthy controls

Mean age in all 114 healthy controls was 46±18 (range 18-80 years). As expected, BP, mainly SBP, carotid diameter and IMT increased with age ($p<0.001$-**Table S2)**.

Examples of changes in the PD-loop with age are presented in **Fig.2A-B**. cPWV increased strongly with age after adjusting for DBP and HR ($r=0.71$, $p<0.001$), ranging from approximately 3.9 m/s to 7.3 m/s in the investigated age-range (**Online Fig.1**). Both ${}_{s}Ds$ and ${}_{d}Ds$ clearly showed an opposite trend, indicating a decrease in wall elasticity with age. Interestingly, carotid *Ds* averaged 10% lower in systole than in diastole ($p<0.001$). Moreover, their relative difference (ΔDs) fell with age, as did H_A and H_I ($p<0.001$ for all) decreasing from approximately 1.77 mmHg·mm and 0.069, respectively, to 0.18 mmHg·mm and 0.005 in the investigated age-range (**Fig.3A-B**). These changes signify a decrease of arterial viscosity with age.

Controls, T2DM and hypertension (Tables 1, 2 and 3)

The calibre (diameter) of the carotid artery increased with age in all groups but N-T2DM, even after adjusting for BP changes. In the multivariate regression analysis (i.e., discarding arbitrary definition of hypertension), T2DM was a significant predictor of increased carotid

diameter (both diastolic and systolic $p<0.01$), independently of BP and HR (**Table 3** and **Fig.4**). Average IMT was 0.65 mm in controls, 0.74 mm in both H-T2DM and hypertensive people and 0.75 in N-T2DM patients (not significant).

While carotid *Ds* was obviously lower in systole than in diastole in all clinical groups ($p<0.001$), Δ*Ds* was significantly greater in hypertensives than in controls ($p<0.05$). Although accentuated in H-T2DMs, Δ*Ds* in N-T2DMs and H-T2DMs did not differ from that of other groups (**Table 2**). As a result, while neither diastolic (cPWV and $_{d}Ds$) nor systolic ($_{s}Ds$) indices of arterial stiffness differed between clinical groups after adjusting for age, sex, HR, DBP, ultrasound machine, and drug treatments; differences among groups were more accentuated in the systolic phase than in diastole. Indeed, MBP significantly contributed to increasing Δ*Ds* (**Table 3**).

Examples of *PD*-loops for representative control, hypertensive, N-T2DM and H-T2DM older adults (>60 years) are displayed in **Fig.2C-D**. H_A and H_I were significantly higher, at over double, in plain hypertensives than in both controls ($p<0.001$ for both) and N-T2DMs ($p<0.05$ for both), but not than in H-T2DMs (**Table 2**). Further, H_A in N-T2DMs was significantly lower than in H-T2DMs ($p<0.05$) and comparable to that of controls. These findings were confirmed by the multivariate regression analysis (**Table 3**) where MBP and antihypertensive treatment, and not T2DM, played a significant role in determining wall viscosity indices.

To test whether Δ*Ds* was indicative of the wall viscosity, we compared differences among clinical groups when adjusting for H_A or H_I. In both cases, the inclusion of hysteresis parameters in the ANCOVA analysis made differences among groups not significant; indicating that Δ*Ds* is 'driving' the hysteresis. Further, Δ*Ds* significantly correlated with both H_A and H_I ($r=0.44$ and 0.49, $p<0.0001$).

DISCUSSION

In this paper we investigated the relationship between carotid pressure and diameter waveforms throughout the cardiac cycle, aiming to improve understanding of the carotid wall's viscoelastic properties. The method here separates effects of the viscous systolic phase from the elastic recoil diastolic phase in healthy control, 'plain' hypertensive and T2DM patients. Our main findings are:

(1) Introducing a technique to separate carotid systolic from diastolic distensibility allowed us to examine the cardiac cycle dependence of distensibility (or its inverse stiffness). Distensibility in both systole and diastole decreased with age in controls. Note that the difference between systolic and diastolic distensibility (ΔDs) is pressure-dependent, being significantly higher in plain hypertensives than controls, whereas T2DMs, at lower BPs here, had intermediate ΔDs.

