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Arterial Stiffness

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Measuring Arterial Stiffness in Animal Experimental Studies

Mark Butlin, Isabella Tan, Bart Spronck, Alberto P. Avolio

ABSTRACT: The arterial wall is a composite material of elastin, collagen, and extracellular matrix with acutely modifiable material properties through the action of smooth muscle cells. Therefore, arterial stiffness is a complex parameter that changes not only with long-term remodeling of the wall constituents but also with acute contraction or relaxation of smooth muscle or with changes in the acute distending pressure to which the artery is exposed. It is not possible to test all these aspects using noninvasive or even invasive techniques in humans. Full characterization of the mechanical properties of the artery and the specific arterial factors causing changes to stiffness with disease or modified lifestyle currently require animal studies. This article summarizes the major in vivo and ex vivo techniques to measure the different aspects of arterial stiffness in animal studies.

VISUAL OVERVIEW: An online visual overview is available for this article.

Key Words: arteries ■ collagen ■ compliance ■ elastin ■ endothelium

Clinically, arterial stiffness is one of the few biological parameters that more than doubles with age.[1] This occurs through a number of different mechanisms, many still poorly understood, that result in elastin fragmentation, collagen cross-linking, and dysfunction of the endothelial lining and vascular smooth muscle cells.[2] Fundamental research of underlying mechanisms of arterial stiffness is still largely at the level of the basic sciences and therefore requires the kind of detailed, invasive, and experimental interventional work that cannot be ethically achieved in humans. Invasive measurement, in particular, is limited to people who are undergoing catheterization procedures as part of routine clinical treatment. This means such studies are mostly limited to people with cardiovascular diseases, and comparison to a healthy cohort is not possible. This also limits the possibility of longitudinal studies. Age-related stiffening research in humans is also limited by arterial stiffening being an aging process spanning over decades. This timescale is not practical nor cost-effective in many research ideas investigating the stiffening of arteries. Yet there is a call for such data, given that increased large artery stiffness has a detrimental impact on arterial blood pulse pressure with age,[3] is a predictor of cardiovascular events and mortality,[4] and is implicated in cognitive impairment and dementia-like diseases.[5] As a result, such research is often done using animals.

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This article provides a broad overview of the approaches and techniques available to quantify and investigate arterial stiffness in animals, drawing on key references that treat the individual techniques in greater detail. Ex vivo techniques generally provide a greater level of information on the mechanistic aspects behind arterial stiffness changes. In vivo techniques are vital to elucidate if small changes in parameters detected in bench-top testing

Correspondence to: Bart Spronck, Department of Biomedical Engineering, CARIM School for Cardiovascular Diseases, Maastricht University, Universiteitssingel 50, Room 3.359, 6229ER, Maastricht, The Netherlands. Email b.spronck@maastrichtuniversity.nl

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Nonstandard Abbreviations and Acronyms

PWV	pulse wave velocity

actually have any impact on the functional stiffness of the vessel. That is, in vivo techniques provide the answer to the question: "Do the alterations in vessel function have a physiologically significant impact?"

IN VIVO MEASUREMENT OF ARTERIAL STIFFNESS

Pulse Wave Velocity

A commonly measured in vivo arterial stiffness parameter, both clinically and experimentally, is the pulse wave velocity (PWV). PWV can be measured by acquiring the cardiovascular pulse (either through flow, pressure, or distension measurement) at 2 locations a known distance (d) apart and measuring the time (pulse transit time [PTT]) for the pulse to travel between those locations (Equation 1).

$$\mathrm{PWV} = \frac{d}{\mathrm{PTT}} \quad (1)$$

PWV is fundamentally related to the incremental material stiffness (E_{inc}), wall thickness (h), radius (r), and blood mass density (ρ) through the Moens-Korteweg equation:

$$\mathrm{PWV} = \sqrt{\frac{E_{inc} \cdot h}{2r\rho}} \quad (2)$$

under assumptions of insignificant change in vessel area, insignificant change in wall thickness and an incompressible vessel wall within the range of forces applied.[6] $E_{inc} \cdot h$ is a measure of structural stiffness. It follows that an increase in PWV can be caused by an increase in material stiffness E_{inc} (eg, through collagen or elastin cross-linking) but also merely by wall thickening (increased h) which increases structural stiffness, but not material stiffness, for a given vessel caliber.[7]

As the distances over which the pulses are acquired are usually relatively small, and the PWV is relatively fast (in the range of 4 to 10 m/s), in animals, the transit times are very small (eg, ≤10 ms for the full length of a rat aorta and in the tens of milliseconds for larger animals). Therefore, high fidelity acquisition of the pulse waveform is required for accurate measurement. For pressure, this is best done with solid-state, pressure tipped catheters placed within the arteries.[8] Dual pressure catheters allow the distance between the pressure sensors to be fixed, removing any error in distance measurement and decreasing variability further. Fluid-filled catheters attached to external pressure sensors must be used with great care to limit damping of the waveform (minimal catheter length, equal length for both catheters, degassed saline, and small volume). These procedures are usually

