Towards the non-invasive determination of arterial wall distensible properties: new approach using old formulae

Original Article

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Abstract

Arterial function and wall mechanical properties are important determinants of hemodynamics in the circulation. However, their non-invasive determination is not widely available. Therefore, the aim of this work is to present a novel approach for the non-invasive determination of vessel's distensibility and elastic modulus.

Simultaneous measurements of vessel's Diameter (D) and flow velocity (U) were recorded to determine local wave speed ($_nC$) in flexible tubes and calf aortas non-invasively using the lnDU-loop method, which was used to calculate the Distensibility ($_nD_s$) and Elastic Modulus ($_nE$), also non-invasively. To validate the new approach, the non-invasive results were compared to traditionally invasive measurements of Dynamic Distensibility (D_{sd}) and Tangential Elastic Modulus (E_m).

In flexible tubes, the average $_nD_s$ is higher and $_nE$ is lower than D_{sd} and E_m by 1.6% and 6.9%, respectively. In calf aortas, the results of $_nD_s$ and $_nE$ agreed well with those of Dsd and Em, as demonstrated by Bland-Altman technique.

The results of $_nD_s$ and $_nE$ are comparable to those determined using traditional techniques. Our results suggest that $_nD_s$ and $_nE$ could be measured *in-vivo* non-invasively, given the possibility of measuring D and U to obtain $_nC$. Further studies are warranted to establish the clinical usefulness of the new approach.

Keywords: Arterial function, Distensibility, Tangential Elastic modulus, Wave speed, lnDU-loop

Introduction

The mechanical properties of the aortic wall have a direct implication on the cardiovascular risk. Arterial hypertension (Heintz et al., 1993), diabetes (Salomaa et al., 1995) and atherosclerosis (Dart et al., 1991) are associated with marked changes in the structure and mechanical properties of large arteries. For example, Vaccarino et al. (2000) found a 10 mmHg increase in pulse pressure, as a measure of arterial stiffness, was correlated with a 12% increased risk of coronary heart disease, a 14% increased risk of congestive heart failure and a 6% increase in overall mortality. Furthermore, arterial stiffness has been shown to be an independent risk factor for cardiovascular events such as primary coronary events, stroke, and mortality (Boutouyrie et al., 2002; Laurent et al., 2003). Therefore, the evaluation of aortic mechanical properties is important in the understanding and early detection of cardiovascular disease.

Several techniques have been developed *in-vivo* and *ex-vivo* to assess the mechanical properties of arteries. For example, Bergel (1961) introduced a classical apparatus to measure the pressure and radius for the determination of segmental distensibility. Humphrey et al. (1993) designed a comprehensive test system by which simultaneous extension, inflation, and torsion experiments on cylindrical segments of vessels could be performed. To avoid the invasive measurements and increase the potential for clinical use, several investigators proposed non-invasive techniques for assessing the mechanical properties of the aorta. Arndt et al. (1968) first reported non-invasive measurements of arterial diameter by means of pulsed ultrasound technique. Tardy et al. (1991) proposed a novel method which estimated the mechanical properties of the peripheral arteries based on the analysis of the arterial diameter against pressure curves derived from ultrasonic and photoplethysmographic measurements.

Wave speed, C, is an important property of an artery and is inversely related to the square root of compliance/distensibility (Merillon et al., 1982). C has been used as a surrogate marker for aortic stiffness (O'Rourke et al., 2002), and has been demonstrated to have a predictive value of risk evaluation in several cardiovascular studies (Boutouyrie et al., 2002; Laurent et al., 2003). C can be determined regionally using the foot-to-foot method (Laurent et al., 2003), and several methods have

been proposed to determine C locally, such as the PU-loop (Khir et al., 2001) and lnDU-loop (Feng and Khir, 2010).

The elastic modulus is a widely used parameter to evaluate the stiffness of a material. However, the non-linear mechanical behaviour of the arterial wall limits its application as a unique value describing arterial stiffness. Nevertheless, arterial distension *in-vivo* ranges in a limited interval of strain levels, and tangential elastic modulus, E_m (the tangent to the stress-strain relationship at a given strain/stress level) can provide valuable insight on the mechanical properties in the physiological pressure range (Panpho et al., 2019). E_m is commonly characterised using *in-vitro* testing of arterial samples subjected to uniaxial/biaxial loading conditions (Haskett et al., 2010). Given the clinical interest, non-invasive methods for determination of E_m have been devised (Payen et al., 2016; Uejima et al., 2019), and the current study is in-part an effort to advance the possibility of using E_m clinically.

The aim of this work is to introduce a novel approach to determine arterial function (distensibility; wave speed) and the mechanical properties (circumferential tangential elastic modulus) of flexible tubes and calf aortas using non-invasive measurements of diameter distension (D) and blood flow velocity (U). We also aim to examine the relative accuracy of the new approach against traditional techniques that use invasive measurements, such as the PU-loop method for determining wave speed and tensile testing for determining the E_m.

Materials and Methods

Ultrasound technologies are now available in almost every cardiac and arterial function clinic around the globe. Relying on the ability of such technologies to measure D and U relatively accurately and non-invasively, we have designed our experiments for measuring these parameters also non-invasively, i.e. without crossing the vessel wall, although using suitable laboratory equipment. Flexible tubes and calf aortas were used in the experiments, noting the mechanical properties of the flexible tubes are non-physiological, and were used to provide validation of the technique.