(2) All viscosity indices, whether as hysteresis area in the *P-D* loop, hysteresis index or difference between distensibility in systole and diastole, also decreased with age in controls.

(3) Despite the age effect, hysteresis was significantly greater in hypertensives than in controls and normotensive T2DMs. Hysteresis in hypertensive T2DMs was significantly greater than in normotensive T2DMs.

(4) Carotid systolic and diastolic diameter were significantly higher in T2DMs than in controls and hypertensives, independently of BP. Conversely, here systolic-diastolic diameter differences were similar in all groups, and between controls and plain hypertensives were mainly caused by BP differences where adjusted diameters were comparable.

The viscoelastic properties of the arterial wall are determined by its complex microstructure and composition [17]. Collagen and elastin provide passive stiffness and elastic properties to the wall, which can be controlled by active contraction of SMCs [17], also modulating the viscous properties of the arterial tissue [9,10]. Therefore, the analysis of the D^2P-loop,

including differences between systolic and diastolic stiffness provide insight into the underlying changes in arterial microstructure associated with ageing, different risk factors and cardiovascular pathologies.

Previous studies have shown the age-related stiffening of elastic arteries using both regional [1] and local [12,25] estimates of PWV. Our findings confirm such results but with cPWV almost doubling across the age range here. Further, although ${}_{s}Ds$ and ${}_{d}Ds$ were determined in the same pressure range, namely the dicrotic notch to diastolic pressure (**Figure 1**), interestingly and in agreement with previous results [15], we found a significantly higher *Ds* in late diastole compared to early systole (~12% relative difference). This means that a given increase in pressure in early systole produces a smaller diameter distension compared to the elastic recoil obtained with a decrease in pressure of the same size in late diastole, suggesting that arterial pressure has to engage both elastic and viscous forces to dilate the vessel in early systole. Furthermore, ΔDs was not constant with age but decreased from ~17% in young controls (<35 years) to 6% in people >50 years.

Here for the first time *in-vivo*, we report a negative correlation between hysteresis of the *PD*-loop and age, suggesting a decrease in the viscous properties of the arterial wall. Our findings agree with those somewhat forgotten by Learoyd and Taylor >50 years ago in an *ex-vivo* study on the human carotid artery [10]. Arterial wall viscosity is commonly associated with the muscular component of the arterial wall as higher viscous properties occur in arteries with greater SMC numbers [9,10]. Therefore, our results likely reflect established microstructural changes of the arterial media related to age, with SMCs migrating to the intima while abandoning their contractile phenotype [26]. Boutouyrie et al. [18] also studied arterial wall viscosity, as the *PD*-loop hysteresis, in mice both *in-vivo* and *in-vitro* under different levels of smooth muscle tone, and found a higher viscosity *in-vitro* independently of the pressure level, suggesting that the viscous effect might be relatively small *in-vivo*.

Using a similar acquisition protocol and signal alignment method to the ones here, Hermeling and colleagues studied the pressure-diameter relationship in 21 patients (age 64±12 years) finding negligible hysteresis [14]. Given the different scope of their work, their small numbers and no clinical background of included subjects, our results indicate that H_I in healthy middle-aged and older individual is indeed relatively small (**Fig.2**). By contrast, in younger subjects, a higher hysteresis area is associated with markedly increased difference between diastolic and systolic distensibility.

