

Endothelium-Derived Nitric Oxide Regulates Arterial Elasticity in Human Arteries In Vivo

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Abstract—Arterial elasticity is determined by structural characteristics of the artery wall and by vascular smooth muscle tone. The identity of endogenous vasoactive substances that regulate elasticity has not been defined in humans. We hypothesized that NO, a vasodilator released constitutively by the endothelium, augments arterial elasticity. Seven healthy young men were studied. A 20-MHz intravascular ultrasound catheter was introduced through an arterial sheath to measure brachial artery cross-sectional area, wall thickness, and intra-arterial pressure. After control was established, indices of elasticity (pressure-area relationship, instantaneous compliance, and stress-strain, pressure-incremental elastic modulus (E_{inc}), and pressure-pulse wave velocity relationships) were examined over 0 to 100 mm Hg transmural pressure obtained by inflation of an external cuff. Thereafter, the basal production of endothelium-derived NO was inhibited by N^G -monomethyl-L-arginine (L-NMMA) (4 and 8 mg/min). Finally, nitroglycerin (2.5 and 12.5 μ g/min), an exogenous donor of NO, was given to relax the vascular smooth muscle. Elasticity was measured under all of these conditions. L-NMMA (8 mg/min) decreased brachial artery area ($P=0.016$) and compliance ($P<0.0001$) and increased E_{inc} ($P<0.01$) and pulse wave velocity ($P<0.0001$). Nitroglycerin (12.5 μ g/min) increased brachial artery area ($P<0.001$) and compliance ($P<0.001$) and decreased pulse wave velocity ($P=0.02$). NO, an endothelium-derived vasodilator, augments arterial elasticity in the human brachial artery. Loss of constitutively released NO associated with cardiovascular risk factors may adversely affect arterial elasticity in humans. (*Hypertension*. 2001;38:1049-1053.)

Key Words: brachial artery ■ elasticity ■ human ■ endothelium-derived relaxing factor

Elasticity of large arteries absorbs the energy of the phasic stroke volume in systole and thereby dampens the arterial pressure wave during its propagation through the arterial tree.¹ The release of stored energy in diastole facilitates the continuous flow of blood to tissues.²

Several indices of arterial elasticity have been used in clinical studies, including compliance, distensibility index, stress-strain relationships, Young's modulus, and pulse wave velocity. Arterial compliance refers to the relationship between arterial dimension and the distending pressure. An increase in compliance corresponds to a decrease in artery stiffness. Arterial compliance changes in a nonlinear fashion with blood pressure. It tends to be greater at lower blood pressures, and for this reason the distensibility index (change in volume/change in pressure \times baseline volume) can lead to erroneous conclusions if the mean distending pressure is shifted by an intervention. Compliance curves and the incremental modulus (E_{inc}) can be used to assess elasticity independent of the blood pressure changes. Recently, a technique to assess arterial elasticity in humans with the use of intravascular ultrasound to measure arterial dimension and inflation of an external blood pressure cuff to generate a range

of distending pressures has been described.³ This approach has been used to assess the effect of different structural components that contribute to arterial elasticity, including collagen, elastin, and vascular smooth muscle.⁴

In addition to the passive contribution of these constituents in the arterial wall, the active component of elasticity in vivo relates to the vascular smooth muscle tone. Prior studies have administered pharmacological vasoactive agents that directly affect smooth muscle tone to study this active component.^{3,5} However, in healthy arteries the continuous production of NO, an endothelium-derived vasodilator, regulates vascular tone and arterial dimension.⁶ Therefore, we investigated whether this endogenous substance plays a significant physiological role in controlling arterial elasticity.

Methods

Seven healthy young men were recruited by newspaper advertisements. Written informed consent was obtained from all subjects, and the study was approved by the Brigham and Women's Hospital Human Research Committee.

Experimental Protocol

All subjects were studied in a quiet room with a constant temperature (23°C). A 20-MHz 3.4F Visions Five-64 intravascular ultrasound

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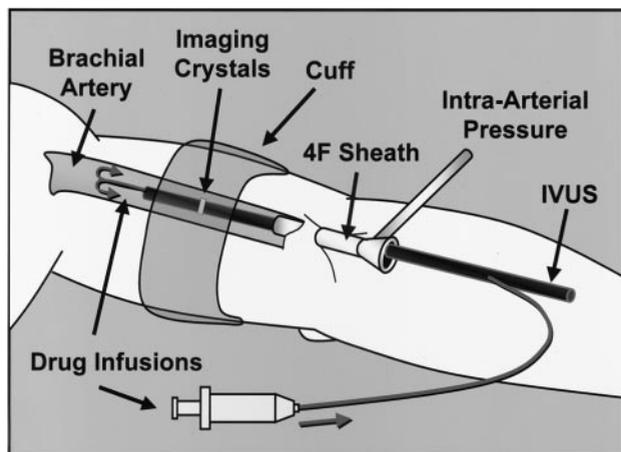