The general approach can be summarised as follows; first, determine C non-invasively in flexible tubes and calf aortas using the lnDU-loop method. The results are then used in the Bramwell-

Hill (Bramwell et al., 1923) and Moens-Korteweg (1878) equations to establish distensibility and elastic modulus (non-invasively), respectively. When testing calf aortas, we hypothesised that, given the pressure dependency of C due to the non-linear mechanical properties of the arterial wall (Spronck et al., 2015), the non-invasive estimation of the elastic modulus provided an estimation of E_m at the pressure level the artery was subjected to during the experiment. To validate our approach, the results were compared to those determined using the traditional dynamic distensibility test and mechanical tensile testing. The flowchart in **Figure 1** explains the steps we followed, and the sections below describe the theoretical and experimental details.

Non-invasive determination of wave speed

The theoretical basis of the lnDU-loop method for the non-invasive determination of wave speed (${}_nC$) has been described previously (Feng and Khir, 2010), can be written as

$$ {}_nC = \pm\frac{1}{2}\frac{dU_{\pm}}{dlnD_{\pm}} \quad (1) $$

Eq.1 describes a linear relation between U and lnD for unidirectional waves, the slope of which indicates wave speed, unit of ${}_nC$ is m/s, '+' and '-' indicate the forward and backward directions. The lnDU-loop method has been validated in previous work (Li and Khir, 2011). Here, we determined the initial linear part by fitting the data corresponding to the early ejection upstroke of the loop, as previously used with the PU-loop (Khir et al., 2001)). ${}_nC$ will be compared with the foot-to-foot wave speed $C_{ftf} = L \,/\, \Delta t$, where L is the distance between two pressure measurement sites, Δt is the time it takes the wave to travel between the two measurements, and wave speed calculated using the PU-loop as previously shown, $C_{PU} = \pm(\frac{1}{\rho}\frac{dP_{\pm}}{dU_{\pm}})$, where dP and dU are the change in pressure and velocity respectively.

Non-invasive determination of distensibility ${}_nDs$

It is well established that C is a function of the tube distensibility (*Ds*) according to the Bramwell-Hill equation (Bramwell et al., 1923)

$$ C^2 = \frac{1}{\rho D_s} = \frac{A\,(\Delta P)}{\rho\,(\Delta A)}\;; \qquad D_{sd} = \frac{\Delta A}{A\,\Delta P} \quad (2) $$

where ρ (kg/m3) is the fluid density, *Ds* is the distensibility of the arterial wall and defined as the fractional change in the vessel cross sectional area (ΔA) in response to the change in pressure (ΔP) with respect to the initial cross sectional area (A). Rearranging Eq.2 gives the non-invasive determination of distensibility (${}_nD_s$);

$$ {}_nD_s = \frac{1}{\rho\ {}_nC^2} \tag{3}$$

Non-invasive determination of Elastic modulus ($_nE$)

Elastic modulus, defined as the ratio of stress to strain, is a material property of the vessel. Moens and Korteweg (1878) arrived independently to the equation that is named after them, which describes the relationship between the physical (wall thickness (h) diameter (D)) and the mechanical properties (wave speed (${}_nC$), Elastic modulus (${}_nE$)). Following (Fung, 1997), relaxing the thin wall assumption allows for determining the elastic modules as

$$ {}_nE = {}_nC^2 \rho \frac{D+h}{h} \tag{4}$$

Eq.4 is used to determine ${}_nE$ for both elastic tubes and calf aortas. Note that both h and D are the dimensions of the inflated unpterturbed tubes/aortas.

Experimental work

Specimens

Ten ascending aortas of matured calves (average 18 months, unknown gender) were obtained from an abattoir, stored at a freezing temperature of -20℃ and allowed to thaw at room temperature for 3 hours before testing without pre-conditioning. All side branches were occluded at their root using wired snares to avoid both leakage and reflections from the small branches. The length of the aorta was measured before mounting in the experimental setup for the wave speed evaluation (average 37.5±3.4cm). Fresh water was used in all of the experiments due to similarity to blood density (difference <5%). As viscosity plays a negligible role in large arteries, we did not consider its effects in the current results.

Determination of wave speed

Set up: The setup of the *in-vitro* experiment for measuring wave speed in flexible tubes was introduced in a previous paper (Li and Khir, 2011). The properties of the flexible tubes used in this work are summarised in **Table 1**. The setup of the *in-situ* experiment for the calf aortas is shown in **Figure 2**.

BCM pump (Cardialcare, Minneapolis, MN, USA) is a flexible diaphragm pulsatile left ventricle assist device that was used to generate a pulse at the inlet of each flexible tube and calf aorta. The BCM was operated by an Intra-Aortic Balloon Pump (Datascope 97XT, Datascope, NJ, USA) and produced pressure and flow waveforms that are similar to those observed *in-vivo* (Khir et al., 2006). Heart rate was set to 80bpm and augmentation was set at the highest level. The inlet and outlet reservoirs were interconnected and the height of the fluid in the reservoirs was adjusted to 100cm above the longitudinal axis of the tube, producing an initial hydrostatic pressure of 10kPa to replicate a physiological diastolic pressure of 75mmHg. Aortas were stretched in the axial direction until horizontal (not bent). This set of experiments were performed at room temperature (~20-24ºC).