PWV is intrinsically pressure-dependent [27]. Therefore, increased PWV is expected in both hypertensives and T2DMs with higher BP than healthy normotensives. However, several studies have reported increased PWV independently of BP. Zhang et al. [6] reported significantly higher regional PWVs in Chinese people affected by T2DM but not hypertension when compared to age-matched healthy controls. Laugesen and colleagues [28] reported a higher average carotid-femoral PWV in T2DM people than in controls with similar 24h ambulatory BP. Here, diastolic indices of arterial stiffness (cPWV and ${}_dDs$), that best describe the purely elastic properties of the arterial wall [13], were, indeed, accentuated (higher cPWV and lower ${}_dDs$) in T2DMs (both N-T2DM and H-T2DM) and hypertensives than in controls, but differences were not significant after adjusting for confounding factors (mainly BP, age, and sex). Similar findings have been reported previously when the elastic exponential *P-D* relationship of the common carotid artery was estimated by iteratively minimising the area of hysteresis in the *PD*-loop of normotensive and hypertensive people [19]. Conversely, all the viscosity indices (H_A, H_I, and ΔDs) were significantly higher in hypertensives than in controls, so that differences in ${}_sDs$, describing a combination of both elastic and viscous wall response, were greater than those in diastolic stiffness, if not significant. Therefore, in agreement with Armentano *et al.* [19], we report increased wall viscosity *in-vivo* in humans associated with hypertension, contributing to increasing wall stiffness in systole. Further, similar differences

were also observed in T2DM, with H_A significantly higher in H-T2DMs than in N-T2DMs, while comparable viscosity indices were found among normotensives (controls vs N-T2DMs) and hypertensives (plain vs H-T2DMs).

Most methods using the 'foot-to-foot' technique, estimate PWV from early systole when viscous forces reach their maximum. Therefore, the positive correlation between H_A and ΔDs found here suggests that reported differences in PWV between hypertensive, T2DM and healthy people might reflect, at least in part, different levels of wall viscosity rather than purely elastic mechanical properties of the arterial wall. Additionally, regional and local PWVs must be compared with caution, as they describe average properties of relatively long sections and properties of single short sections of the arterial tree, respectively. Therefore, changes in local PWV might not reflect changes in regional PWV as ageing and pathologies act differently in different regions of the arterial tree [12,19].

The finding of higher diastolic and systolic carotid diameters in T2DM patients compared to controls and hypertensive patients, independently of BP, confirm a previous observation of our group [29]. We also reported a direct relation of matrix metalloproteinases 12 (MMP-12) with interleukin 6 and 8 (IL-6 and IL-8), and a direct correlation of MMP-12 with carotid inter-adventitial diameter independent of age and blood pressure. With MMP-12a being a potent elastase highly expressed in macrophages, these previous findings support the hypothesis that changes here in carotid diameter and distensibility in T2DM patients may depend in part on enhanced expression of the inflammatory mediators inducing MMPs activation with a consequent extracellular matrix alteration.

How the current results are related to or even induce changes in the microvasculature so characteristic of T2DM was not studied here but it is established that carotid and systemic PWV is related to ophthalmic blood flow [30] and retinopathy, as well as consistently with stroke,

brain function and cognitive decline [7,31]. The same relationship applies in other arterial beds, including the kidney [32] and lower limbs /feet.

Study limitations

While our clinical matching of patient age and sex was reasonable, it was not perfect which might have affected the results, although further adjusting for these features was carried out. Furthermore, hypertension was differentially controlled in the plain hypertensives and H-T2DMs. As a result, only plain hypertensives had significantly higher BP compared to all other groups. While this fact reflected also on inter-group differences in unadjusted cPWV (considerably increased in plain hypertensives), these differences were considerably reduced after statistical adjustments (mainly DBP), suggesting that, in agreement with previous studies [19,33], increased BP unlikely has chronic effects on CCA stiffness.

Accurate simultaneous acquisition of *P* and *D* waveforms on the two contralateral carotid arteries was not possible for all patients; in ~19% of the total number of recruited subjects the two signals were acquired sequentially but minimising the intercurrent time between acquisitions. The estimation of the wave speed via the D^2P-loop method requires the correct alignment of only the late diastolic portion of the *P* and *D* waveforms, which can be achieved also on sequentially acquired data. Our preliminary analysis showed that the accuracy of the estimation of cPWV was unaffected by the type of analysis (sequential or simultaneous recordings) conducted on the simultaneously acquired data. However, H_A is determined by a precise alignment over the entire length of the heartbeat. As this objective is poorly achievable with sequential waveforms, we excluded such participants from analysis of arterial wall viscosity.