Figure 1. Study setup. The intravascular ultrasound (IVUS) catheter was inserted into the brachial artery with the ultrasound crystals centered under an arm cuff. The drugs were infused proximal to the imaging crystals through the guidewire channel of the IVUS catheter. The sheath side arm was used to simultaneously measure intra-arterial pressure.

catheter (Endosonics) was inserted into the brachial artery via a 4F arterial sheath. The side arm of the arterial sheath was connected to a Statham P23 pressure transducer (Gould Statham Instruments), and the arterial waveforms were recorded onto a physiological recorder (Gould) together with the ECG. The pharmacological agents were infused into the guidewire channel of the intravascular ultrasound catheter to enter the brachial artery approximately 10 mm upstream of the ultrasound crystals (Figure 1).

A control solution of 5% dextrose was infused with a Harvard pump (Harvard Apparatus) at 1 mL/min for 8 minutes, followed by the NO synthase inhibitor *N*²-monomethyl-L-arginine (L-NMMA) at 4 (16 μ mol/min) and 8 mg/min (32 μ mol/min) for 4 minutes each, then nitroglycerin, a NO donor, at 2.5 and 12.5 μ g/min for 4 minutes each.

A 12-cm blood pressure cuff was placed around the upper arm and centered over the ultrasound crystals on the intravascular ultrasound catheter. This cuff was inflated by 10-mm Hg increments to generate a range of transmural distending pressures (intra-arterial minus cuff pressure) below the diastolic pressure during each of the 5 infusions.³

Image Analysis

The intravascular ultrasound images were digitized from the videotape. The lumen cross-sectional area and wall thickness were measured by computerized planimetry (TapeMeasure, INDEC Systems) by 1 operator blinded to the drug infusions and stage in the cardiac cycle. Diastolic images from the cardiac cycle were measured to avoid any errors due to the inertial and viscous behaviors of the arterial wall during systole.³ The images from 4 cardiac cycles were averaged for each pressure increment. The arterial thickness was measured adjacent to a vein.³

Calculations of Elastic Properties

The method for estimating arterial elasticity has been described previously.^{3,5} Briefly, the transmural pressure versus cross-sectional area data were plotted for each of the 5 infusions. Each curve was fitted to the formula of Langewouters et al,⁷ $A = a[0.5 + 1/\pi \tan^{-1}(P/c - b/c)]$, where *A* is cross-sectional area, *P* is transmural pressure, and *a*, *b*, and *c* are 3 independent parameters that characterize each pressure-area curve, by using nonlinear least-squares regression methods. Brachial artery instantaneous compliance, circumferential wall stress, wall strain, E_{inc} , and pulse wave velocity were calculated as described previously.^{3,5}

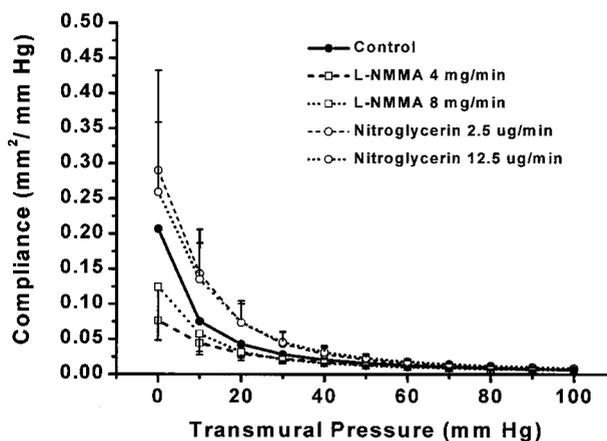
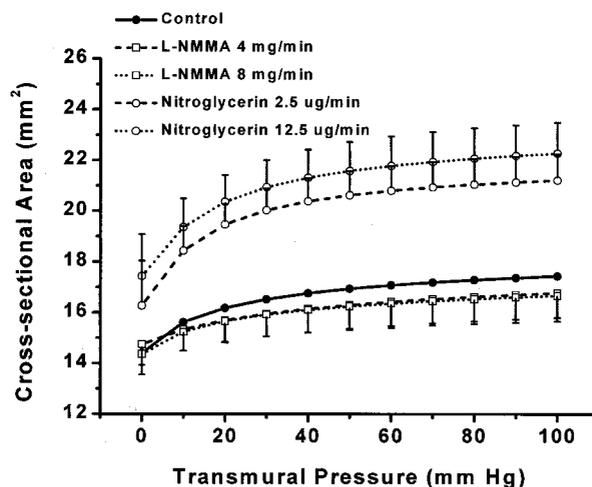