Highlights

- Arterial stiffness is a complex parameter changing with wall remodeling and with acute changes in distending pressure or smooth muscle tension.
- This article summarizes in vivo and ex vivo arterial stiffness measurement techniques for animal studies.
- These techniques allow quantification of the pressure dependency of arterial stiffness and assessment of active (smooth muscle and vascular endothelium) and passive (elastin and collagen) contributions to arterial stiffness.

performed in the anesthetized animal (Figure 1). Where a dual pressure catheter is not used, the distance between the 2 separate catheters must be measured to calculate PWV. This can be done postmortem by leaving the catheters in place, dissecting to the artery of interest to visualize the arterial path and the catheter tips within it, and placing a wetted suture along the length between the 2 catheters. Marking off this length on the wetted suture, the suture can be removed, and the linear distance measured using a ruler (for large animals) or Vernier calipers (for small animals). Repeating this several

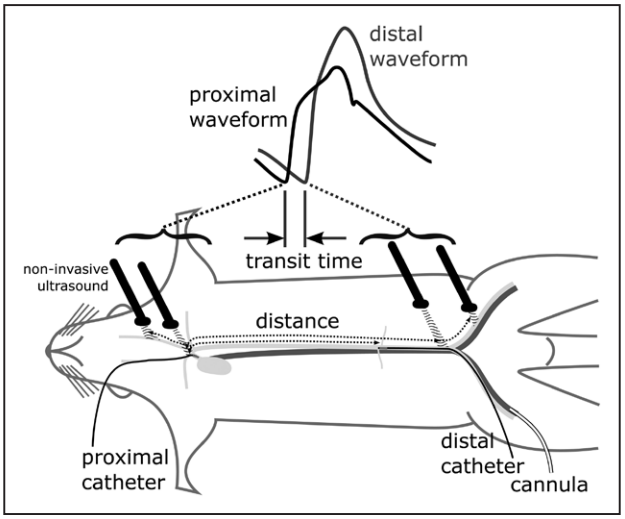

Figure 1. Sites of pulse measurement for the purposes of pulse wave velocity measurement in animals here demonstrated schematically for a rat but transferrable to other animals.
Pressure tipped catheters or fluid-filled catheters externalized to pressure sensors, introduced via the carotid and femoral artery can be introduced into the aortic arch and descending aorta (alternatively, a dual pressure sensor catheter can be introduced into the descending aorta removing the need for measurement of distance between sensors). Noninvasive ultrasound measurement of flow or diameter to obtain a pulse waveform is achievable at the carotid, aortic arch, abdominal aorta, and femoral sites (also elsewhere in larger animals). Dividing the measured distance between 2 vascular sites with the transit time from the waveforms at those sites allows measurement of pulse wave velocity. A venous cannula allows systemic infusion of blood pressure altering drugs.

times increases the confidence in the measurement of the tortuous path of the artery using this technique.

The size of the catheter used, especially in small-animal studies, needs to be considered to ensure that it is not substantially altering the parameters being measured. Computational modeling shows that two 1F (0.33 mm) diameter catheters placed in the mouse ascending and abdominal descending aorta of diameter between 1.2 and 1.6 mm changes local blood pressure resulting in an aortic PWV change of 0.3 m/s[9]. A 1.2F (0.4 mm diameter) dual pressure catheter along the length of the descending aorta in the same scenario increased pressure but decreased aortic blood flow, with a resulting PWV decrease of 0.4 m/s[9]. These effects are not important when manipulating pressure to obtain PWV at all physiological pressures but should be considered when using only anesthetized, baseline conditions in the analysis for mice studies. Likely more important in in vivo mouse studies is wall shear stress, which changes substantially in the region of the catheter[9] and may have effects on local endothelial function. Although a 1.2F catheter occludes ≈8% of the mouse aortic cross-sectional lumen area, the same effects would not be substantial for the same size catheters used in the adult rat aorta (≈2% to 3% occlusion) nor in larger animal studies.

Noninvasive measurement of PWV is possible using ultrasound or tonometric[10] acquisition of the pulse in the anesthetized animal. Ultrasound can be used to acquire distension of blood flow waveforms at 2 vascular sites for noninvasive assessment of PWV. This affords greater flexibility than applanation tonometry as it is more feasible in smaller animals, such as rats and mice, and allows interrogation of most major vessels as the vessel does not need to be close to the body surface as it does for tonometry. Although these noninvasive techniques allow acquisition of high-quality waveforms for transit-time measurement, distances need to be estimated using body surface measurements, which has a greater error than invasive techniques when comparing different animals or longitudinal data where animal growth is significant.