A limitation of this study is the use of two different ultrasound systems for the acquisition of the diameter distension waveform in the two centres (Pisa and Massa). As previously

mentioned in the Methods, different raw data filtering used by the two companies likely introduced different delays of the acquired signals with respect to the ECG waveforms, complicating the signal alignment. To evaluate the effect this might have on the estimated viscosity indices, we analysed the dataset with simultaneous measures taken only with Aloka device and found very similar results to those obtained for the entire cohort (see **Table 4 vs Tables 1 and 2**). Furthermore, we previously reported the existence of systematic difference in the echotracking achieved using the two ultrasound systems [34]. For this reason, the ultrasound system has been considered as a potential confounder for all the parameters that were directly or indirectly derived from the diameter distension waveform. Only ΔD was significantly affected by the ultrasound system (**Table 3**), while no significant interaction was observed with cPWV and viscosity parameters.

Conclusions

Analysing the systolic-diastolic dependence of arterial distensibility allows assessment of the viscous and elastic properties of the arterial wall. While arterial stiffness increases with age, carotid wall viscosity falls in middle-aged and older healthy adults. However, despite its decline with age, wall viscosity was positively associated with rising BP in those with or without hypertension, and in T2DM, where carotid diameters were also increased. The increased arterial wall viscosity, likely related to the SMC status, suggests a new mechanism for arterial stiffening as determined by systolic metrics with 'traditional' (foot-to-foot) PWV. Further studies can examine whether changes in viscoelastic wall properties are related to and efficiently predict clinical events such as stroke, other cerebral events and cardiovascular disease.

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TABLES

Table 1. Haemodynamic features comparing: healthy older controls, hypertensives, and hypertensive and normotensive type-2 diabetes mellitus (T2DM) patients.

	Control (>50 years) **N=55**	**Hypertensive** **N=69**	**Normotensive T2DM** **N=25**	**Hypertensive T2DM** **N=99**
Male [%]	49	58	64	68
Age [Years]	61 ± 8	65 ± 10	62 ± 9	65 ± 7
	$51 - 80$	$38 - 81$	$41 - 82$	$45 - 80$
HR [bpm]	61.1 ± 8.8	61.7 ± 9.0	68.9 ± 9.5	70.2 ± 10.9
SBP_b [mmHg]	125.2 ± 19.8	144.5 ± 20.0	129.2 ± 13.9	128.8 ± 19.1
SBP_c [mmHg]	113.4 ± 18.2	131.0 ± 17.5	117.4 ± 13.4	116.7 ± 17.2
DBP [mmHg]	73.7 ± 8.9	81.5 ± 9.2	79.2 ± 10.2	76.8 ± 9.6
MBP [mmHg]	91.4 ± 11.4	103.4 ± 11.2	96.4 ± 10.8	94.7 ± 11.8
PP [mmHg]	39.7 ± 15.0	49.5 ± 14.7	38.2 ± 9.0	39.9 ± 13.0
Aloka:Esaote	22: 33	24: 45	21: 4	90: 9