Simultaneous waveforms of pressure (P), external diameter (D_o) and flow rate (Q), from which U was determined, were measured in a location approximately 20cm proximal to the outlet (**Figure 2b**). D_o was measured using a pair of ultrasonic crystals (Sonometrics Corporation, Ontario, Canada) with a resolution of 0.024mm and unloaded wall thickness was measured using a digital caliper after the experiment. P and Q were measured using high-fidelity 6F tipped catheter pressure transducer (Millar Instruments, Texas, USA) and ultrasonic flow probe (Transonic System, Inc, NY, USA), respectively. All data were sampled at 500Hz using Sonolab (Sonometrics Corporation) and analysed using Matlab (The Mathworks, MA, USA).

The dynamic distensibility (D_{sd}) was calculated as shown above (Eq.2), where ΔA and ΔP are respectively the difference between systolic and diastolic A and P.

Mechanical determination of Tangential Elastic modulus (E_m)

E_m was determined using uniaxial tensile test (Model 5540, Instron Corporation, Norwood, MA, USA), and the experiments were performed at room temperature (~20-24ºC).

Flexible tubes: Samples from the flexible tubes were cut into the standardized cross-section. The specimen was slowly stretched until a small increase in load was observed and initial specimen length was noted. The specimens were stretched until specimen failure at a crosshead rate of 10mm/min (**Figure 3a, b**).

Calf aorta: The protocol for this test comprises the following steps:

Immediately following the *in-situ* experiments, symmetrical rings at the measurement sites were dissected. Each ring was free of arterial branches or irregular sections. Measurements of width (w_0), thickness (h_0) and circumference were taken several times using digital calliper and averaged; diameter was calculated from the circumference. Samples were kept wet by spraying water onto them.

The sample was placed and preloaded until 0.005N was reached. 3 cycles from 0 to 60mmHg, 60 to 160mmHg and 30 to 200mmHg at loading rate of 10mm/min (Dobrin, 1978), were applied sequentially (**Figure 3c, d**). Pressure-equivalent stress levels were estimated using the Laplace's formula (Burton, 1954),

$$\sigma = \frac{PD}{2h} \tag{5}$$

using the deformed diameter ($D = D_0(1+\epsilon)$ where D_0 is the unloaded internal diameter). Stresses were calculated using the Cauchy's formulation (i.e. assuming the incompressibility of the arterial wall) (Duprey et al., 2010).

$$\sigma = \frac{F}{A_0}(1+\epsilon) \tag{6}$$

where F is the applied load, $A_0 = w_0 h_0$ the unloaded cross-sectional area, and ϵ the strain.

E_m was calculated at the loading part of the last cycle of each test as the slope of the tangent to the non-linear stress-strain relationship. Considering the initial 75mmHg pressure and ≈20mmHg pulse pressure in the wave speed experiment, E_m was evaluated at a stress levels equivalent to pressures ranging from 70 to 90mmHg at intervals of 5mmHg.

Wall thickness in Eq.5 was assumed constant throughout the experiments and Table 2 includes the formulae used in the determination of all of the measured and calculated parameters.

Statistical analysis

All of the *in-situ* measurements were taken twice for each sample and the results averaged. Then, results were averaged across samples and presented as mean ±SD. Student's t-test were performed using SPSS version 22 to compare the distensibility and elastic modulus calculated by different methods. For the wave speed *C,* differences between the three methods, i.e. lnDU-loop, PU-loop and foot-to-foot, first evaluated using repeated measures ANCOVA, and then pairwise comparison was performed using Student's t-test as detailed before. $P<0.05$ was considered statistically significant. Bland-Altman technique (Martin Bland and Altman, 1986) was used to establish the agreement between different techniques, and the limits of agreement was taken as ±2SD of the mean difference.

Results

Examples of the measured D and U waveforms are presented in **Figure 4a** (*in-vitro*) and **Figure 4b** (*in-situ*).

***In-vitro* results**

The results of ${}_nC$, D_{sd}, ${}_nD_s$, ${}_nE$ and E_m in flexible tubes are shown in **Table 1**. As expected, ${}_nC$ increased with increasing h and decreased with increasing D.

The average difference between D_{sd} and ${}_nD_s$ is 0.35MPa^{-1} (limits of agreement: -19.9 to 20.6MPa^{-1}), with ${}_nD_s$ being slightly higher (1.6%) than D_{sd}.

The results indicate that ${}_nE$ is 6.9% smaller than E_m, and the average difference between the two methods is -0.28MPa (limits of agreement: -1.77 to 1.22MPa). Overall, the two techniques showed good agreement, as most of the points lie in proximity of the identity line (**Figure 5a)**, However, large differences between the two techniques in a few samples contributed to increasing the limits of agreement in the Bland-Altman plot (**Figure 5b**).

***In-situ* results**

The dimensions of calf aortas were obtained after the water experiment. At the measurement site, the internal diameter ranges from 22.1 to 29.1mm (average: 24.7±2.1mm), h ranges from 4.5 to 6.4mm (average: 5.4±0.5mm).

