Brief Review

Nitric Oxide and the Regulation of Large Artery Stiffness
From Physiology to Pharmacology

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The association between arterial stiffening and aging is well described and can be observed in almost all populations worldwide. A number of other cardiovascular risk factors including diabetes and cigarette smoking are also associated with increased large artery stiffness, often referred to as “premature arterial stiffening.” It is now apparent that the aortic pulse wave velocity (PWV), a measure of arterial distensibility, predicts outcome in a variety of different populations, including hypertensives,1 diabetics, 2 individuals with end-stage renal disease (ESRD),3 and even in older adults.4 Indeed, in some populations, aortic PWV is a better predictor of future events than peripheral blood pressure.4 Moreover, arterial stiffening may be more than just a marker of cardiovascular risk; stiffening may also play a more direct role in the development of atherosclerotic plaques. Thus, arterial stiffness would appear to be a novel therapeutic target for the prevention of excess cardiovascular morbidity and mortality.

Factors Influencing Large Artery Stiffness

To exploit such an exciting prospect fully, it is necessary to understand the factors regulating arterial stiffness. Traditionally, stiffness of a vessel was viewed as simply a function of the structural elements of the vessel wall and distending (mean arterial) pressure. However, the large arteries also have a generous coat of smooth muscle, which can alter the distribution of stresses between the elastic and collagenous fibers of the vessel wall and thus alter arterial stiffness. Because smooth muscle tone is influenced by a number of circulating and local vasoactive mediators, arterial stiffness may be actively regulated, and indeed modifiable, at least in the short-term. The muscular arteries have a rich sympathetic innervation, and catecholamines are known to alter smooth muscle tone. Moreover, removal of the vascular endothelium in animals alters large artery stiffness,5,6 suggesting that endothelial-derived substances regulate arterial stiffness in vivo. However, the endothelium releases a number of different mediators including nitric oxide (NO), endothelin-1 (ET-1), and C-type natriuretic peptide, which can alter smooth muscle tone and thus also potentially regulate large artery stiffness.

NO and Endothelial Function

NO not only influences vascular tone but is also an important anti-atherogenic molecule. Vasomotor endothelial function can be assessed with a variety of techniques in the peripheral or coronary circulation,7,8 although existing techniques are not widely applicable because of a number of limitations. Nevertheless, endothelial dysfunction, characterized by decreased bioavailability of NO, in resistance and conduit arteries is a predictor of cardiovascular risk and outcome.9 Conditions associated with endothelial dysfunction such as hypercholesterolemia10–12 and diabetes13–15 are also associated with increased arterial stiffness, and a number of therapeutic interventions that improve endothelial function also reduce arterial stiffness,16 suggesting that NO may itself regulate large arterial stiffness.

Although controversy exists as to whether hypertension per se is associated with endothelial dysfunction,17,18 endothelial function in unselected hypertensive subjects predicts outcome.19 In a recent study involving 262 never-treated hypertensive patients, pulse pressure, a surrogate of large artery stiffness, was the strongest independent predictor of the response to the endothelium-dependent agonist acetylcholine,20 accounting for 34% of the observed between-patient variation. After adjustment for other variables, each 1-mm Hg increase in pulse pressure was associated with a 9% reduction in the response to acetylcholine. A further study involving normal subjects and patients with stable coronary artery disease also demonstrated a significant inverse correlation between endothelial function, as assessed by flow-mediated dilatation of the brachial artery, and tonometry-measured characteristic impedance of the proximal aorta—a more direct index of large artery stiffness.21

Further evidence linking NO and large artery function comes from observations made in patients with ESRD. Pulse pressure is also a strong predictor of total and cardiovascular risk in such patients,22 but traditional risk factors such as age, hypertension, dyslipidemia, diabetes, and smoking, which are
associated with endothelial dysfunction, cannot fully account for the advanced state of arterial stiffening and the high rate of cardiovascular morbidity and mortality. However, accumulation of naturally occurring nitric oxide synthase (NOS) inhibitors may offer a potential explanation. Plasma concentration of asymmetrical dimethylarginine (ADMA), a potent endogenous competitive inhibitor of NOS, can reach levels of 7.5 times normal in patients with ESRD. Endothelial dysfunction in uremic children is related to plasma ADMA levels. More recently, ADMA was noted to be an independent predictor of total and cardiovascular mortality, and of the severity of carotid atherosclerosis in hemodialysis patients. Multivariate analysis showed that a 2-μmol/L increase in plasma ADMA (an almost doubling of normal values) was associated with a 37% increase in risk for fatal and nonfatal cardiovascular events. Thus, ADMA may represent an independent risk factor in ESRD, leading to further reduction in NO, endothelial dysfunction, arterial stiffening, and predisposition to increased cardiovascular events.