IMT [mm]*	0.69 ± 0.13	0.74 ± 0.13	0.75 ± 0.13	0.74 ± 0.15
${}_{s}D$ [mm]†	7.90 ± 0.88	8.17 ± 0.87	8.24 ± 0.58	8.63 ± 1.10
${}_{d}D$ [mm]‡	7.43 ± 0.84	7.69 ± 0.85	7.80 ± 0.54	8.17 ± 1.08
ΔD [mm]§	0.47 ± 0.13	0.48 ± 0.14	0.43 ± 0.12	0.45 ± 0.15
cPWV [m/s]‡	5.61 ± 1.07	6.47 ± 1.29	5.96 ± 1.28	5.98 ± 1.27
${}_{d}Ds$ [MPa^{-1}]‡	36.14 ± 13.24	28.48 ± 11.71	31.19 ± 11.14	31.47 ± 14.40
${}_{s}Ds$ [MPa^{-1}]‡	33.32 ± 11.23	24.82 ± 10.75	28.27 ± 9.79	28.31 ± 12.88
ΔDs [%]	5.8 ± 13.0	13.2 ± 10.3	8.7 ± 7.8	9.5 ± 9.1
H_A [mmHg mm]*	0.32 ± 0.34	0.96 ± 0.85	0.36 ± 0.23	0.69 ± 0.68
H_I [-]	0.022 ± 0.020	0.048 ± 0.037	0.023 ± 0.015	0.039 ± 0.027

Data are unadjusted mean ± standard deviation. Symbols indicate the covariates included in the ANCOVA for each parameter (age and sex were included for all). Adjusted parameters are presented in Table 2.

Additional parameter-specific adjustment: *Ultrasound machine (US); †US, SBP, HR; ‡US, DBP, HR; §US, PP, HR; the main outcome parameters were also adjusted for treatments (antihypertensives, statin, oral antidiabetics and insulin).

cPWV=local carotid pulse wave velocity, DBP=diastolic blood pressure, $_{d}D$=carotid diameter at DBP, $_{d}Ds$=distensibility in late diastole, $\Delta D = {}_{s}D - {}_{d}D$, $\Delta Ds\% = ({}_{d}Ds - {}_{s}Ds)/{}_{d}Ds$=change in Distensibility as a %, H_A=hysteresis area, and H_I=hysteresis index, HR=heart rate, IMT=intima media thickness, MBP=mean blood pressure, PP=pulse pressure, SBP_b=brachial systolic blood pressure, SBP_c=carotid systolic blood pressure, $_{s}D$=carotid diameter at SBP_c, $_{s}Ds$= distensibility in early systole.

Table 2. Results of the ANCOVA analysis; between Controls, Hypertensives and hypertensive and normotensive type-2 diabetes mellitus (T2DM) patients.

	Control (>50 years) **N=55**	**Hypertensive** **N=69**	**Normotensive T2DM** **N=25**	**Hypertensive T2DM** **N=99**
HR [bpm]	60.1 [57.5 – 62.7]	61.9 [59.6 – 64.2]	68.5 [64.7 – 72.3] †§	70.8 [68.8 – 72.7] ‡#
SBP_b [mmHg]	127.7 [122.9 – 132.6]	144.0 [139.7 – 148.2] ‡	130.4 [123.8 – 137.5] ‖	127.4 [123.8 – 131.0] #
SBP_c [mmHg]	115.6 [111.2 – 120.0]	130.6 [126.7 – 134.4] ‡	118.4 [111.9 – 124.8] ‖	115.6 [112.3 – 118.8] #
DBP [mmHg]	73.9 [71.4 – 76.4]	81.7 [79.5 – 83.8] ‡	78.9 [75.2 – 82.5]	76.7 [74.8 – 78.5] ‖
MBP [mmHg]	92.0 [89.4 – 94.7]	103.0 [100.4 – 105.6] ‡	96.3 [91.9 – 100.7]	93.7 [91.6 – 96.0] #
PP [mmHg]	41.7 [38.4 – 45.0]	48.9 [46.0 – 51.8] †	39.5 [34.7 – 44.4] ‖	38.9 [36.4 – 41.3] #
IMT [mm]	0.70 [0.66 – 0.74]	0.72 [0.69 – 0.76]	0.77 [0.71 – 0.83]	0.74 [0.70 – 0.77]
$_sD$ [mm]	8.07 [7.82 – 8.32]	8.04 [7.82 – 8.27]	8.30 [7.96 – 8.64]	8.61 [8.41 – 8.81] *‖
$_dD$ [mm]	7.59 [7.34 – 7.83]	7.63 [7.41 – 7.86]	7.84 [7.51 – 8.17]	8.13 [7.93 – 8.32] *§
ΔD [mm]	0.46 [0.42 – 0.50]	0.44 [0.41 – 0.47]	0.46 [0.40 – 0.51]	0.49 [0.45 – 0.52]
cPWV [m/s]	5.97 [5.51 – 6.43]	6.31 [5.93 – 6.70]	6.02 [5.47 – 6.56]	5.88 [5.49 – 6.26]

