Original Article

Effect of Systemic Nitric Oxide Synthase Inhibition on Arterial Stiffness in Humans

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Stiffening of large elastic arteries impairs the buffering function of the arterial system and contributes to cardiovascular disease. The aim of this study was to determine whether endothelium-derived nitric oxide (NO) modulates the stiffness of large elastic arteries in humans. Seven apparently healthy adults (60±3 years, 2 males and 5 females) underwent systemic α-adrenergic blockade (phentolamine) and systemic NO synthase inhibition using N^G-monomethyl-L-arginine (L-NMMA) in sequence. Phentolamine was given first to isolate contribution of NO to arterial stiffness by preventing reflex changes in sympathetic tone that result from systemic NO synthase inhibition, and also to compare arterial stiffness at a similar mean arterial pressure. Mean arterial blood pressure decreased ($p<0.05$) after phentolamine infusion but returned to baseline levels after L-NMMA infusion. The carotid β-stiffness index (*via* simultaneous ultrasound and applanation tonometry on the common carotid artery) did not change after the restraint of systemic α-adrenergic nerve activity (9.8±1.2 *vs.* 9.1±1.1 U) but increased ($p<0.05$) after NO synthase inhibition (12.6±2.0 U). These results suggest that NO appears to modulate central arterial stiffness in humans. (*Hypertens Res* 2007; 30: 411–415)

***Key Words*: arterial compliance, endothelium, sympathetic nervous system**

Introduction

Large elastic arteries in cardiothoracic and carotid regions expand and recoil during cardiac contraction and relaxation, thereby damping fluctuations in arterial pressure and blood flow (*1*). In sedentary humans, the stiffness of arteries in the cardiothoracic region increases with advancing age (*2–4*). The stiffening of large elastic arteries impairs the buffering function of the arterial system and leads to cardiovascular dysfunction *via* elevated systolic blood pressure, augmented left ventricular afterload, decreased coronary blood flow, and blunting of arterial baroreflex sensitivity (*5–7*).

Arterial stiffness is modulated by vasoconstrictor tone exerted by the smooth muscle cells. Nitric oxide (NO) bioavailability is thought to be one of the most potent modulators of smooth muscle tone. While animal studies have consistently demonstrated that NO synthase inhibition increases measures of arterial stiffness (*8*, *9*), human studies remain controversial. Although local intra-arterial infusion of drugs that inhibit NO release is an ideal protocol with which to examine this issue, such a procedure is difficult to perform in the human aorta. As such, most studies have used systemic infusion of N^G-monomethyl-L-arginine (L-NMMA) (*10*, *11*). However, these studies are difficult to interpret because the changes in arterial stiffness indices were accompanied by

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Table 1. Selected Physiological Characteristics of Subject

Variables	Mean±SEM	Range
Height (cm)	161±2	153–172
Body mass (kg)	60±4	42–72
Body mass index (kg m^{-2})	22.9±1.2	17.9–26.9
Body fat (%)	28.4±2.0	21.5–34.7
$V_{O_2\,max}$ (mL kg^{-1} min^{-1})	28.8±2.3	18.7–35.8
Plasma glucose (mmol L^{-1})	5.1±0.1	4.5–5.7
Plasma total cholesterol (mmol L^{-1})	5.8±0.3	4.7–7.0
Plasma HDL cholesterol (mmol L^{-1})	1.6±0.1	1.4–2.2
Plasma LDL cholesterol (mmol L^{-1})	3.6±0.2	2.7–4.6
Plasma triglyceride (mmol L^{-1})	1.3±0.3	0.5–2.4

$V_{O_2\,max}$, maximal oxygen consumption; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

increases in mean arterial blood pressure as well as reflex reductions in sympathetic vasoconstrictor tone that could independently affect arterial stiffness. Indeed, Stewart *et al*. (*11*) concluded that the effects of systemic infusion of a NO blocker on pulse wave velocity (PWV) could be explained primarily by changes in mean arterial blood pressure. Another issue that makes the interpretation of these studies difficult is the use of indirect measures of arterial stiffness that are influenced by factors other than arterial stiffness. For example, increases in the augmentation index previously observed after L-NMMA (*10*, *11*) may have been caused by increases in systemic vascular resistance rather than increases in arterial stiffness, as the augmentation index reflects not only arterial stiffness but also wave reflection (*12*).