At the measurement site, the average ${}_nC$ is 3.80±0.41m/s. The average C_{PU}, and C_{ftf} are 3.87±0.43m/s and 4.08±0.73m/s, respectively. Repeated measures ANOVA indicated that there was a significant difference between wave speed measures ($p<0.05$) and pairwise comparison yield significant difference between ${}_nC$ and C_{PU}. The limit of agreement was ±1.34 m/s between C_{PU} and C_{ftf}, ±1.32 m/s between ${}_nC$ and C_{ftf}, and ±0.16 m/s between C_{PU} and ${}_nC$ (**Figure 6**). We note that large limits of agreement between either of the loops methods (C_{PU} or ${}_nC$) and foot-to-foot were caused by a single artery, where C_{ftf} was ~2 m/s higher than C_{PU} or ${}_nC$. There was a small difference between ${}_nC$ and C_{PU} (-0.08±0.08), which was significant ($P<0.05$), but not between ${}_nC$ and C_{ftf} ($P=0.31$). **Figure 7a** shows C calculated using different techniques.

Non-invasive determination of distensibility and elastic modulus

Average ${}_nD_s$ is 71.5±14.4MPa^{-1} and average D_{sd} is 69.2±13.7MPa^{-1}. The average difference is 1.2MPa^{-1} ($P=0.51$, limits of agreement: -19.2 to 23.9MPa^{-1}), **Figure 7b**.

Average ${}_nE$ is 0.179±0.036MPa and average E_m is 0.171±0.030MPa and 0.178±0.031MPa at the experimental diastolic pressure 75mmHg and at 80 mmHg, leading to an average difference between these two methods of 0.008MPa (limits of agreement: -0.088 to 0.104MPa) ($P=0.622$) and 0.002MPa (limits of agreement: -0.094 to 0.097MPa) ($P=0.917$), respectively, and indicating that ${}_nE$ closely matched E_m in proximity of the diastolic pressure, (**Figure 7c**). The results of both techniques are in good agreement as demonstrated using the scatter plot with identity line and Bland-Altman plot, **Figure 5c** and **d**.

Discussion

In this work we demonstrated the viability of a novel approach for the determination of arterial function and wall mechanical properties non-invasively.

The basic measurements of D and U were taken simultaneously at the same location. The novel approach relies chiefly on the determination of ${}_nC$, using the lnDU-loop; which was previously validated against invasive measurements (Li and Khir, 2011). Wave speed determined non-invasively

was used in the Bramwell-Hill equation (Bramwell et al., 1923) to establish arterial function; distensibility, and in the Moens-Korteweg equation to establish wall mechanical property: i.e. elastic modulus. The results were validated against classical invasive techniques, and our main finding demonstrate the good agreement between non-invasive and invasive techniques for determining $_nC$, $_nD_s$ and $_nE$.

Arterial function and mechanical properties are important determinants of blood pressure. Most current clinical techniques for determining arterial function refer to wave speed as the parameter of interest, and use non-invasive measurements of pressure (Mackenzie et al., 2002) or flow (Wentland et al., 2014) at two different sites, applying the foot-to-foot method to determine wave speed. The current gold standard technique uses MRI measurement (Huybrechts et al., 2011); however, availability of MRI limits its applicability in the clinical setting. To determine arterial distensibility and mechanical properties, most techniques rely on the relationship between area/diameter and pressure changes, Eq.2 (Godia et al., 2007; Mackenzie et al., 2002). However, central pressure (ascending aorta) cannot be accurately measured directly non-invasively and may only be derived from peripheral recordings (e.g. carotid and femoral arteries) using transfer functions, which may introduce inaccuracies. Furthermore, wave speed measured using MRI present regional values of wave speed, indicating an average distensibility between the two measurement sites. Therefore, the applicability of earlier techniques to a specific site *in-vivo*, the ascending aorta for example, remains limited.

The technique presented in the current work is based on the non-invasive measurements of flow velocity and diameter at a single site, which could be routinely obtained using ultrasound technologies currently widely available. This means the function and mechanical properties can be determined at any arterial site accessible by ultrasound measurements, providing local information, which would be particularly useful for the assessment/diagnosis of arterial stiffness.

All of the parameters assessed non-invasively in this work showed good agreement with the corresponding values measured invasively both *in-vitro* and *in-situ*. Limit of agreement intervals between wave speed estimates are smaller than those reported *in-vivo* by Di Lascio (2014). Agreement between methods is within ±10% (in the discussion limits of agreement are expressed as percent of the

ratio between difference and average of the measures obtained with the two methods) except for one C_{ftf} value with higher deviation from its corresponding C_{PU} and ${}_nC$. It is worth considering that, from Eq.3-4, a ±10% error in the estimation of ${}_nC$ produces a ~±20% error in the estimation of ${}_nE$ and ${}_nD_s$. In flexible tubes, no significant difference was found between invasive and non-invasive E and D_s (-6.9% and 1.5%, respectively), and the size of the difference between the two methods is in the order of the data experimental noise. In the calf aortas, average differences between the invasive and non-invasive measurements were 1.1% and 3.4% for E and D_s, respectively, with limit of agreement ±54% and ±30%, similar to differences found in flexible tubes and comparable to the size of the experimental noise and consequent errors in the estimation of ${}_nC$.