${}_{d}Ds$ [MPa^{-1}]	34.8 [28.3 − 41.3]	30.3 [28.3 − 35.9]	32.0 [25.4 − 38.6]	31.0 [26.7 − 35.3]
${}_{s}Ds$ [MPa^{-1}]	32.4 [26.8 − 38.1]	26.7 [21.8 − 31.6]	29.5 [23.8 − 35.2]	27.5 [23.8 − 31.3]
ΔDs [%]	4.8 [0.5 − 10.1]	13.8 [9.4 − 18.1] *	6.8 [1.3 − 12.4]	10.1 [6.5 − 13.6]
H_A [mmHg mm]	0.14 [−0.20 − 0.49]	0.95 [0.65 − 1.23] ‡	0.20 [−0.17 − 0.54] §	0.80 [0.58 − 1.04] **
H_I [-]	0.018 [0.003 − 0.032]	0.048 [0.036 − 0.060] ‡	0.018 [0.003 − 0.034] §	0.041 [0.032 − 0.051]

Data are presented as adjusted mean [95% confidence interval].

cPWV=local carotid pulse wave velocity, DBP=diastolic blood pressure, ${}_{d}D$=carotid diameter at DBP, ${}_{d}Ds$=distensibility in late diastole, ΔD= ${}_{s}D$- ${}_{d}D$, $\Delta Ds\%$=(${}_{d}Ds$ - ${}_{s}Ds$)/${}_{d}Ds$ =change in Distensibility as a %, H_A=hysteresis area, and H_I=hysteresis index, HR=heart rate, IMT=intima media thickness, MBP=mean blood pressure, PP=pulse pressure, SBP_b=brachial systolic blood pressure, SBP_c=carotid systolic blood pressure, ${}_{s}D$=carotid diameter at SBP_c, ${}_{s}Ds$= distensibility in early systole.

*p<0.05, †p<0.01, and ‡p<0.001 with control. §p<0.05, ‖p<0.01, and #p<0.001 with hypertensive. **p<0.05, ††p<0.01 with normotensive T2DM.

Table 3. Results of the multivariate regression analysis.

Outcomes →	IMT	${}_{s}D$	${}_{d}D$	ΔD	cPWV	${}_{d}Ds$	${}_{s}Ds$	ΔDs	H_A	H_I
Tested variables										
Sex	–	$-0.31 \pm 0.06^{\ddagger}$	$-0.31 \pm 0.06^{\ddagger}$	–	–	–	–	–	–	–
Age	$0.37 \pm 0.06^{\ddagger}$	$0.22 \pm 0.06^{\ddagger}$	$0.22 \pm 0.06^{\ddagger}$	–	$0.27 \pm 0.06^{\ddagger}$	$-0.31 \pm 0.07^{\ddagger}$	$-0.31 \pm 0.07^{\ddagger}$	–	–	–
US	–	–	–	$0.22 \pm 0.08^{\dagger}$	–	–	–	–	–	–
HR	–	–	–	$-0.18 \pm 0.09^{*}$	–	–	–	–	$-0.22 \pm 0.11^{\dagger}$	–
MBP	–	$0.12 \pm 0.06^{*}$	$0.13 \pm 0.06^{*}$	–	$0.40 \pm 0.06^{\ddagger}$	$-0.38 \pm 0.07^{\ddagger}$	$-0.43 \pm 0.06^{\ddagger}$	$0.25 \pm 0.08^{\dagger}$	$0.31 \pm 0.10^{\ddagger}$	$0.22 \pm 0.08^{\dagger}$
T-HTN	–	–	–	–	–	–	–	–	$0.21 \pm 0.08^{\dagger}$	$0.25 \pm 0.08^{\dagger}$
T2DM	–	$0.21 \pm 0.08^{\dagger}$	$0.20 \pm 0.08^{\dagger}$	–	–	–	–	–	–	–

Data are presented as beta ± standard error, with $^{*}p<0.05$, $^{\dagger}p<0.01$, and $^{\ddagger}p<0.001$.