Figure 2. Mean pressure versus area curves (top) and instantaneous compliance curves (bottom) for each infusion. Inhibition of basal endothelium-dependent NO with high-dose L-NMMA significantly reduced brachial artery area ($P=0.016$) and compliance ($P<0.0001$) over the whole pressure range. Nitroglycerin significantly increased brachial artery area ($P<0.001$) and compliance ($P<0.05$) over the whole arterial pressure range.

Statistical Analysis

The curves generated by each infusion were compared by general linear mixed effects models in SAS. Area, compliance, and strain were log transformed to generate more linear relationships with the covariates. Interaction terms were used to assess nonparallel shifts in the curves. We performed post hoc pairwise comparisons of the infusions compared with control using the Dunnett-Hsu correction for multiple comparisons, with statistical significance set at $P<0.05$.

An expanded Methods section can be found in an online data supplement available at <http://www.hypertensionaha.org>.

Results

Patient Population

We studied 7 healthy young men age 23 to 33 years (mean \pm SD age, 28 \pm 4 years). Mean \pm SD values were as follows: systolic blood pressure, 116 \pm 11 mm Hg; diastolic pressure, 67 \pm 5 mm Hg; total cholesterol, 169 \pm 33 mg/dL; LDL cholesterol, 98 \pm 34 mg/dL; and body mass index, 23.8 \pm 2.2 kg/m².

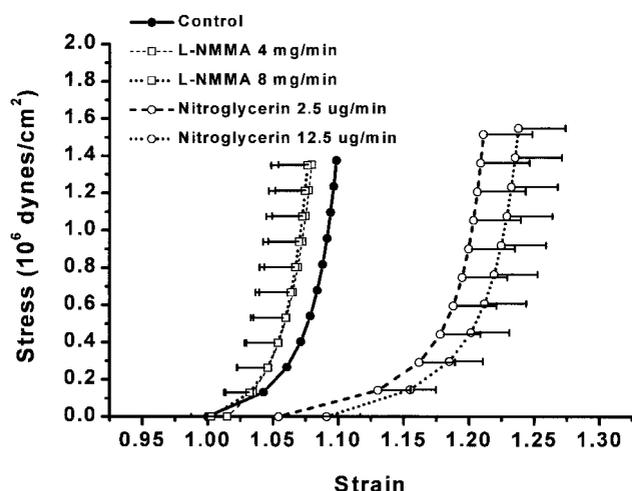


Figure 3. Mean wall stress and strain curves with control infusion and with the 2 concentrations of L-NMMA and nitroglycerin. High-dose L-NMMA significantly shifted the curve to the left ($P<0.002$), and nitroglycerin shifted the curve to the right ($P<0.001$).

Effects of NO on Brachial Artery Pressure-Area Relationship and Compliance

Figure 2 (top) shows the transmural pressure versus cross-sectional area relationships. There were significant nonparallel differences among the various experimental conditions on the pressure-area curves ($P<0.02$). Compared with control infusion, there was an increase in cross-sectional area with nitroglycerin (12.5 $\mu\text{g}/\text{min}$; $P<0.001$) and a reduction in area with L-NMMA (8 mg/min; $P=0.016$) across all transmural pressures.

The transmural pressure versus compliance relationships are shown in Figure 2 (bottom). There was a significant nonparallel difference between the experimental conditions on the pressure curves ($P<0.001$). Compared with control, nitroglycerin increased compliance (12.5 $\mu\text{g}/\text{min}$; $P<0.0001$), and L-NMMA reduced compliance (8 mg/min; $P<0.0001$). In a separate analysis that confined the examination of the effects of the infusions to the physiological range of transmural pressures (60 to 100 mm Hg), compliance increased with nitroglycerin (12.5 $\mu\text{g}/\text{min}$; $P<0.001$) and decreased with L-NMMA (8 mg/min; $P<0.05$).

Effects on Stress, Strain, E_{inc} , and Pulse Wave Velocity

Wall stress versus strain relationships are shown in Figure 3. Nitroglycerin significantly shifted the curves to the right (12.5 $\mu\text{g}/\text{min}$; $P<0.001$), and L-NMMA shifted the curve to the left (8 mg/min; $P<0.02$).