The lnDU-loop method relies on the linear relationship between U and lnD in early systole when the assumption of unidirectional forward-travelling waves is reasonable. Therefore, we postulate that, given the non-linearity of the arterial wall stress-strain relationship, the wave speed obtained with this method would provide an insight into arterial stiffness (E_m) at the early systolic phase of the cardiac cycle; i.e. at stress level equivalent to the diastolic pressure. Our results seem to support this postulation; the highest agreement between ${}_nE$ and E_m was found at 80mmHg, where the experiments diastolic pressure was set at 75mmHg and the pulse pressure was ≈20mmHg. In any case, ${}_nE$ and E_m did not differ significantly at any of the investigated pressure levels.

The nonlinearity of the arterial wall stress-strain relationship is well established (Burton, 1954). Therefore, it may be misleading to consider a single value of the E_m, which needs to be determined as a function of the temporal stress/pressure values. Therefore, given the dynamic distensibility of the arterial wall, D_{sd}, with every heartbeat, a more meaningful estimate of ${}_nE$ or E_m would be at the strain/stress levels corresponding to early systolic pressure range. In addition, ${}_nE$ or E_m is a stiffness index, differently from wave speed, has the advantage of being independent on the geometrical features (i.e. h and D) and could represent a more powerful indicator of the mechanical status of the arterial tissue to be used in clinical practice.

Previous attempts to non-invasively estimate the arterial wall elastic modulus *in-vivo* relied on stress-strains relationships using pressure and diameter acquired with applanation tonometry and

ultrasound scanning, respectively (Aggoun et al., 2000; Khamdaeng et al., 2012; Pagani et al., 1979). While this method provided results (Aggoun et al., 2000; Khamdaeng et al., 2012) comparable to those presented in our study, its clinical applicability remains limited to superficial arteries, such as the carotid and the femoral artery. More recently, Franquet et al. (2013) developed a technique based on MRI acquisition of the artery cross-sectional area and brachial pressure measurements. The method involves tuning the elastic parameters of an artery cross-section finite elements model to match the *in-vivo* time-deformation acquired with MRI, when the measured brachial pressure is prescribed as input. While the method elegantly estimates E, it employs a huge assumption; the pressure in the studied artery equals that of the brachial artery, neglecting the distal pressure amplification characterising the arterial tree. Further, the cost of MRI represents a major limitation for using this technique in large cohorts.

Experimental Considerations

Although, the wall of large arteries is known to be anisotropic, the circumferential direction of the wall, pertaining to distensibility, is more relevant to arterial function. Therefore, the tensile tests for the calf aortas were conducted in the circumferential direction only.

It has previously been reported that the results of the lnDU-loop method for determining $_nC$ could be affected by large reflections (Borlotti et al., 2014), when the diameter and velocity measurements are taken close to reflection sites. The measurement site in the current experiments is relatively far from the reflection site, the interface between the calf aorta or the flexible tubes and the connecting tubes to the reservoir 2 (**Figure 2**). If the measurements were taken closer to the reflection site, the results may have been affected, and their accuracy may not hold. Segers et al (2014) proposed a technique for correcting errors incurred by reflections to the PU-loop, however the technique requires invasive measurements of pressure, which would limit its use in the clinical setting. The relationship between the size of reflection and the consequent wave speed inaccuracy remains an open question, which together with a non-invasive correction factor to the loops technique present worthy challenges that require addressing in future work.

The wall thicknesses of calf aortas were measured after the experiments, as we did not have any means of taking these measurements dynamically. Therefore, average h of our measurements might be slightly larger than those *in-vivo*. However, we expect these differences to be insignificant (Wells et al., 1998).

Limitations

The applicability of the *in-situ* data presented here to normal vessels *in-vivo* has some limitations: for example, the mechanical properties of blood vessels *in-vivo* are strongly influenced by the tethering to surrounding tissues and by the tone of the smooth muscles in the vessel wall, which in turn depend on the humoral and neural factors. Such factors were not studied in this investigation, although we expect their effect might be too small and will not significantly affect the results or their interpretations. Another minor limitation is the use of tap water instead of a blood mimicking fluid; water-glycerine mix, or physiological saline solution. The imbalance in electrolytes between intracellular and experimental fluid might have altered the elastic properties of the arterial tissue, although this is expected to induce negligible effect on the agreement between the two techniques.

The temperature clearly affects the behaviour of the arterial wall (here room and not physiological body temperature), although the changes might be too small. However, the purpose of this study was to compare the results of two techniques. Since, both experiments have been performed at the same temperature, comparing the results remain valid and should not be affected by the said temperature. It has also been shown that freezing affects the mechanical properties of the arterial wall. However, the effect of freezing on the passive mechanical properties of the arterial wall is small and should not affect the validity of the study. Additionally, the comparison between techniques was performed on the same samples, thus further mitigating against risk of possible bias due to freezing.

Conclusions

The novel approach developed in this work using the non-invasively determined wave speed by lnDU-loop method makes it possible to establish arterial function; distensibility, and wall properties; Em. The results of the current work, evaluated in flexible tubes and calf aortas, agreed well with those determined using traditional invasive techniques; dynamic distensibility test and tensile test, which provide confidence in the viability of the new technique. The non-invasive nature and encouraging results obtained in this work warrant clinical investigation to establish the usefulness of the proposed novel approach.