Accordingly, the aim of the present study was to test the hypothesis that endothelium-derived NO modulates the stiffness of large elastic arteries in humans. To account for reflex changes in sympathetic tone as well as increases in mean arterial blood pressure that result from systemic NO synthase inhibition, we administered a systemic α-adrenergic blocker (phentolamine) prior to the infusion of the NO synthase inhibitor. Furthermore, we directly measured arterial stiffness (β-stiffness index) by using a combination of ultrasound imaging with simultaneous applanation tonometry. The β-stiffness index was chosen for the present study because it is an index of arterial stiffness that minimizes effect of distending pressure on arterial stiffness (*13*).

Methods

Subjects

Seven apparently healthy sedentary adults (60±3 years, 2 men and 5 postmenopausal [*i.e.*, estrogen-deficient] women) were studied (Table 1). None of the subjects were current smokers or were currently taking any medications including hormone replacement therapy. All subjects performed an incremental cycling exercise test to assess their cardiovascular disease risk and to obtain maximal oxygen consumption as previously described (*14*). None of the subjects had any apparent cardiovascular disease as assessed by medical history and physical examination. All potential risks and procedures of the study were explained to the subjects, who gave their written informed consent to participate in the study. The study was reviewed and approved by the local Institutional Review Board.

Experimental Protocol

All measurements were performed an overnight fast that also included abstinence from caffeine. Subjects were studied under supine resting conditions in a quiet, temperature-controlled room (24–26°C). Hemodynamics and arterial stiffness were measured three times: at baseline, with systemic α-adrenergic blockade and with systemic α-adrenergic blockade and systemic NO synthase inhibition (*15*). Baseline measurements were made after 15 min supine rest. Then, subjects received a systemic α-adrenergic blockade (phentolamine mesylate, 0.1428 mg kg^{-1} i.v. bolus over 2 min and a subsequent continuous [0.01428 mg kg^{-1} min^{-1}] i.v. infusion). The second measurement was performed 5 min after the commencement of phentolamine infusion. Subsequently, subjects underwent systemic NO synthase inhibition (L-NMMA, 3 mg kg^{-1} i.v. bolus over 5 min and a subsequent continuous [0.05 mg kg^{-1} min^{-1}] i.v. infusion) under α-adrenergic blockade condition. The last measurement was performed 10 min after the commencement of L-NMMA infusion. The infusion procedure and the dose of the systemic drug infusion were consistent with previous studies (*15*, *16*) reporting that the dosage of blockade was adequate to block α-adrenergic system activity and NO production.

β-Stiffness Index

Arterial stiffness was obtained with a combination of ultrasound imaging of the common carotid artery and simultaneous applanation tonometry of the contralateral carotid artery. A longitudinal image of the carotid artery was measured with an ultrasound machine equipped with a high-resolution linear-array transducer (axial resolution of 0.06 mm) as previously described (*4*, *17*). Carotid arterial pressure waveforms were obtained with arterial applanation tonometory incorporating an array of 15 micropiezoresistive transducers (FormPWV/ABI, Colin Medical Technology, Komaki, Japan), and were calibrated by equating the carotid mean arterial and diastolic blood pressure to the brachial values (*18*). The β-stiffness index was calculated using the equation $[\ln(P_1/P_0)]/[(D_1 - D_0)/D_0]$, where D_1 and D_0 are the maximal and minimum arterial diameters and P_1 and P_0 are the highest and lowest blood pressures, respectively (*13*). At each time point, the average of 10 heart cycles was analyzed. To exclude inter-investigator variability, one investigator analyzed all ultrasound images and blood pressure waveforms. The coefficients of variation for the two trials were

0.7±0.2%, 3±1%, 4±1% and 5±2% for carotid artery diameter, systolic blood pressure, diastolic blood pressure and arterial compliance, respectively.

Arterial Pulse Wave Velocity, Blood Pressure and Heart Rate

Arterial PWV was obtained by an automatic device (Form PWV/ABI, Colin Medical) as previously described (*19*). Aortic (carotid-femoral artery) PWV was calculated from the distance traveled between the two arterial recording sites and its time delay, which was measured automatically with the foot-to-foot method. Its coefficient of variation for the two trials was 4±1%. Brachial blood pressure and heart rate were also measured with oscillometric pressure sensor cuffs and the electrocardiograph equipped with this automatic device. Automatic oscillometric blood pressure was used to eliminate potential investigator bias associated with the auscultation.