Figure 4 shows the isometric and isobaric E_{inc} relationships. At constant E_{inc} , strain was significantly higher with nitroglycerin (12.5 $\mu\text{g}/\text{min}$; $P<0.0001$) and was significantly reduced with L-NMMA (8 mg/min; $P<0.002$). Under isobaric conditions, there was a significant increase in E_{inc} with L-NMMA (8 mg/min; $P<0.0001$), while nitroglycerin returned E_{inc} to baseline.

Figure 5 shows the pulse wave velocity versus transmural pressure relationships. There was a significant increase in

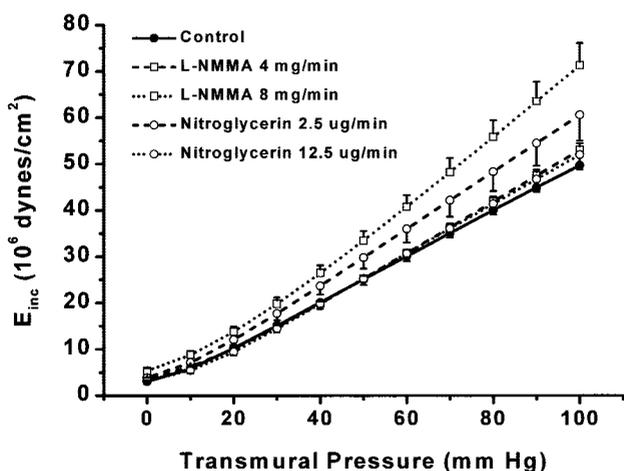
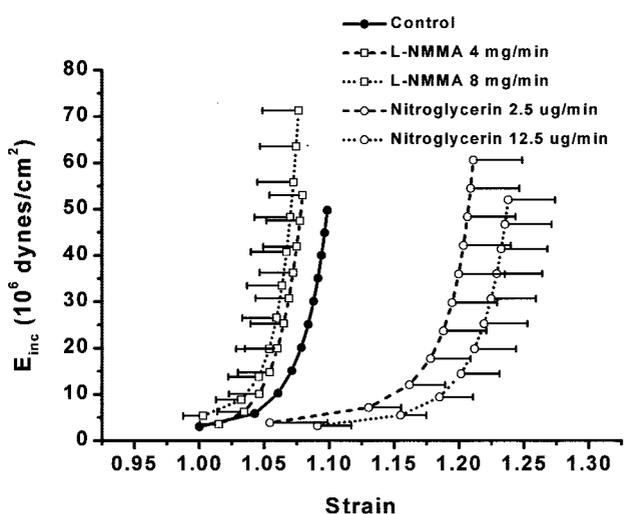


Figure 4. Isometric (top) and isobaric (bottom) E_{inc} with control and with the 2 concentrations of L-NMMA and nitroglycerin. At constant E_{inc} , strain was significantly reduced with inhibition of endothelium-dependent NO with both doses of L-NMMA ($P<0.01$) and significantly increased with nitroglycerin ($P<0.001$). Under isobaric conditions, there was a significant increase in E_{inc} with high-dose L-NMMA ($P<0.0001$) and low-dose nitroglycerin ($P<0.01$), but high-dose nitroglycerin returned E_{inc} to control.

pulse wave velocity with L-NMMA (8 mg/min; $P<0.0001$) and a decrease in pulse wave velocity with nitroglycerin (12.5 $\mu\text{g}/\text{min}$; $P=0.02$).

Discussion

The novel finding of this study is that the constitutive release of NO contributes to arterial elasticity in healthy humans. We used intravascular ultrasound to assess elasticity of the brachial artery by measuring its cross-sectional area and wall thickness over a range of luminal distending pressures, generated by inflation of an external blood pressure cuff. Inhibition of NO synthase with L-NMMA consistently affected all measures of arterial elasticity. It reduced arterial compliance, shifted the stress-strain and E_{inc} -stress curves to the right, increased E_{inc} under isometric and isobaric conditions, and increased pulse wave velocity.

