In Vivo Human Brachial Artery Elastic Mechanics
Effects of Smooth Muscle Relaxation

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Background—The effects of smooth muscle relaxation on arterial wall mechanics are controversial. We used a new, in vivo, noninvasive technique to measure brachial artery wall mechanics under baseline conditions and following smooth muscle relaxation with nitroglycerin (NTG).

Methods and Results—Eight healthy, normal subjects (6 male, 2 female; age 30±3.1 years) participated in the study. The nondominant brachial artery was imaged through a water-filled blood pressure cuff using an external ultrasound wall-tracking system at baseline and following 0.4 mg sublingual NTG. Simultaneous radial artery pressure waveforms were recorded by tonometry. Transmural pressure (TP) was reduced by increasing water pressure in the cuff. Brachial artery area, unstressed area, compliance, stress, strain, incremental elastic modulus (Einc), and pulse wave velocity (PWV) were measured over a TP range from 0 to 100 mm Hg. Baseline area versus TP curves generated 30 minutes apart were not significantly different. NTG significantly shifted area versus TP (P<0.0001) and compliance versus TP (P<0.001) curves upward, whereas the Einc versus TP (P<0.05) and PWV versus TP (P<0.01) curves were shifted downward. NTG also significantly shifted stress versus strain (P<0.01) and Einc versus strain (P<0.01) curves to the right.

Conclusions—We conclude that brachial artery elastic mechanics can be reproducibly measured over a wide range of TP and smooth muscle tone using a new noninvasive ultrasound technique. Smooth muscle relaxation with NTG increases isobaric compliance and decreases isobaric Einc and PWV in the human brachial artery. (Circulation. 1999;100:41-47.)

Key Words: arteries ■ elasticity ■ nitroglycerin ■ muscle, smooth ■ ultrasonics ■ vasodilation

Large arteries buffer the pulsatile flow and pressure waves generated by intermittent left ventricular contraction. In addition, large arteries affect the transmission rate or pulse wave velocity (PWV) of both incident and reflected pressure waves. These mechanical functions of large arteries are important determinants of the pulsatile components of left ventricular afterload. Many noninvasive techniques have been developed to measure the arterial elastic mechanics in humans. Although each technique has its relative advantages, there are some important limitations. Most of the techniques measure arterial elastic mechanics over a limited pressure range (at mean pressure or between systole and diastole). Many techniques cannot separate the direct effects of a drug on arterial wall mechanics from the indirect effects related to a change in blood pressure. Because vessels are not as stiff at pressures below the physiological range, it may be easier to separate diseased from normal vessels by studying the vessels at low transmural pressure (TP). Additionally, determination of arterial diameter at very low or zero TP allows for the calculation of true vessel strain and incremental elastic modulus (Einc), a measure of intrinsic wall stiffness.

The first goal of this study was to describe and demonstrate the reproducibility of a new external ultrasound technique for the noninvasive, in vivo measurement of brachial artery elastic mechanics over a wide pressure range. The second goal of this study was to use this technique to study the effects of smooth muscle relaxation on brachial artery elastic mechanics. Previous in vivo studies in animals and humans have differed in their conclusions regarding the effects of smooth muscle relaxation on arterial stiffness. Smooth muscle relaxation has been shown to cause an increase, a decrease, or little change in Einc. Studies involving smooth muscle contraction have also shown conflicting results. Although smooth muscle relaxation increases stiffness by tensing the collagen and elastin fibers in parallel with the smooth muscle, it decreases stiffness by reducing tension in the smooth muscle itself and in the series elastic component. The net effect of smooth muscle relaxation on arterial stiffness depends on the balance of these competing effects. With this new technique, we can measure the Einc of the arterial wall under baseline conditions and, following nitroglycerin (NTG), to determine the direct effects of smooth muscle relaxation on in vivo arterial stiffness.
Methods

Study Population
We studied 8 normal human subjects age 28 to 36 (mean±SEM, 30±1.1 years). There were 6 male and 2 female subjects. All subjects were without cardiovascular disease, as determined by history, physical examination, routine blood tests, and ECG. Mean blood pressure was 114±2/64±2 mm Hg. Body weight was 72±3 kg and forearm volume was 890±57 mL. Written informed consent was obtained from all subjects. The study was approved by the Human Rights in Research Committee at the University of Minnesota.