Genetic Studies

Although a number of animal studies have examined the effect of “knocking out” the endothelial nitric oxide synthase (eNOS) gene, few have reported data on diastolic or pulse pressures. Recently, data concerning 24-hour blood pressure in eNOS knockout mice and controls, assessed by telemetry, have been reported. There was a modest increase in average blood pressure in the knockouts animals relative to controls (104±2 versus 119±1 mm Hg). However, this change was accompanied by a more pronounced increase in pulse pressure of 23%, suggesting that NO was having a greater effect on large arterial stiffness than peripheral resistance. The investigators also observed a sustained increase of 10 mm Hg in pulse pressure in the control but not knockout animals after inhibition of NO production with Nω-nitro-L-arginine methyl ester (L-NAME). To date, PWV has not been assessed in eNOS knockout animals. However, increased PWV has been reported in apoE knockout mice, which are known to exhibit endothelial dysfunction, relative to controls. Although the knockouts had systemic blood pressure similar to that of the controls, they had extensive aortic atherosclerotic lesions, making interpretation difficult. Human studies have focused on polymorphisms of the eNOS gene, but the data are conflicting. In the largest study, Lacolley et al were unable to demonstrate an effect of either of the 2 eNOS polymorphisms on PWV in normotensive or hypertensive subjects. Conversely, in a smaller study, Mourad et al demonstrated that the presence of the G298T polymorphism was associated with a steeper relationship between age and PWV, but only in women.

Systemic Studies

Systemic infusions of drugs that promote or inhibit NO release have been used to investigate the role of NO in regulating large artery stiffness. Many studies have clearly demonstrated that NO donors, such as glyceryl trinitrate (GTN), reduce augmentation index, a composite measure of arterial stiffness and wave reflection, independently of any effect on blood pressure in healthy subjects, and reduce augmentation index in those with a range of cardiovascular risk factors including hypertension and hypercholesterolemia. Such an effect tends to reduce pulse pressure amplification, and thus aortic pressure decreases by more than does brachial artery pulse pressure. NO donors also reduce other measures of large artery stiffness in hypertensive individuals, and in animals, but not always independently of changes in distending pressure. Although glyceryl trinitrate reduces aortic PWV in animals, again independently from changes in blood pressure, such observations have not been repeated in humans.

The contribution of basal NO to resting large artery stiffness has been assessed by infusion of various inhibitors of NOS, including L-NMMA, and L-NAME. Systemic infusion of L-NMMA increases augmentation index in healthy normal volunteers, and L-NAME produces similar effects on various windkessel-derived indices of arterial stiffness. However, these studies are difficult to interpret because the changes in stiffness are invariably accompanied by increases in mean arterial pressure (MAP) and reflex reductions in heart rate. More recently, Stewart et al attempted to overcome these limitations by infusing L-NMMA, and also noradrenaline and dobutamine, to control for changes in MAP. They assessed large artery stiffness more directly by measuring the carotid–femoral PWV, which is inversely related to distensibility. Surprisingly, they were unable to demonstrate any direct effect of NO production on large artery stiffness, instead concluding that systemic inhibition of NO had no effect on carotid–femoral PWV over that attributable to an increase in MAP per se. Oddly, they did detect a pressure-independent reduction in aortic PWV after infusion of saline as a control vehicle.

Stewart’s observations are at variance with animal data in which bolus injection of L-NAME results in an increase in aortic PWV, measured intraarterially, which is not observed after injection of phenylephrine as a control constrictor. Perhaps, more interestingly, the same investigators also demonstrated that the increase in aortic PWV after chronic NO inhibition was even greater, which suggests some degree of vascular remodeling may have occurred. Therefore, conditions associated with decreased NO bioavailability, such as diabetes and hypercholesterolemia, may effect aortic stiffness in the longer-term by structural modification, thus providing a mechanism linking endothelial dysfunction to an increased risk of cardiovascular events. NO is known to alter the synthesis of a number of important matrix proteins, which provides 1 possible explanation for these observations. Although species differences could clearly account for the discrepant observations between humans and animals, an alternative explanation is the different methodological approaches used. In particular, because aortic PWV changes by only ~6% per decade of life, it is highly likely that inhibiting NO production will produce a relatively modest change in the PWV. Such a change may well be below the level of detection for surface-based measurement systems, such as that used by Stewart et al, unless large numbers of patients are studied. In addition, although MAP changed similarly in the human studies, differences in cardiac output or ejection duration may confound interpretation of changes in the PWV.
Finally, Stewart et al did not take into consideration the potential role of counter-regulatory mechanisms such as ET-1 or the sympathetic nervous system.