T-HTN=antihypertensive treatment, cPWV=local carotid pulse wave velocity, DBP=diastolic blood pressure, ${}_{d}D$=carotid diameter at DBP, ${}_{d}Ds$=distensibility in late diastole, $\Delta D = {}_{s}D - {}_{d}D$, $\Delta Ds\% = ({}_{d}Ds - {}_{s}Ds)/{}_{d}Ds$ =change in Distensibility as a %, H_A=hysteresis area, and H_I=hysteresis

index, HR=heart rate, IMT=intima media thickness, MBP=mean blood pressure, PP=pulse pressure, SBP_b=brachial systolic blood pressure, SBP_c=carotid systolic blood pressure, ${}_sD$=carotid diameter at SBP_c, ${}_sDs$= distensibility in early systole, T2DM=type 2 diabetes mellitus. Dichotomic variables: sex: male=0, female=1; US: Aloka=0, Esaote=1; T-HTN: untreated=0, treated=1; T2DM: no=0, yes=1.

Table 4. Comparison of viscosity parameters between controls, hypertensives, patients with type-2 diabetes mellitus without hypertension (N-T2DM), and patients with T2DM and hypertension (H-T2DM). Only patients whose waveforms were acquired with Aloka have been included. Data are mean ± standard deviation and adjusted values [95% confidence intervals]. Parameter-specific adjustments are indicated in the table using symbols.

	Control (>50 years) **N=22**	**Hypertension** **N=20**	**N-T2DM** **N=21**	**H-T2DM** **N=89**	**All T2DM** **N=110**
Male [%]	55	59	62	69	68
Age [years]	60 ± 7	56 ± 9	60 ± 8	65 ± 7	62 ± 8
${}_{d}Ds$ [MPa^{-1}] §	37.54 ± 14.73 $36.8\ [28.8 - 44.9]$	33.66 ± 10.36 $31.3\ [23.8 - 38.8]$	30.39 ± 10.74 $33.6\ [26.4 - 40.9]$	31.80 ± 14.39 $31.7\ [27.7 - 35.7]$	31.53 ± 13.73 $32.3\ [29.4 - 35.2]$
${}_{s}Ds$ [MPa^{-1}] §	34.51 ± 12.52 $34.3\ [27.4 - 41.2]$	29.51 ± 9.90 $27.5\ [21.1 - 34.0]$	27.67 ± 9.62 $31.6\ [25.4 - 37.8]$	28.62 ± 12.86 $28.2\ [24.8 - 31.6]$	29.46 ± 12.14 $29.2\ [26.7 - 31.7]$
ΔDs [%] ‖	5.8 ± 14.9 $4.2\ [-2.4 - 10.9]$	12.6 ± 9.8 * $13.2\ [7.0 - 19.3]$	8.4 ± 7.9 $5.1\ [-0.9 - 11.2]$	9.4 ± 9.1 $10.4\ [7.0 - 13.8]$	9.2 ± 8.9 $8.8\ [6.4 - 11.3]$
H_A [mmHg mm]‡	0.24 ± 0.27	0.87 ± 0.97 *	0.36 ± 0.23 †	0.67 ± 0.67	0.61 ± 0.63 †