In theory, it would also be possible to measure PWV using telemetry (over short periods of time, where animal growth affecting distance between the catheters is negligible), although the authors are not aware of any studies that have reported this to date. PWV in conscious, free-moving rats has been studied with externalized, fluid-filled catheters.[11,12] Conscious, unrestrained measurement of PWV overcomes the disadvantages of the depressive effects of anesthesia on the cardiovascular system[13,14] for invasive measurement of PWV and the effect of stress induced by restraining in noninvasive, conscious measurement of PWV by ultrasound or tonometry in animals.

Although body surface estimation of vascular length is used as standard practice in noninvasive PWV assessment in humans, the errors in body surface measurement of distances is more critical in animal studies because (1) there is minimal study of the accuracy of noninvasive estimates of arterial distances compared with measured arterial distances in animals; (2) errors in estimation of arterial length in small animals is much more critical than in humans as the path length is greatly smaller, resulting in larger relative error for variation in estimates of arterial length. The problem with relative errors in path length estimation in noninvasive assessment of PWV in small animals greatly reduces the sensitivity of the analysis compared with invasive techniques, even if perfect noninvasive pulse waveforms are acquired.

All methods require the calculation of the delay (transit time) in the pulse between the 2 measured locations. Cross-correlation techniques can be used but are not robust when the 2 sites contain waveforms of different shape, which is often the case. Location of a fiducial point on the cardiovascular waveform to measure transit time, usually the foot of the waveform, avoids this problem. Mitchell et al[15] showed in rats that this foot-to-foot method was accurate and reproducible and more reliable than techniques that use the whole waveform. Historically, locating the foot of the waveform was done by hand. Automating the process allows for a user-independent measurement and analysis of larger data sets (eg, 5 minutes of rat data generates in the order of 1500 pulses to be analyzed). The algorithm used to reliably and repeatably find the foot of the waveform depends on the waveform shape and thus the animal being studied. The more acute diastolic foot of the mouse and rat pressure waveform is well defined by the peak of the second time derivative of pressure. In larger animals, where the diastolic foot of the pressure waveform is more rounded, methods using tangents fitted during late diastole, or early systole, or both, are more robust in locating the waveform foot without user input.[6]

As arterial stiffness is pressure dependent, pressure needs to be accounted for when comparing study groups. In animal studies, this is possible by acutely manipulating blood pressure. Administration of pharmacological agents to alter blood pressure while PWV is measured allows comparison at the same pressure across a full range of physiological pressures. In rat studies, phenylephrine and sodium nitroprusside, although vasoactive, have been shown to obtain a wide physiological range of blood pressure with minimal direct effect on large artery (aortic) stiffness[16] when infused intravenously at 30 μg/(kg·mL) if taking the data following the infusion where blood pressure returns from the extreme back to resting baseline pressure (Figure 2). By acutely altering pressure, arterial stiffness can be compared at the same level of pressure in animal groups that have different resting pressures, for example, normotensive and hypertensives groups, where arterial stiffness comparison would not otherwise be able to be done due to the acute pressure dependency of arterial stiffness. It also allows

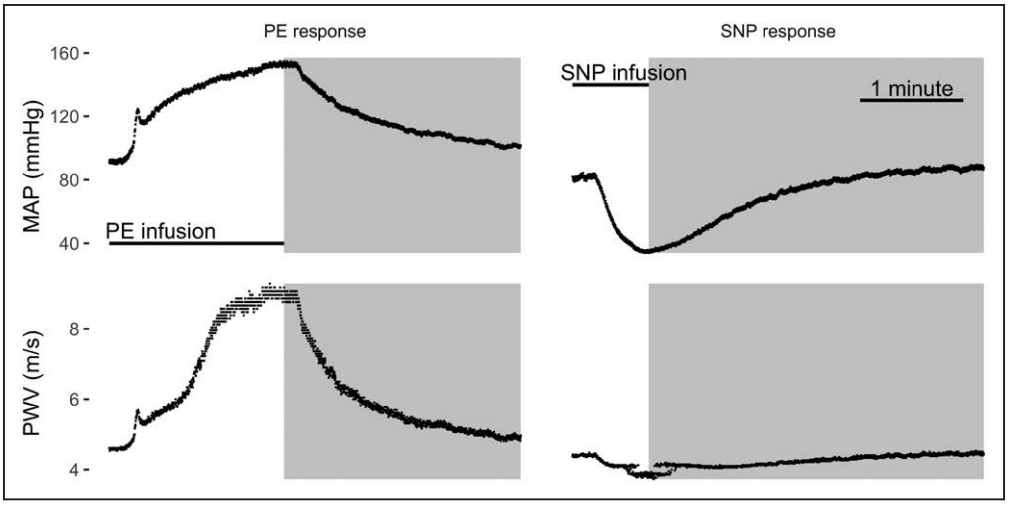

Figure 2. Infusion of phenylephrine (PE) and sodium nitroprusside (SNP) allows measurement of aortic pulse wave velocity (PWV) across a full physiological range of blood pressure, here shown in a rat.
In rats, when taking the return to baseline data (shaded area), the direct effect of the vasoactive drugs, infused at 30 µg/(kg·mL), on the large arteries is minimal.[16] MAP indicates mean arterial pressure.