Measurement of Brachial Artery Pressure and Diameter

The nondominant brachial artery was imaged through a water-filled vinyl blood pressure cuff using a pulsed ultrasound echo-tracking system (WTS, PIE). A 7.5 MHz linear array ultrasound transducer was positioned over the brachial artery of the nondominant arm approximately 4 to 6 cm proximal to the antecubital fossa. A 2D, longitudinal B-mode image of the artery was obtained which clearly showed the anterior and posterior walls. An M-line was selected perpendicular to the artery and the resulting radio frequency signal over 6 to 8 cardiac cycles was digitized and stored in memory. Markers were manually placed on the highest amplitude radio frequency signals, which represented the anterior and posterior walls, and these signals were tracked over time (Figure 1A).

Blood pressure waveforms were simultaneously recorded (Figure 1B) from the radial artery using applanation tonometry (N500, Nellcor Inc). The radial waveforms were calibrated to oscillometric cuff readings taken every 3 minutes from the contralateral arm. By treating the early wavefront as a region that maintains its identity in the propagated wave1 and neglecting both inertial and viscous behavior, the timing of the pressure waveform was corrected for the slight phase delay by matching the foot of the pressure waveform with the foot of the diameter waveform. At higher water cuff pressure (lower TP), the radial artery pressure waveform was distorted. Therefore, the radial artery waveform obtained with 0 mm Hg pressure in the water-filled cuff was used for all pressure-area calculations. The pressure in the water cuff was measured using a pressure transducer calibrated with a mercury manometer.

![Figure 1. Ultrasound recordings of anterior and posterior wall motion (A). The distance between the 2 walls of the artery is plotted vs time. B, Simultaneous radial artery pressure and brachial artery diameter waveforms.](image-url)
Brachial artery intima-media thickness (IMT) was measured using a technique similar to that validated for measurement of carotid artery IMT.13 We obtained high-resolution, longitudinal, ultrasonic B-mode images of the brachial artery using a 10 MHz water bath transducer and an ultrasound machine (Phase 2, Biosound Inc). Images were recorded on S-VHS videocassettes for later analysis. IMT and diameter were measured at end diastole as determined from an ECG. The IMT was measured as the average distance between the 5 points from the leading edge of the lumen-intima interface to the leading edge of the media-adventitia interface of the far wall. The lumen diameter for each frame was measured as the average distance between 5 points from the trailing edge of the near wall lumen-intima interface to the leading edge of the media-adventitia interface of the far wall. The 5 frames were averaged to determine the diameter and IMT. All measurements were performed by an individual who was blinded to the experimental conditions. Wall cross sectional area (WCSA) was calculated as:

\[
WCSA = \pi [(R_{\text{out}})^2 - (R_{\text{in}})^2],
\]

where \(R_{\text{out}}\) = outer radius = diameter/2, and \(R_{\text{in}}\) = inner radius = \(R_{\text{out}} - \text{IMT}\).

Wall thickness measurements at all other cuff pressures were then calculated assuming constant WCSA.14

### Calculation of Arterial Elastic Properties

Ensemble pressure and area waveforms were generated from approximately 20 beats at each 20 mm Hg step using custom software. The diastolic portion of the data were fit to Langewouters’ arc tangent model,15

\[
A = a [0.5 + 1/\pi \cdot \tan^{-1} (\text{TP}/c - b/c)]
\]

where \(A\) is brachial artery area and \(a\), \(b\), and \(c\) are parameters describing the area versus pressure curves. All individual area-pressure data were fit to this arc tangent model with values of \(r > 0.90\) using the Levenberg-Marquardt algorithm.

Brachial artery compliance \((C)\) was calculated as the first derivative area versus pressure curves:

\[
C = a/(\pi \cdot c)[1/(\text{TP}/c - b/c^2)]
\]

Circumferential wall stress \((\sigma)\) was calculated as follows:

\[
\sigma = \text{TP} \cdot r_e/\text{IMT}
\]

where \(r_e\) is mid-wall radius. Brachial artery outer radius \((r_{\text{out}})\) was calculated as follows:

\[
r_{\text{out}} = (A/\pi)^{1/2}
\]

assuming that the brachial artery cross-section is circular. Mid-wall radius was calculated as \(r_{\text{out}} - \text{IMT}/2\). Circumferential strain \((\epsilon)\) was calculated as follows:

\[
\epsilon = r_e/r_i
\]

where \(r_e\) is the effective unstressed mid-wall radius (the mid-wall radius at 0 mm Hg TP) under baseline conditions. \(E_{\text{inc}}\) was calculated as 75% of the slope of the stress-strain curves:

\[
E_{\text{inc}} = 0.75 \cdot \delta z/\delta e
\]

This method of calculating \(E_{\text{inc}}\) is similar to that used by others16 and assumes that the arterial wall is isotropic and has a Poisson’s ratio of 0.5. PWV was calculated using the Mocns-Korteweg equation17:

\[
\text{PWV} = [(E_{\text{inc}} \cdot \text{IMT})/(2 \rho \cdot r_e)]^{1/2}
\]

where \(\rho\) is blood density (1.055 g/mL).