	0.29 [−0.14 − 0.71]	1.04 [0.65 − 1.43]	0.22 [−0.17 − 0.59]	0.66 [0.44 − 0.87]	0.53 [0.38 − 0.68]
H_I [-] ‖	0.022 ± 0.023	0.047 ± 0.046 *	0.023 ± 0.015	0.038 ± 0.027	0.036 ± 0.026
	0.018 [−0.001 − 0.037]	0.048 [0.030 − 0.065]	0.016 [−0.001 − 0.033]	0.041 [0.031 − 0.050]	0.034 [0.027 − 0.040]

HR=heart rate, SBP_b=brachial systolic blood pressure, SBP_c=carotid systolic blood pressure, DBP=diastolic blood pressure, MBP=mean blood pressure, PP=pulse pressure, IMT=intima media thickness, ${}_sD$=carotid diameter at SBP_c, ${}_dD$=carotid diameter at DBP, $\Delta D = {}_sD - {}_dD$, cPWV=local carotid pulse wave velocity, ${}_dDs$=distensibility in late diastole, ${}_sDs$= distensibility in early systole, $\Delta Ds\% = ({}_dDs - {}_sDs)/{}_dDs$ =change in Distensibility as a %, H_A=hysteresis area, and H_I=hysteresis index.

*p<0.01 with controls, and †p<0.05 with hypertensives, and ‡p<0.001. §p<0.05, ‖p<0.01, and #p<0.001 with hypertensive. **p<0.05, ††p<0.01 with normotensive T2DM.

Parameter-specific adjustment: ‡Age, sex, ultrasound machine (US) and treatments (antihypertensives, statin, oral antidiabetics and insulin); § Age, sex, US, DBP, HR and treatments; ‖Age, sex, and treatments.

FIGURE LEGENDS

Figure 1 – Graphical representation of the analysis performed on the D^2P and PD-loops. A: example of simultaneously acquired pressure and diameter heartbeats at the left and right common carotid artery, respectively. B: ${}_{d}Ds$ and cPWV were estimated from the linear regression of late diastole of the D^2P-loop (the descending limb of the loop, red dashed line). ${}_{s}Ds$ was estimated in early systole (the ascending limb of the loop, blue dashed line). Note: both ${}_{s}Ds$ and ${}_{d}Ds$ are calculated at the same pressure range (i.e. from diastolic pressure to the pressure at the dicrotic notch). Large light-blue arrows indicate the loop direction. C: The H_A is the area enclosed in the PD-loop. H_I is calculated as H_A normalised to PP and ΔD. The pressure and diameter waveforms used in all three panels are taken from a 26 year old subject included in the study.

cPWV: carotid PWV; D: diameter; ΔD: systolic D – diastolic D; H_A: hysteresis area; H_I: hysteresis index; P: pressure; PP: pulse pressure; ${}_{s}Ds$: distensibility in systole.

Figure 2 - Examples of PD-loops for A) three healthy controls: 26, 55, and 78 years (left, middle and right respectively) and C) a healthy control, a hypertensive patient, a N-T2DM patient, and a H-T2DM patient (all >60 years). In panel B and D, the same pressure-diameter loops of panel A and C, respectively, were plotted by subtracting diastolic diameter and pressure from the diameter and pressure waveform, respectively, in order to force the onset of each loop from the origin (0,0) position and hence allow for a better comparison between the loops. In healthy controls, the slope of the PD-loop increased with age, while the hysteresis, clearly visible in the young loop, was much reduced in middle-aged and old individuals (Panel B). Note the increase in diameter (and pressure) with age (Panel A). Conversely, in hypertensives and H-T2DMs hysteresis persists also in older individuals (Panel C).

D: diameter; H-T2DM: hypertensive type 2 diabetes; N-T2DM: normotensive type 2 diabetes; *P*: pressure.

Figure 3 – Relationship between H_A (A) and H_I (B) and age in all the controls (N=114) included in this study.

H_A: hysteresis area; H_I: hysteresis index.

Figure 4 - Changes in systolic (A) and diastolic diameter (B) with age in the diabetic (T2DMs, N=124) and non-diabetic subjects (controls >50 years + hypertensives, N=124) included in this study.

T2DM: type 2 diabetes mellitus.