the investigation of the pressure sensitivity of arterial stiffness (the slope of arterial stiffness with blood pressure changes), which has been shown to change with chronic hypertension decreasing the pressure sensitivity of arterial stiffness.[17]

With in vivo measurements, beat-to-beat blood pressure and thus PWV values can be obtained, resulting in a large number of data points for analysis. Conventionally, data points are often averaged across several cardiac cycles using data binning, whereby PWV values are grouped together according to defined intervals (eg, in 10 mm Hg) of blood pressure (bins), then averaged for each bin. This data binning can resolve uneven weighting of data, as there may be more data points (pulses) in some pressure ranges compared with others. Comparisons of PWV values can then be performed across the blood pressure bins, or at the same blood pressure bin, using statistical tests such as ANCOVA. It is important to note that the relationship between blood pressure and arterial stiffness, and thus PWV, is by nature curvilinear.

As such, although simple linear regression may be used for small ranges of blood pressure, a second-order polynomial should otherwise be fitted to the data. Furthermore, since linear regression is based upon the assumption that data points are independent, linear mixed models, as opposed to repeated-measures ANOVA, can be used to both account for the relationship between blood pressure and PWV in individual animals, as well as for comparisons between groups of interest. More modern statistical methods can deal with unevenly weighted data and, at the same time, harness the statistical power of having many data points (pulses) without the requirement of binning data. Linear mixed models have these advantages as well as being more apt to handle missing and unbalanced data.[18] An explanation of how linear mixed models can be used in animal cardiovascular research can be found in the technical report by Gonçalves et al.[19] Alternatively, robust statistical methods can also be used.[20,21]

Compliance and Distensibility

PWV, as introduced above, is measured by determining the transit time between 2 measurement sites. Another technique to assess stiffness that is commonly used in humans is determination of arterial compliance and distension. By combining the change in diameter over the cardiac cycle with a measure of systolic and diastolic blood pressure, compliance (C) can be calculated as

$$C = \frac{A_s - A_d}{P_s - P_d} \quad (3)$$

where A_s and A_d are the systolic and diastolic arterial lumen cross-sectional areas, respectively, and P_s and P_s the systolic and diastolic blood pressure, respectively. Compliance values will differ considerably between small and large vessels (and, hence, also with body size). This can be resolved by normalizing compliance to A_d, yielding distensibility (D):

$$D = \frac{C}{A_d} \quad (4)$$

Subsequently, a local measure of PWV can be obtained using the Bramwell-Hill equation[22]:

$$PWV_{dist} = \sqrt{\frac{1}{\rho \cdot D}} \quad (5)$$

with ρ the blood mass density, typically taken to be 1050 kg/m^3.

The lumen cross-sectional area measurements are typically obtained using ultrasound wall tracking and thus can often be obtained noninvasively. Calculation of compliance also requires blood pressure measurement at the same site, and this is usually obtained from invasive catheter measurements, similar to the case of transit-time PWV measurement (Section Pulse Wave Velocity). For longitudinal studies, an externalized arterial cannula could be used to assess pressure or pressure telemetry, with the limitation that pressure may not be measured at the precise location of interest and where the vessel diameter is being measured using ultrasound. Cuff-based blood pressure measurements, for example, tail-cuff blood pressure in rodents, do not provide the accuracy of pulse pressure measurement required to assess compliance[23] and are not useful in that context.

Calculated (Compliance Based) Versus Measured PWV

PWV measured using the pulse transit time (Section Pulse Wave Velocity) yields an integrated measure of arterial stiffness over the segment of vessel being interrogated. PWV calculated from distensibility (Section Compliance and Distensibility) is a measure of arterial stiffness at the single location where area and pressure are measured.

Arterial stiffness metrics, whether PWV, compliance, or distensibility, intrinsically vary with blood pressure[24–26] as well as heart rate.[27–29] However, the relationship between blood pressure and heart rate with PWV measured using pulse transit time and PWV calculated from distensibility differ; hence, the 2 methods of arriving at PWV are not interchangeable. This can be attributed to the way that compliance is normally calculated (Equation 3). In particular, this equation uses an approximate, linearized derivative of pressure to diameter using the systolic and diastolic points of pressure and diameter, which leads to an overestimation of PWV that gets progressively pronounced with increased pulse pressure.[30]

PWV measured using transit time is measured at the point of diastolic blood pressure (using the foot-to-foot technique). Therefore, if pressure and diameter are measured continuously and compliance calculated using the incremental measurements close to diastolic pressure (as opposed to using the systolic and diastolic points of pressure and diameter), PWV calculated by compliance and PWV measured using transit time may be more closely correlated. This exercise has not been carried out to date. Despite these problems, PWV calculated from compliance using systolic and diastolic points is likely to persist in human studies due to the ease of measuring systolic and diastolic blood pressure noninvasively, as opposed to obtaining the full pressure waveform. Caution must be used in comparing these results to measured (transit time) PWV in animal and human studies.