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Conflict of interest statement

YL, AG, IBW and AWK have nothing to disclose.

References

Aggoun, Y., Sidi, D., Levy, B.I., Lyonnet, S., Kachaner, J., Bonnet, D., 2000. Mechanical properties of the common carotid artery in Williams syndrome. Heart 84, 290–293.

Arndt, J.O., Klauske, J., Mersch, F., 1968. The diameter of the intact carotid artery in man and its change with pulse pressure. Pflugers Arch. Gesamte Physiol. Menschen Tiere 301, 230–240.

Bergel, D.H., 1961. The dynamic elastic properties of the arterial wall. J. Physiol. 156, 458–469.

Borlotti, A., Li, Y., Parker, K.H., Khir, A.W., 2014. Experimental evaluation of local wave speed in the presence of reflected waves. J. Biomech. 47, 87.

Boutouyrie, P., Tropeano, A.I., Asmar, R., Gautier, I., Benetos, A., Lacolley, P., Laurent, S., 2002. Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: A longitudinal study. Hypertension 39, 10–15.

Bramwell, J.C., Hill, A. V., McSwiney, B.A., 1923. The velocity of the pulse wave in man in relation to age as measured by the hot-wire sphygmograph. Heart 10, 233–255.

Burton, A.C., 1954. Relation of structure to function of the tissues of the wall of blood vessels. Physiol. Rev. 34, 619–642.

Dart, A.M., Lacombe, F., Yeoh, J.K., Cameron, J.D., Jennings, G.L., Laufer, E., Esmore, D.S., 1991. Aortic distensibility in patients with isolated hypercholesterolaemia, coronary artery disease, or cardiac transplant. Lancet 338, 270–273.

Di Lascio, N., Bruno, R., Stea, F., Bianchini, E., Gemignani, V., Ghiadoni, L., Faita, F., 2014. Non-invasive assessment of carotid PWV via accelerometric sensors: validation of a new device and comparison with established techniques. Eur. J. Appl. Physiol. 114, 1503–1512.

Dobrin, P.B., 1978. Mechanical properties of arteries. Physiol. Rev. 58, 397–460.

Duprey, A., Khanafer, K., Schlicht, M., Avril, S., Williams, D., Berguer, R., 2010. In Vitro Characterisation of Physiological and Maximum Elastic Modulus of Ascending Thoracic Aortic Aneurysms Using Uniaxial Tensile Testing. Eur. J. Vasc. Endovasc. Surg. 39, 700–707.

Feng, J., Khir, A.W., 2010. Determination of wave speed and wave separation in the arteries using diameter and velocity. J. Biomech. 43, 455–462.

Franquet, A., Avril, S., Le Riche, R., Badel, P., Schneider, F.C., Boissier, C., Favre, J.P., 2013. Identification of the in vivo elastic properties of common carotid arteries from MRI: A study on subjects with and without atherosclerosis. J. Mech. Behav. Biomed. Mater. 27, 184–203.

Fung, Y.C., 1997. Biomechanics. Springer New York, New York.

Godia, E.C., Madhok, R., Pittman, J., Trocio, S., Ramas, R., Cabral, D., Sacco, R.L., Rundek, T., 2007. Carotid artery distensibility: A reliability study. J. Ultrasound Med. 26, 1157–1165.

Haskett, D., Johnson, G., Zhou, A., Utzinger, U., Vande Geest, J., 2010. Microstructural and biomechanical alterations of the human aorta as a function of age and location. Biomech. Model. Mechanobiol. 9, 725–736.

Heintz, B., Gillessen, T., Walkenhorst, F., vom Dahl, J., Dörr, R., Krebs, W., Hanrath, P., Sieberth, H.G., 1993. Evaluation of segmental elastic properties of the aorta in normotensive and medically treated hypertensive patients by intravascular ultrasound. J. Hypertens. 11, 1253–1258.

Humphrey, J.D., Kang, T., Sakarda, P., Anjanappa, M., 1993. Computer-aided vascular experimentation: A new electromechanical test system. Ann. Biomed. Eng. 21, 33–43.

Huybrechts, S.A.M., Devos, D.G., Vermeersch, S.J., Mahieu, D., Achten, E., de Backer, T.L.M., Segers, P., van Bortel, L.M., 2011. Carotid to femoral pulse wave velocity: a comparison of real travelled aortic path lengths detrmined by MRI and superficial measurments. J. Hypertens. 29, 1577–1582.

Khamdaeng, T., Luo, J., Vappou, J., Terdtoon, P., Konofagou, E.E., 2012. Arterial stiffness identification of the human carotid artery using the stress-strain relationship in vivo. Ultrasonics 52, 402–411.

Khir, A.W., O'Brien, A., Gibbs, J.S.R., Parker, K.H., 2001. Determination of wave speed and wave separation in the arteries. J. Biomech. 34, 1145–1155.

Khir, A.W., Swalen, M.J., Segers, P., Verdonck, P., Pepper, J.R., 2006. Hemodynamics of a pulsatile left ventricular assist device driven by a counterpulsation pump in a mock circulation. Artif. Organs 30, 308–312.