Body Fat

Percent body fat was determined by the bioelectric impedance method (*17*, *20*).

Blood Samples

A blood sample was collected from the antecubital vein using venipuncture after an overnight fast. Plasma concentrations of glucose, lipids and lipoproteins were determined by the use of the standard enzymatic technique, as previously described (*21*).

Statistical Analyses

ANOVA and ANCOVA with repeated measures were used to determine the effects of systemic α-adrenergic blockade and systemic NO synthase inhibition on arterial stiffness. All data are reported as means±SEM. Statistical significance was set *a priori* at $p<0.05$.

Results

As shown in Fig. 1, mean arterial blood pressure decreased after phentolamine infusion ($p<0.05$), but returned to baseline after L-NMMA infusion. Accordingly, the α-adrenergic blockade increased heart rate, and the subsequent addition of NO synthase blockade reduced heart rate. Carotid systolic blood pressure was lowered by phentolamine infusion ($p<0.05$, Table 2) but returned to the baseline level after L-NMMA infusion. Carotid artery lumen diameters were not affected significantly by the administration of either phentolamine or L-NMMA.

Neither the carotid β-stiffness index nor aortic PWV changed after phentolamine infusion, and both increased ($p<0.05$) after the L-NMMA infusion (Fig. 1). Because there was an increase, albeit nonsignificant, in mean arterial blood pressure after L-NMMA infusion, we performed ANCOVA with change in mean arterial blood pressure as the covariate.

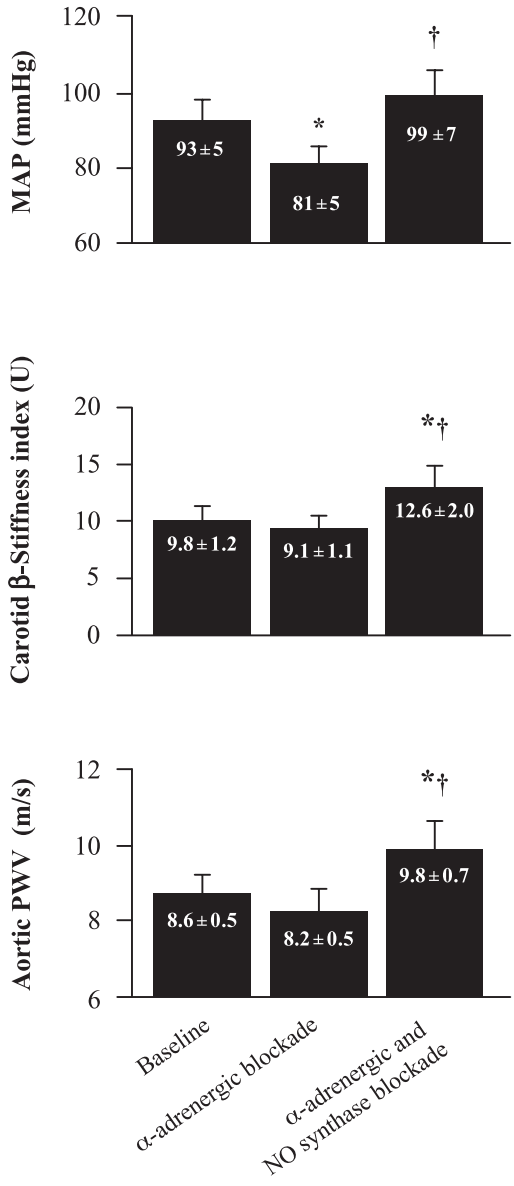

Fig. 1. *Mean arterial blood pressure (MAP), carotid β-stiffness index and aortic pulse wave velocity (PWV) before treatment (baseline), during systemic α-adrenergic blockade and during systemic α-adrenergic and nitric oxide (NO) synthase blockade. *$p<0.05$ vs. baseline. †$p<0.05$ vs. α-adrenergic blockade.*

The increase in arterial stiffness after L-NMMA remained strong ($p=0.05$) after the influence of mean arterial blood pressure was statistically accounted for.