Endothelial Function

Although impairing endothelial function with endothelial active drugs during simultaneous measurement of vessel diameter provides an in vivo measure of endothelial function, more often used is a simplified protocol comparing blood pressure changes following infusion of acetylcholine (inducing endogenous nitric oxide activation) and sodium nitroprusside (an exogenous nitric oxide donor).[31] In animal studies, more common than in vivo measurements of endothelial function is ex vivo quantification (Section Contribution of Cellular Components to Arterial Stiffness).

EX VIVO MEASUREMENT OF ARTERIAL STIFFNESS

In vivo quantification of arterial stiffness does not effectively separate the contribution of the cellular (endothelial function, smooth muscle function) and noncellular (elastin fragmentation, collagen cross-linking, vascular wall remodeling) components to arterial stiffness. Ex vivo animal studies do enable this separation. Histological methods are important in quantifying the structure and geometry of the vessel and may assist in explaining changes in vascular stiffness. This section, however, concentrates on methods of assessing vascular stiffness per se.

Contribution of Cellular Components to Arterial Stiffness

Ex vivo characterization of the function (endothelial and smooth muscle contribution to dynamic changes in vessel stiffness) of microvessels has been the defining life achievement of Mulvany and Aalkjaer.[32] Many of the same principles of functional testing of microvessels can also be applied to large vessels. Translating to large vessels is simply a process of allowing for greater time (due to dynamics of diffusion across tissue) and using appropriate equipment (hooks instead of wires in typical myography equipment for small-animal large arteries or use of organ bath apparatus with force transducers for large arteries of large animals). Pressure myography is also an option for both small and large vessels.

The smooth muscle response can be characterized using dose-response curves to phenylephrine (contraction) and sodium nitroprusside (dilation). Endothelial function can be interrogated using acetylcholine (causing dilation through endothelial release of nitric oxide) and N(ω)-nitro-L-arginine methyl ester (L-NAME; causing contraction by blocking the endothelial nitric oxide release).[33] The same concentrations of phenylephrine and sodium nitroprusside in an ex vivo bath and in vivo in blood appear to alter vascular stiffness ex vivo, but not significantly in vivo.[16] The reasons for this observation have not been studied, but it is suspected that it is

due to the kinetics of drug absorption being substantially different with the ex vivo vessel being immersed in the fluid, and the in vivo vessel only having the luminal surface exposed to the drug-containing fluid with rapid uptake of the drugs at vascular sites distal to the large arteries being studied.[16]

Pressure myography is truer to the in vivo setting than wire myography in that a uniform pressure is applied to the inner wall of the vessel. In addition, the measured diameter in response to the applied fluid pressure does not need normalization when using pressure myography.[34] Wire and other tensile myography techniques require the measured force to be normalized to the size of the vessel, either by measurement of the cross-sectional area that the force is applied to (vessel segment length (l) and wall thickness (h), resulting in a stress (σ) in Pascals, Figure 3), or to the mass and longitudinal length of the vessel segment (resulting in units N/m per kilogram). A more meaningful method of expressing the result is to relate the wall stress to distending pressure (Figure 3), as detailed in the literature, including by Noordergraaf.[35] Taking half the cylindrical ring of vessel, as drawn in Figure 3B, the force derived from wall tension is related to the stress by

$$F_{wall} = 2\sigma lh \quad (6)$$

The force acting against this is the difference between the pressure inside (P_i) and outside (P_o) the vessel and that force is equal to

$$F_{transmural} = 2P_i r_i l - 2P_o r_o l \quad (7)$$

for internal and external vessel radius of r_i and r_o, respectively. If the vessel wall is not breaking, then the 2 forces must be equal and thus

$$\sigma h = P_i r_i - P_o r_o \quad (8)$$

Wall tension (T) is the force per unit length, and thus multiply wall stress by wall thickness gives tension (Equation 9).

$$T = \sigma h \quad (9)$$

Given the equated forces in Equation 8, pressure can be expressed in terms of wall tension:

$$T = P_i r_i - P_o r_o \quad (10)$$

For a thin-walled vessel, wall thickness is small compared with the internal and external radius. Thus $r_i \simeq r_o$. Under this assumption, a single vessel radius (r) can be used:

$$T = r(P_i - P_o) \quad (11)$$

$$= r\Delta P \quad (12)$$

where ΔP is the transmural pressure. This is a corollary of the Laplace equation. Therefore, for a vessel ring mounted on 2 rods, wires, or pins, the equivalent transmural pressure (ΔP) from the measured force can be calculated knowing the radius (r) and longitudinal length (l) of the vessel segment (Equation 13, providing pressure in Pascals when using SI units of Newtons and meters for force and vessel dimensions, with conversion to mm Hg obtained by dividing by 133.3 Pa/mm Hg).