Laurent, S., Katsahian, S., Fassot, C., Tropeano, A.-I., Gautier, I., Laloux, B., Boutouyrie, P., 2003. Aortic Stiffness Is an Independent Predictor of Fatal Stroke in Essential Hypertension. Stroke 34, 1203–1206.

Li, Y., Khir, A.W., 2011. Experimental validation of non-invasive and fluid density independent methods for the determination of local wave speed and arrival time of reflected wave. J. Biomech. 44, 1393–1399.

Mackenzie, I.S., Wilkinson, I.B., Cockcroft, J.R., 2002. Assessment of arterial stiffness in clinical practice. QJM 95, 67–74.

Martin Bland, J., Altman, D.G., 1986. STATISTICAL METHODS FOR ASSESSING AGREEMENT BETWEEN TWO METHODS OF CLINICAL MEASUREMENT. Lancet 327, 307–310.

Merillon, J.P., Motte, G., Masquet, C., Azancot, I., Guiomard, A., Gourgonf, R., 1982. Relationship between physical properties of the arterial system and left ventricular performance in the course of aging and arterial hypertension. Eur. Heart J. 3, 95–102.

O'Rourke, M.F., Staessen, J.A., Vlachopoulos, C., Duprez, D., Plante, G.E., 2002. Clinical applications of arterial stiffness; definitions and reference values. Am. J. Hypertens.

Pagani, M., Mirsky, I., Baig, H., Manders, W.T., Kerkhof, P., Vatner, S.F., 1979. Effects of age on aortic pressure-diameter and elastic stiffness-stress relationships in unanesthetized sheep. Circ. Res. 44, 420–9.

Panpho, P., Brendan, G., Chim, Y.H., Davies, H., Field, M., Madine, J., Akhtar, R., 2019. Macro- and Micro-mechanical Properties of the Ovine Aorta: Correlation with Regional Variations in Collagen, Elastin and Glycosominoglycan Levels. Artery Res. 25, 27–36.

Payen, T., Palermo, C.F., Sastra, S.A., Chen, H., Han, Y., Olive, K.P., Konofagou, E.E., 2016. Elasticity mapping of murine abdominal organs in vivo using harmonic motion imaging (HMI).

Phys. Med. Biol. 61, 5741–5754.

Salomaa, V., Riley, W., Kark, J.D., Nardo, C., Folsom, A.R., 1995. Non-insulin-dependent diabetes mellitus and fasting glucose and insulin concentrations are associated with arterial stiffness indexes: The ARIC study. Circulation 91, 1432–1443.

Segers, P., Swillens, A., Taelman, L., Vierendeels, J., 2014. Wave reflection leads to over- and underestimation of local wave speed by the PU- and QA-loop methods: theoretical basis and solution to the problem. Physiol. Meas. 35, 847.

Spronck, B., Heusinkveld, M., Vanmolkot, F., Roodt, J.O., Hermeling, E., Delhaas, T., Kroon, A., Reesink, K., 2015. Pressure-dependence of arterial stiffness: potential clinical implications. J. Hypertens. 33, 330–338.

Tardy, Y., Meister, J.J., Perret, F., Brunner, H.R., Arditi, M., 1991. Non-invasive estimate of the mechanical properties of peripheral arteries from ultrasonic and photoplethysmographic measurements. Clin. Phys. Physiol. Meas. 12, 39–54.

Uejima, T., Dunstan, F.D., Arbustini, E., Łoboz-Grudzień, K., Hughes, A.D., Carerj, S., Favalli, V., Antonini-Canterin, F., Vriz, O., Vinereanu, D., Zamorano, J.L., Popescu, B.A., Evangelista, A., Lancellotti, P., Lefthériotis, G., Kozakova, M., Palombo, C., Fraser, A.G., 2019. Age-specific reference values for carotid arterial stiffness estimated by ultrasonic wall tracking. J. Hum. Hypertens.

Vaccarino, V., Holford, T.R., Krumholz, H.M., 2000. Pulse pressure and risk for myocardial infarction and heart failure in the elderly. J. Am. Coll. Cardiol. 36, 130–138.

Wells, S.M., Langille, B.L., Adamson, S.L., 1998. In vivo and in vitro mechanical properties of the sheep thoracic aorta in the perinatal period and adulthood. Am. J. Physiol. Circ. Physiol. 274, H1749–H1760.

Wentland, A.L., Grist, T.M., Wieben, O., 2014. Review of MRI-based measurements of pulse wave velocity: a biomarker of arterial stiffness. Cardiovasc. Diagn. Ther. 4, 193–206.

Table 1: Materials, dimensions, wave speeds and mechanical properties of the flexible tubes.