The parameters found by nonlinear regression were used to compute the area and stress corresponding to the measured values of TP and strain, respectively. A versus TP and stress versus strain curves from 0 to 100 mm Hg were then generated at 2 mm Hz.
increments for each individual. Points on the fitted curves for each condition were averaged and mean area versus pressure and stress versus strain curves for baseline and NTG conditions were determined.

Statistics
Statistical analysis was performed using SPSS for Windows 95. Baseline reproducibility of wall thickness and \( r \) were assessed using paired \( t \) tests. Reproducibility of IMT and diameter measurements was also assessed using the Bland-Altman technique. Curves generated at baseline and following NTG administration were compared using ANOVA with 2 repeated variables (condition and pressure). Statistical significance was defined as \( P < 0.05 \). Data are presented as mean ± SEM.

Results
Figure 2 shows arterial diameter waveforms for an individual at 4 different transmural (and cuff) pressures under baseline and NTG conditions. As cuff pressure increased and TP decreased, the change in arterial diameter over a given cardiac cycle markedly increased.

Figure 3 shows the mean area versus TP relationship for the brachial artery on 2 occasions 30 minutes apart in the 8 normal subjects. There was no significant difference in the 2 curves. Mean unstressed mid-wall radius was 1.53 ± 0.09 mm and mean WCSA was 4.37 ± 0.54 mm\(^2\). Reproducibility studies of unstressed mid-wall radius, IMT, and B-mode diameter showed mean differences of \( -0.01 ± 0.1 \) mm (0.6% of the mean), \( 0.001 ± 0.004 \) mm (0.3% of the mean), and \( -0.028 ± 0.036 \) mm (0.7% of the mean), respectively (\( P = NS \)). Plots of the average IMT or diameter versus the difference in IMT or diameter (not shown) revealed no relation between average values and the difference between values.

Sublingual NTG had no effect on heart rate (from 59 ± 4 to 62 ± 5 bpm, \( P = NS \)) or on systolic pressure (from 114 ± 2 to 113 ± 2 mm Hg, \( P = NS \)), however, diastolic pressure decreased slightly (from 64 ± 2 to 58 ± 2 mm Hg, \( P < 0.05 \)).

Figure 4 shows the area versus TP curves at baseline and following 0.4 mg sublingual NTG. NTG significantly (\( P < 0.0001 \)) shifted the baseline area versus TP curve upward in a nonparallel fashion. At 100 mm Hg, brachial artery area increased by 6.46 mm\(^2\) (38% of baseline). NTG also significantly (\( P < 0.001 \)) shifted the compliance versus TP curve (Figure 5) upward with an increase in compliance at 100 mm Hg of 0.008 mm\(^2\)/mm Hg (94% of baseline). Figure

\[ \text{Area (mm}^2\text{)} \]

\[ \text{Transmural Pressure (mm Hg)} \]

\[ \text{Baseline vs NTG} \]

\[ *P < 0.0001 \]

\[ \text{Compliance vs pressure curves at baseline (dotted) and after 0.4 mg sublingual NTG (solid). NTG significantly increased compliance by an average of 38% over the entire pressure range as compared with baseline.} \]

\[ \text{E}\text{inc vs pressure curves at baseline (dotted) and after 0.4 mg sublingual NTG (solid). NTG significantly decreased E}\text{inc by an average of 42% over the entire pressure range as compared with baseline.} \]

\[ \text{Stress vs strain curves at baseline (dotted) and after 0.4 mg sublingual NTG (solid). NTG significantly shifted the baseline stress-strain curve rightward resulting in a large decrease in isometric wall stress.} \]

\[ *P < 0.01 \]
Figure 8.  

6 shows the effects of NTG on Einc. NTG significantly (P<0.05) shifted the Einc versus TP curve downward. The decrease in Einc at 100 mm Hg was 11.8\times10^6\text{ dynes/cm}^2 (35\% of baseline). NTG significantly (P<0.01) decreased PWV. The decrease in PWV was 5.04 m/sec at 100 mm Hg (33\% of baseline).