Discussion

The salient finding of the present study is that the carotid β-stiffness index increased significantly after systemic NO synthase inhibition. These changes in central arterial stiffness

Table 2. Hemodynamic and Artery Indices

Variables	Baseline	α-Adrenergic blockade	α-Adrenergic and NO synthase blockade
Heart rate (bpm)	59±4	70±5*	61±4†
Brachial SBP (mmHg)	123±6	109±5*	133±9†
Brachial DBP (mmHg)	73±3	65±3*	79±5*,†
Brachial PP (mmHg)	50±4	44±3	53±5†
Carotid SBP (mmHg)	113±6	99±4*	123±9†
Carotid PP (mmHg)	40±5	34±3	43±5
Carotid diastolic diameter (mm)	6.1±0.4	6.0±0.3	6.0±0.3
Carotid systolic diameter (mm)	6.4±0.4	6.3±0.3	6.2±0.3

Values are means±SEM. NO, nitric oxide; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure. *$p<0.05$ *vs*. baseline; †$p<0.05$, *vs*. α-adrenergic blockade.

were not associated with changes in mean arterial blood pressure. Our present results indicate that systemic NO production appears to modulate central arterial stiffness in humans.

Wilkinson *et al*. (*10*) demonstrated in humans that intravenous infusion of L-NMMA significantly increased the estimated aortic augmentation index and the timing of the reflected waveform. However, a concomitant elevation of mean arterial blood pressure, as well as the use of the augmentation index, makes it difficult to interpret NO synthase inhibition on central arterial stiffness. Indeed, Stewart *et al*. (*11*) showed a strong relation between changes in aortic PWV with L-NMMA administration and corresponding changes in mean arterial blood pressure; they concluded that changes in mean arterial blood pressure could explain the increase in aortic PWV with the systemic inhibition of NO release. The results of the present study give a more definitive insight into contribution of NO to large artery stiffness. To minimize the influence of mean arterial blood pressure induced by systemic NO synthase inhibition on measures of arterial stiffness, we measured the β-stiffness index for the present study (*13*). The carotid β-stiffness index did not change with phentolamine infusion and was significantly elevated with NO synthase inhibition. These changes were not associated with changes in mean arterial blood pressure. Our present results suggest that the stiffness of central arteries may be modulated by NO bioavailability in humans.

In contrast to some human studies (*22*), Joannides *et al*. (*23*) showed an increase in arterial distensibility with basal NO inhibition. The paradoxical increase in arterial stiffness that Joannides *et al*. (*23*) observed may be due to the effect of reflex reductions in sympathetic vasoconstrictor tone induced by NO synthase inhibition (*24*). To isolate contribution of NO to arterial stiffness by preventing reflex changes in sympathetic tone, we administered an α-adrenergic blocker concomitant with a NO synthase inhibitor. Systemic NO synthase inhibition significantly increased central arterial stiffness. These results suggest that the paradoxical increase in arterial stiffness previously observed by Joannides *et al*. (*23*) may be attributed to the reflex decrease in sympathetic vasoconstrictor tone.

Although the potential contribution of sympathoinhibition on arterial stiffness was not a major aim of this study, our experimental design allowed us to examine it. We found that neither the β-stiffness index nor aortic PWV changed after phentolamine infusion, despite the fact that mean arterial blood pressure decreased significantly. Our present results agree with a previous finding that the activation of sympathetic nervous system activity induced by lower body negative pressure did not change aortic wall mechanics in humans (*25*). Because of the conflicting findings reported in the literature (*26*), further investigations into the potential contribution of sympathetic nervous system activity to arterial stiffness are warranted.

The present study has several important limitations. First, only a small number of subjects were studied. Thus, it is possible that we could not detect some of the changes because of a lack of statistical power. Indeed, there was a modest increase in mean arterial blood pressure after L-NMMA, although the change was not statistically significant. Second, NO bioavailability is known to decline with advancing age (*27*). Because we used healthy but older subjects, our results may underestimate contribution of NO to the modulation of central arterial stiffness.

In summary, the simultaneous restraints of systemic α-adrenergic nervous activity *via* phentolamine and NO synthase *via* L-NMMA augmented central arterial stiffness. Our present results suggest that NO bioavailability may modulate central arterial stiffness.

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