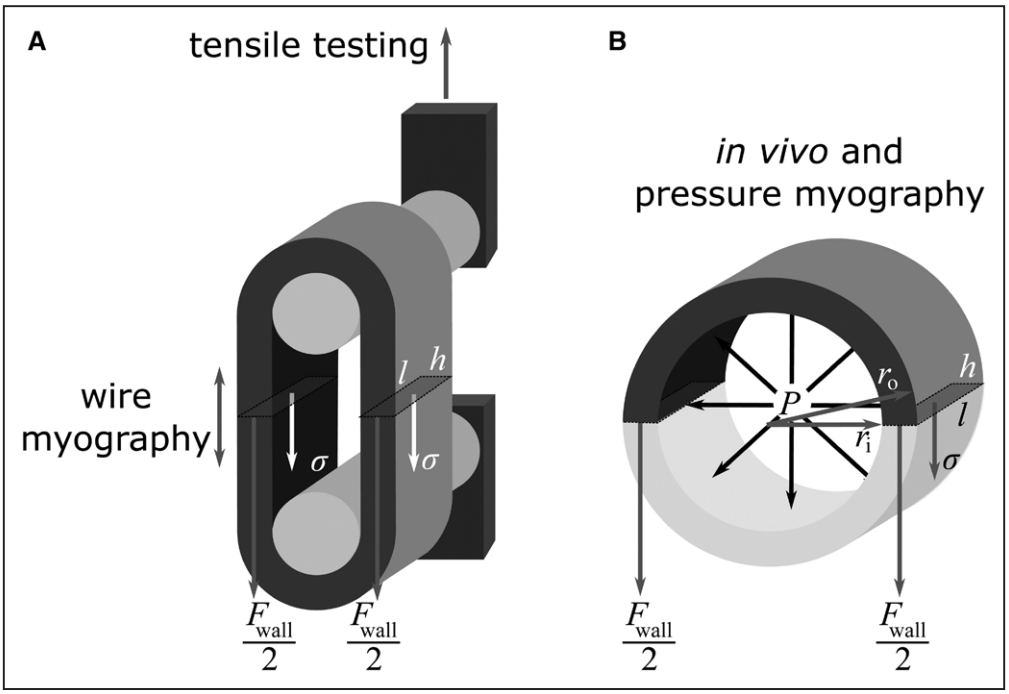

Figure 3. Force (F), whether induced in the case of myography, or applied in the case of tensile testing, needs to be normalized to the area (in figure, indicated by dotted line, defined by wall thickness, h multiplied by longitudinal vessel length, l) that the force is applied.
This stress (σ) can be converted to an equivalent distending pressure (P) as would be experienced in vivo (Equation 13).

$$\Delta P = \frac{F}{2rl} \quad (13)$$

Contribution of the Extracellular Matrix to Arterial Stiffness

Tensile testing is a fundamental tool for quantifying material stiffness. For larger vessels in larger animals, a strip of vessel can be cut, mounted, and pulled in the circumferential direction (the longitudinal direction may also be tested but would be a secondary result to circumferential testing). For smaller animals and smaller vessels, the vessel can be mounted as a ring with 2 metal pins passing through the lumen of the vessel to apply the pulling force (Figure 3).

There are no set rules on how to conduct tensile testing. However, there are some key points to consider. First, it should be ensured that smooth muscle cells are not actively contributing to the measured force or initial measurements in the unloaded state. This could be done by ensuring the smooth muscle has been put through conditions to ensure cell death or by testing under conditions that create maximal smooth muscle relaxation, such as soaking in high dose sodium nitroprusside. Second, viscoelastic effects on the test should be minimized. Measures to minimize viscoelastic effects include precycling the tissue between 2 low force points a number of times until force/extension hysteresis disappears and always applying extension at a slow rate.[36]

To take a rat aortic ring as an example: two 26 gauge needles can be passed through the lumen of most rat aortas of most ages to allow a force to be applied to the inner surface of an aortic ring. Precycling between forces equivalent to a distending pressure of 0 and 180 mm Hg (Equation 13) between 8 and 10 times will usually remove any force/extension hysteresis within that range. Stretching at a rate of 2 mm/min to breaking point is suitably slow to minimize viscoelastic effects on the force/extension curve.