Figures 7 and 8 show stress versus strain and Einc versus strain curves at baseline and following NTG. NTG resulted in a significant (P<0.01) rightward shift of these curves and, thus, a large decrease in isometric arterial wall stress and stiffness.

Discussion

We describe a new noninvasive ultrasound technique for measuring in vivo brachial artery elastic properties over a wide range of pressure and smooth muscle tone. Using this technique, we demonstrate that smooth muscle relaxation with NTG improves brachial artery elastic properties. NTG increases brachial artery compliance and decreases PWV by both increasing brachial artery cross-sectional area and by decreasing brachial artery stiffness as measured by Einc. NTG also shifts the stress-strain and Einc-strain curves rightward, reducing isometric wall stress and Einc.

Measurement of Arterial Elastic Properties

A unique aspect of the technique described for measuring arterial elastic mechanics is the use of a pressurized water-filled cuff surrounding the brachial artery. This allows for the reproducible measurement of brachial artery wall mechanics over a wide range of TP without changing systemic pressure or activating reflex neurohormonal mechanisms. TP is calculated as arterial pressure minus water cuff pressure. We have previously validated this calculation using intravascular ultrasound and an air-filled cuff.9

We used the posterior wall IMT as our measure of wall thickness, because full wall thickness cannot readily be measured by external ultrasound. We obtained a mean wall thickness-to-radius ratio of 0.14. This value is consistent with data obtained in animal studies showing wall thickness-to-radius ratios of 0.11 to 0.15 for large peripheral muscular arteries.17 True wall thickness should also include adventitia thickness. If our wall thickness measurements were increased by 10\%, Einc would change by 9.1\% at 100 mm Hg. Any errors in wall thickness, and hence WCSA, would change arterial elastic values similarly under baseline and NTG conditions. Therefore, although the absolute values would change, the effects of drugs on arterial elastic properties would be nearly identical.

The radial artery pressure waveform was used as an approximation of the brachial artery pressure waveform because brachial artery pressure could not be noninvasively measured simultaneously with brachial artery diameter. Previous studies in our laboratory with intra-brachial catheters showed no significant changes in brachial artery pressure waveform morphology as a consequence of external cuff pressure. The radial and brachial artery waveforms are similar in shape, but the radial waveform is slightly delayed because of pulse wave transmission. Correction for this time delay between the brachial and radial arteries was accomplished by aligning the foot of the radial pressure waveform with the foot of the diameter waveform.1 19 The exact timing of the pressure and diameter waveforms is not critical for the accurate measurement of arterial elastic mechanics in this study. Only diastolic portions (from the dicrotic notch to end diastole) of the individual A versus TP curves were used to generate the complete curves. Because changes in pressure and diameter occur slower in diastole, small phase lags do not appreciably alter the area-pressure relation during this part of the cardiac cycle. The mechanical properties described are thus static properties of the brachial artery and do not include the (much smaller) viscous and inertial properties of the artery.20

The technique described for measuring brachial artery elastic mechanics offers many advantages. Brachial artery elastic mechanics are measured over a pressure range between 0 and 100 mm Hg. Most other techniques measure arterial elastic mechanics at a single pressure (usually mean pressure) or over a pressure range between systole and diastole. Although the lower end of the pressure range is not physiological, there are reasons for studying the artery at low TP. Brachial artery compliance increases markedly as TP decreases. The demonstration of a drug effect on arterial compliance is more apparent at low TP. Similarly, the ability to differentiate normal from diseased vessels may be improved at lower TP.

The measurement of arterial size at 0 mm Hg TP is of particular importance and is not feasible using any other noninvasive technique. We define the arterial radius at 0 mm Hg under baseline conditions as the effective unstressed radius (ro). This value should be similar, but not identical, to the unstressed radius measured in vitro because there is still some small residual stress at 0 mm Hg TP.21 The values of ro determined with the noninvasive ultrasound technique used in this study are highly reproducible. The mean value of ro was 71\% of the arterial radius at 100 mm Hg, which is comparable to the value of 84\% obtained in older, normal human subjects using intravascular ultrasound.22 By measuring ro under baseline conditions, we were able to calculate the true arterial strain, which is the ratio of the radius at any given pressure and ro. This allowed for the...
calculation of $E_{inc}$, an intrinsic measure of arterial wall stiffness. A single value of $r_o$ was used for the calculation of arterial strain under baseline and NTG conditions. We chose to use a single value for $r_o$ as measured under baseline conditions because, as Dobrin argues, this method of computing strains corresponds to the behavior of arteries in vivo and treats each vessel as a single relaxed artery with superimposed vascular smooth muscle.22