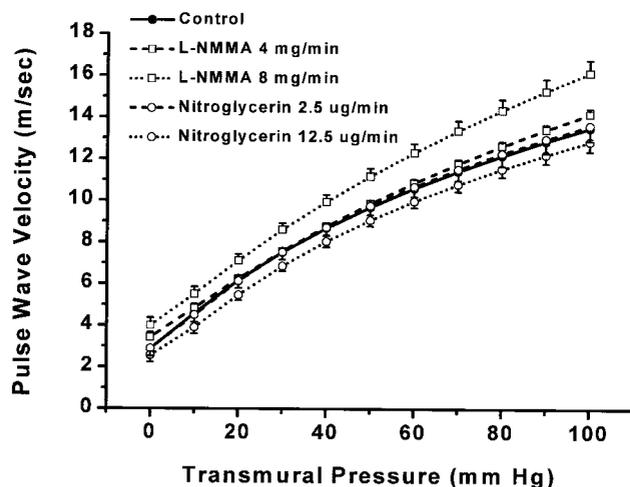


Figure 5. Effect of L-NMMA and nitroglycerin on pulse wave velocity over the pressure range. High-dose L-NMMA increased pulse wave velocity ($P < 0.001$), and high-dose nitroglycerin decreased pulse wave velocity ($P = 0.02$).

Although the methodology we used is invasive, it yields rigorous measures of arterial elasticity *in vivo* by relating changes in artery caliber to changes in pressure over a wide range of transmural pressures. Furthermore, our analysis is derived from diastolic images that assess the pure elastic behavior of the artery and not the viscous or inertial behavior exhibited during the rapid stretch of systole.³ The consistent results among several measures of elasticity, each emphasizing a somewhat different aspect of arterial stiffness,³ and the dose-response effects strengthen our results and conclusions.

A previous study found that the basal release of NO paradoxically reduced arterial compliance.⁸ In that study compliance was measured by external ultrasound with wall tracking over a more restricted range of arterial pressure, and a lower concentration of L-NMMA (10^{-6} mol/min) was used. In contrast, we evaluated elasticity across a broad range of distending pressures generated by the use of an external cuff, and we used a higher concentration of L-NMMA previously shown to nearly completely inhibit NO bioavailability.⁹ By using area and pressure measurements from diastole, we also avoided the viscous and inertial components of arterial distention during the rapid changes in systole, thereby more accurately defining the changes in arterial elasticity with loss of NO.

Arterial elasticity has 2 major elements: a passive component that reflects the structural composition of the artery wall and an active component related to arterial tone.⁴ The latter reflects the anatomic "in series" linkage of vascular smooth muscle cells with connective tissue.¹⁰ Accordingly, pharmacological vasoconstrictors typically reduce and vasodilators augment arterial elasticity.^{3,5,11,12} The locally released vasoactive substances that might regulate arterial elasticity in healthy humans have not been well defined. Because NO, an endothelium-derived vasodilator, is released constitutively, we postulated that it improves the elastic behavior of arteries and increases arterial compliance. The findings in the present study confirm this hypothesis. Reduced bioavailability of endothelium-derived NO impaired compliance, whereas the

administration of nitroglycerin, an exogenous NO donor, reversed the loss of compliance induced by L-NMMA.

Elasticity of the arterial wall facilitates healthy behavior of the cardiovascular system. In systole, elasticity reduces left ventricular wall stress by damping the rise in peak systolic pressure.¹⁰ The energy thus stored is released in diastole, facilitating organ perfusion throughout the cardiac cycle. A decrease in compliance reduces the diastolic component of blood flow that is particularly important in the coronary arteries and increases the wave reflection from peripheral arteries to accentuate aortic systolic pressure and left ventricular load. Hence, it is perhaps not surprising that loss of arterial elasticity, in its simplest form assessed by widening of arterial pulse pressure, is associated with a marked increase in cardiovascular morbidity and mortality.¹³⁻¹⁷

Potential Clinical Implications

Cardiovascular risk factors, including hypertension, diabetes, and dyslipidemia, change the composition and thickness of the arterial wall and reduce the bioavailability of constitutive NO.⁶ Risk factors also reduce arterial compliance and distensibility¹⁸⁻²¹ as early as the first decade of life, well before structural changes in arteries occur.¹⁸ The findings in the present study suggest that the reduction in NO contributes to a loss of arterial elasticity in this setting. While risk factor modification may take years to favorably alter arterial structure, loss of constitutive NO can be corrected more rapidly.⁶ Thus, future investigations should delineate whether risk factor modification or other therapies directed at improving endothelial vasodilator function can improve arterial elasticity over the same, relatively rapid time course with which they restore NO. In addition, future studies need to define the contribution of NO to elasticity in other arteries, including the aorta.

In conclusion, using a rigorous approach, we have demonstrated that constitutive release of NO contributes to the physiological regulation of elasticity in healthy human brachial arteries *in vivo*. Future studies should delineate the role of NO in regulating elasticity of other arterial beds and document the effect of risk factor modification on improving arterial elastic properties through augmenting NO.

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