As with wire myography, force needs to be normalized for the cross-sectional area to which the force is applied to group the data and compare between study groups. Equation 13 applies to rings of arterial tissue to convert the applied force into an equivalent in vivo distending pressure. Extension needs to be normalized to a starting length to calculate strain. For larger vessels, where a strip of tissue is being tested, this is trivial to measure. For small vessels, usually tested as a vessel ring, the starting length is half the circumference of the unloaded sample. The unloaded circumference can be measured using standard light microscopy and a graticule, or by using the length measured during tensile testing where the first uptake of minimal load is detected.

It can be instructive in tensile testing to convert length extension to equivalent circumference and radius to express strain (ε) as

$$\varepsilon = \frac{\Delta r}{r} \quad (14)$$

The resultant material stiffness (E_{inc}) at each increment of tension is (Figure 4):

$$E_{inc} = \frac{d\sigma}{d\varepsilon} \quad (15)$$

The stress/strain curve is usually well represented by a third-order polynomial fit. Breaking stress and breaking strain are usually noted and compared between study groups. The stress/strain curves themselves can also be statistically compared or a comparison can be made at a nominal level of strain or stress. It is often loosely interpreted that very low levels of strain load predominantly elastin fibers and equate to evaluating the elastin contribution to stiffness. It follows that comparison at high levels of strain predominantly load the stiffer collagen fibers and equates to evaluating the collagen contribution to stiffness.[37,38]

Although tensile testing is usually applied to the vessel as a whole, digestion of components of the vessel wall and tensile testing of the remaining material can isolate the mechanical contribution of elements of the arterial wall. For example, smooth muscle and collagen can be digested in sodium hydroxide, leaving elastin in place.[39] Alternatively, elastase (and collagenase) can specifically remove elastin

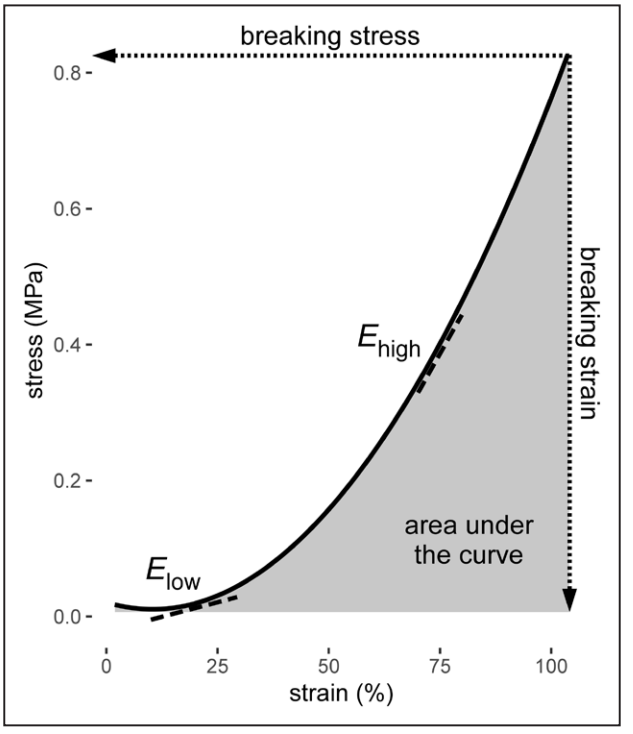

Figure 4. A typical stress-strain curve for arterial tissue, the example here being a bovine retinal artery ring.
The slope of the curve is the incremental Young modulus (E), the material stiffness of the vessel at that strain. Comparison of stress-strain curves in animal studies might be made by comparing breaking stress, breaking strain, the area under the curve, the curve as a whole using mixed model analysis, or the Young modulus at nominal strains (here shown nominally at 20%, E_{low}, and 75%, E_{high}, strain).

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(and collagen).[40] Tensile testing of such digested vessels gives the mechanical strength of the remaining components.

Scanning acoustic microscopy, nanoindentation, atomic force microscopy, and high-force magnetic micromanipulation,[41] although mechanical tests far removed from the conditions in vivo, permit mechanical testing of the individual layers and components within the vessel wall.

The possibility of interrogating the stiffness, and even viscoelasticity[42] of the substructure of the arterial wall, provides a level of detail not available when testing the artery as a whole such as in tensile testing. It is important in these methods to report all methodological conditions to permit interpretation of the data. For example, measured stiffness using atomic force microscopy varies significantly with the shape and size of the indenter used.[43]

Importance of Loading Regimen

Just like in vivo stiffness metrics such as PWV depend on blood pressure, ex vivo arterial stiffness metrics are also strongly dependent on the biomechanical loading state in which measurements are taken. In vivo, arteries are loaded biaxially, that is, stretched both longitudinally and circumferentially. Stresses in those directions are coupled: longitudinal stretching not only affects longitudinal but also circumferential stress and stiffness and vice versa.[44]

In atomic force microscopy, a sample is typically assessed in an (axially as well as circumferentially) unloaded condition. Such measurements yield stiffness metrics (Young modulus) that are 2 orders of magnitude smaller than those obtained under in vivo loading conditions.[44]

Wire myography and tensile testing are typically performed at an in vivo relevant circumferential stretch. However, in the longitudinal (axial) direction, the vessel is typically left unloaded. Custom rigs can be made to load and test square samples of vascular tissue biaxially,[45] permitting properties to be quantified in the circumferential and longitudinal direction simultaneously.