Roach and Burton have demonstrated that under very low pressure or stress, the elastic modulus of the arterial wall is very close to the elastic modulus of elastin alone, because little or no collagen bears stress under this condition.23 Cox has shown a significant correlation between the initial slope of the stress-strain curve at zero stress and the elastin content.24 The mean elastic modulus of elastin ($E_{inc}$ at 0 stress) in this study was approximately $1.0 \times 10^6$ dyn/cm². This value is consistent with in vivo data from our laboratory using intravascular ultrasound12 and from animal studies which estimate the elastic modulus of elastin to be between 1 and $10^6$ dynes/cm².24–26

Measuring brachial artery wall mechanics over a wide pressure range provides the opportunity to compare the vessel isometrically under different levels of smooth muscle tone. With other techniques that measure arterial elastic mechanics between systolic and diastolic pressures, equivalent strains cannot be acquired under significantly different levels of smooth muscle tone. Modeling of the contributions of elastin, collagen, and smooth muscle to arterial wall stress and $E_{inc}$ can best be achieved by isometric analysis. Application of arterial wall models to determine constitutive equations for the contributions of the different wall components to total measured wall mechanics has been performed in vitro,23,24 in conscious dogs,10 and in human subjects in vivo using intravascular ultrasound.12 The technique described in this study is the first noninvasive tool (to our knowledge) that can be used for this purpose in human subjects in vivo.

Effects of Smooth Muscle Relaxation on Arterial Elastic Properties

Smooth muscle relaxation with NTG produced a 38% increase in brachial artery area at 100 mm Hg, which is similar to values reported by others using B-mode techniques.27 This was associated with a 94% increase in compliance at this pressure. $E_{inc}$ decreased by 35% at 100 mm Hg and PWV decreased by 33%. The improvements in arterial compliance and PWV with NTG are similar to those seen in other studies using different techniques.28 They are also similar to our findings using intravascular ultrasound.9,12

One difference between the results of the present study and our intravascular ultrasound study is that $E_{inc}$ did not change significantly with intra-arterial NTG in that study. The explanation for this difference in findings may be related to the fact that the subjects in the present study were younger, the study included women, and the amount of vasodilation obtained in this study was significantly greater. NTG increased brachial artery area 38% at 100 mm Hg in this study, whereas it increased brachial artery area 22% at the same pressure in our previous intravascular study.9 Also, the invasive nature of our previous study may have altered basal vascular tone.

A decrease in isobaric $E_{inc}$ following vasodilation has not been consistently found. Peterson has shown that the vasodilator acetylcholine decreases isobaric $E_{inc}$ in the femoral artery of dogs.6 In contrast, Gou7 has found vasodilation to produce the opposite effect. This difference may again be a result of the type of artery studied, the amount of vasodilation induced, and the methods used. Vasodilation decreases arterial stiffness by reducing tension generated by the smooth muscle and connective tissue elements in series with the smooth muscle.26 However, vasodilation increases arterial stiffness by tensing the collagen and elastin fibers in parallel with the smooth muscle. In the present study, the reduction in measured arterial stiffness with NTG likely occurred because the decrease in smooth muscle (and associated series elastic component) contribution to arterial stiffness during vasodilation outweighed the increase in parallel collagen and elastin contribution to arterial stiffness. In different vessels, at different pressures, or in different species, the relative weights of these opposing factors may vary, resulting in different effects of smooth muscle relaxation on $E_{inc}$. In any given study, $E_{inc}$ or any other measure of intrinsic wall stiffness must be measured, as the effects of a drug on arterial stiffness cannot necessarily be inferred from the effects of the drug on arterial compliance.29,30

Conclusions

Brachial artery elastic properties including area, effective unstressed area, compliance, stress, strain, $E_{inc}$, and PWV can be reproducibly measured over a wide pressure range using a new noninvasive technique that involves imaging the brachial artery through a pressurized water-filled cuff. With this technique, we demonstrate that smooth muscle relaxation increases isobaric arterial compliance and decreases isobaric PWV in normal human subjects as a result of both increased arterial size and decreased arterial stiffness. NTG also markedly decreases isometric arterial wall stress and $E_{inc}$. This technique offers promise for noninvasive studies of the short- and long-term effects of drugs and disease on in vivo arterial elastic mechanics.

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References


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