Material	D (mm)	h (mm)	$_nC$ m/s	D_{sd} (M Pa^{-1})	$_nD_s$ (M Pa^{-1})	$Diff_{Ds}$ (M Pa^{-1})	E_m (MPa)	$_nE$ (MPa)	$Diff_E$ (MPa)
Silicone	8	1	22.3	1.9	2.0	0.1	4.88	4.48	-0.41
		2	26.7	1.1	1.4	0.3	4.25	3.56	-0.68
		3	33.5	1.0	0.9	-0.1	4.16	4.11	-0.05
	10	1	20.0	2.9	2.5	-0.4	4.83	4.40	-0.43
		2	25.3	1.4	1.6	0.2	4.71	3.84	-0.87
		3	29.9	1.0	1.1	0.1	4.61	3.87	-0.74
	16	2.4	22.4	1.6	2.0	0.4	4.72	3.85	-0.87
		3	25.1	1.4	1.6	0.2	3.97	3.99	0.02
Rubber	16.7	1.5	23.9	1.9	1.8	-0.1	5.08	6.70	1.62
	20.6	1.5	20.7	2.1	2.3	0.2	6.28	6.31	0.03
Latex	8.5	0.1	5.2	48.9	37.6	-11.3	0.96	1.56	0.60
	24.2	0.27	3.1	118.2	103.4	-14.8	0.90	0.61	-0.29
	32.3	0.27	2.6	118.3	148.0	29.7	2.67	1.16	-1.51
	Average difference					0.3			-0.28
	Limit of agreement					-19.9 – 20.6			-1.78 – 1.22

D: internal diameter, h: wall thickness, $_nC$: wave speed determined by *ln*DU-loop, D_{sd}: distensibility calculated from the dynamic test, $_nD_s$:distensibility calculated from $_nC$, E_m: tangential elastic modulus given by the tensile test, $_nE$:Elastic modulus calculated from $_nC$, $Diff_{Ds}=D_{sd}-{}_nD_s$, $Diff_E=E_m-{}_nE$.

Table 2 - Formulae used in the determination of all of the measured and calculated parameters

	Invasive testing	Non-invasive testing
D	$D = D_0(1+\epsilon)$	$D = \sqrt{D_e^2 - 4D_0h_0 - 4h_0}$
h	$h = h_0$	$h = \frac{D_e - D}{2}$
σ	$\sigma = \frac{F}{w_0 h_0}(1+\epsilon)$	N/A
Laplace equation	$\sigma = \frac{PD_0(1+\epsilon)}{2h_0}$	N/A
ϵ	$\epsilon = \frac{L - L_0}{L_0 + \frac{\pi\delta}{2}}$	N/A
E_m	$E_m = \left.\frac{d\sigma}{d\epsilon}\right\|_P$	N/A
Ds	$Ds = \frac{\Delta A}{A\,\Delta P}$	N/A
${}_nE$	N/A	${}_nE = {}_nC^2\rho\frac{D+h}{h}$
${}_nDs$	N/A	${}_nDs = \frac{1}{\rho c^2}$

D_e: unperturbed loaded external diameter
D: unperturbed loaded internal diameter
h: unperturbed loaded wall thickness
E_m: tangential elastic modulus
σ: Cauchy stress
ϵ: strain
${}_nC$: non-invasive wave speed
δ: diameter of the tensiometer holding pin
L_0: Initial distance between tensiometer holding pin
L: Distance between tensiometer holding pin
w_0: Initial width of the specimen ring
D_0: Unloaded diameter
h_0: Initial wall thickness

Figures Captions

Figure 1: Flow chart for the experimental design.

Figure 2: (a) A schematic diagram of the experimental setup. R_{es1} and R_{es2} are the inlet and outlet reservoirs, which provide the initial pressure to the system, and keep the system air-free. Pressure and flow were measured using transducer tipped catheters, ultrasonic flow meter and probes, respectively. Diameter was measured using a pair of ultrasound crystals. (b) The detailed diagram of the aorta and the measurement site. The average length of the aorta was 37.5±3.4 cm. Two flexible tubes were inserted into the aorta to connect the aorta to the water tank, and tied with the cable ties. Distance was measured in the preparation.

Figure 3: Test Sequences for flexible tubes (a, b) and calf aortas (c, d).

Figure 4: (a) diameter and velocity waveforms in rubber tube 16.7 mm in diameter and 1.5 mm wall thickness at 50 cm away from the inlet of the tube in vitro; (b) diameter and velocity waveforms at upper thoracic of the calf aorta in situ.

Figure 5: The agreement between Elastic modulus determined by the *ln*DU-loop and tensile test is assessed by scatter plot and Bland-Altman method in flexible tubes (a, b) and calf aortas (c, d). In a and c, the dash line indicates the line of equality between the two parameters. In b and d, the dashed horizontal line indicates the average difference of Elastic modulus determined by the two methods. The upper and lower solid horizontal indicate ±2SD.

Figure 6: Bland-Altman plots comparing different methods for the estimation of C in calf aortas: lnDU-loop ($_nC$), PU-loop (C_{PU}), and foot-to-foot (C_{ftf}) methods. Dashed lines indicate the average difference between methods and horizontal solid lines show ±2SD.

Figure 7: (a) wave speed (b) distensibility and (c) Elastic modulus results. C_{ftf}: foot-to-foot wave speed, ${}_nC$: *ln*DU-loop wave speed, C_{PU}: PU-loop wave speed, ${}_nD_s$: distensibility determined non-invasively, D_{sd}: dynamic distensibility, ${}_nE$: Elastic modulus determined non-invasively, E_m: tangential elastic modulus from tensile test. E_m is reported for values of pressure ranging from 70 to 90 mmHg.