In the case of pressure myography, the longitudinal axis is fixed, and the vessel can be mounted at its in vivo axial stretch.[46] Methods have also been outlined for measuring changes in the longitudinal force at the in vivo axial length in techniques of pressure myography.[47]

Whether length is fixed or free has important implications and can lead to 2-fold differences in observed circumferential stress and material stiffness.[44] Furthermore, sensitivity to vasoactive stimuli may differ between uniaxial (eg, wire myography) and biaxial testing (eg, pressure myography with the artery mounted at its in vivo length) regimens.[48] In conclusion, caution should be exercised when comparing stiffness metrics obtained using different techniques.

VISCOELASTICITY

The arterial wall has viscoelastic properties[49] and, therefore, in dynamic (pulsatile) loading conditions, the rate of change of the stress applied to the vessel will affect the lag of strain and the subsequent apparent stiffness. It is suspected that the effect is substantial enough to see changes in arterial stiffness with changes in heart rate seen in normal physiology.[27–29,50] It is, therefore, important to consider viscoelasticity when measuring stiffness under conditions of changing, or different, cyclic rates (heart rates).

The area within the arterial pressure–diameter curve of a single cardiac cycle measured in vivo provides information on viscoelasticity. In animal studies, frequency of loading can be controlled by pacing the heart,[27] but it is harder to control the load (pulse pressure) within each cycle at varying mean pressures in the intact circulation. Ex vivo assessment of viscoelasticity allows for better control over both frequency and load. Langewouters et al's[51,52] work of the 1980s used ex vivo techniques and contributed substantially to the understanding of viscoelasticity of arteries at different sites and with aging.

More recently, high-frequency small-displacement strain has been proposed as a method of assessing vascular stiffness response, although more in the context of cross-bridge state of vascular smooth muscle cells.[53] A more physiologically comparable ex vivo test, that echoes the work of Langewouters et al[51,52], uses customized organ bath equipment to apply strain or force oscillations of a more physiological magnitude and frequency and pressure-diameter loops generated as shown by Leloup et al[54] that could be used for quantification of viscoelasticity ex vivo. Such dynamic testing of arterial stiffness, whether in vivo where large differences in heart rate are observed or ex vivo where changes in oscillation frequency are used, should address viscoelasticity alongside the measurement of stiffness.

SUMMARY

The techniques available for arterial stiffness assessment in animal studies permit a very detailed characterization of vessel mechanics. Ex vivo characterization through myography and tensile testing can assess the active (smooth muscle–derived and endothelium-derived relaxation) and passive (noncellular components) contribution to vessel stiffness. Scanning acoustic microscopy, nanoindentation, and atomic force microscopy can provide more detail on the contribution of individual vessel constituents to the artery stiffness. Methodological details, and reporting of those methodological details, are very important in all ex vivo quantification of arterial stiffness, as methodological differences can have substantial impacts on the absolute measures of arterial function and stiffness.

Although statistical significance might be reached in comparison of ex vivo quantification of arterial stiffness, it is important to also assess whether there is physiological significance in these differences and this

can be assessed in vivo at equivalent pressures using measures of PWV and compliance noting, however, that compliance and PWV are not directly interchangeable. Animal studies permit the quantification of in vivo arterial stiffness across a wide physiological range of blood pressure, allowing comparison of arterial stiffness under acute conditions of hypotension and hypertension, as well study of the effect of normal diurnal variability in blood pressure on arterial stiffness.

These techniques for assessment of arterial stiffness allow measurement not only of the resting stiffness of the artery, as assessed in humans, but also a highly comprehensive quantification of the pressure dependency of arterial stiffness and the assessment of the contribution of active (smooth muscle and vascular endothelium) and passive (elastin and collagen) components to arterial stiffness.

ARTICLE INFORMATION

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Affiliations

From the Department of Biomedical Sciences, Faculty of Medicine, Health and Human Sciences, Macquarie University, Sydney, NSW, Australia (M.B., I.T., A.P.A.); Department of Biomedical Engineering, School of Engineering & Applied Science, Yale University, New Haven, CT (B.S.); and Department of Biomedical Engineering, CARIM School for Cardiovascular Diseases, Maastricht University, the Netherlands (B.S.).

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Disclosures

None.

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