**Local Studies**

More definitive evidence for the role of NO in regulating large artery stiffness comes from local intra-arterial infusion of 1-NNMMA and GTN. Such techniques overcome many of the methodological limitations of systemic infusions, because the drug doses used are much lower and, if infusion periods are relatively short, MAP and heart rate are unusually unaffected. Such an approach can be further enhanced by direct, high-fidelity, intravascular measurement of PWV, using pressure or flow waveforms, or distensibility or compliance, using ultrasound. Using such techniques, endothelium-derived NO has been shown to regulate large arterial compliance, using pressure or flow waveforms, or distensibility or compliance, using ultrasound. Similar observations have been made in the human coronary circulation using ultrasound-derived measurement of distensibility, and in the human brachial artery for elasticity, compliance, and PWV.

Conversely, drugs that lead to an increase in local NO levels such as glyceryl trinitrate and acetylcholine, reduce arterial stiffness in human muscular arteries. Together, these data suggest that basal, simulated, and exogenous NO act to reduce large artery stiffness in vivo, independently of any change in blood pressure. Other endothelium-derived and circulating mediators, including ET-1, have also been shown to regulate large arterial stiffness in vivo, further emphasizing the potential importance of the vascular endothelium in the functional regulation of arterial stiffness and highlighting the potential for cross-talk between vasoactive axes. Therapeutic intervention aimed at increasing the bioavailability of NO may, therefore, be useful in conditions associated with age-related or premature arterial stiffening.

**Therapeutics**

**Lifestyle Modification**

Exercise increases NO production possibly via upregulation of eNOS, and regular aerobic endurance exercise attenuates age-related reductions in central arterial compliance and reduces the genetic susceptibility to increased central pressure augmentation. Exercise training also improves arterial compliance in patients with congestive cardiac failure, a condition associated with endothelial dysfunction. However, aerobic exercise training failed to modify large arterial stiffness in a group of patients with isolated systolic hypertension, and a more recent study suggested that resistance training may paradoxically increase arterial stiffness in healthy middle-aged men. Further studies will be needed to better define the type of exercise and which patient groups will benefit most.

Obesity is increasingly common and is associated with both endothelial dysfunction and increased arterial stiffness. Although weight reduction reduces pulse pressure, only 1 study has investigated its effect on indices of stiffness such as PWV. Toto-Moukouo et al reported that weight loss in obese hypertensive subjects was associated with a reduction in arterial stiffness, but this was confounded by a concomitant decrease in blood pressure, making interpretation of the data difficult. Dietary intervention with compounds that improve endothelial function such as phytoestrogens have also shown promise as agents that can reduce arterial stiffness when introduced into the diet.

**Pharmacological Therapy**

Antihypertensive agents may influence arterial stiffness in a number of ways: indirectly via a reduction in MAP, or directly via an effect on the various components of the arterial wall. Theoretically, the ideal antihypertensive agent should reduce blood pressure and stiffness. In subjects with ESRD treated with antihypertensive drugs, survival is greatest in those subjects in whom therapy reduces PWV and MAP, rather than MAP alone. Isolated systolic hypertension is a condition characterized mainly by increased large arterial stiffness rather than a raised peripheral vascular resistance. Such patients are at increased cardiovascular risk, compared with patients with essential hypertension, and benefit from blood pressure reduction. However, effective blood pressure reduction in this increasingly large patient group is often difficult. Optimal treatment of isolated systolic hypertension requires a minor reduction in peripheral resistance, but more importantly a major reduction in large artery stiffness and early wave reflection. Available antihypertensive drugs with such properties include nitrates, angiotensin-converting enzyme inhibitors, angiotensin receptor antagonists, and aldosterone antagonists, which reduce wave reflection and arterial stiffness. Phosphodiesterase 5 inhibitors and collagen cross-link breakers may also offer newer, but complimentary, approaches. The β-adrenergic antagonist nebivolol, which vasodilates via NO release and improves endothelial function, reduces large arterial PWV independently of any effect on blood pressure and heart rate. In addition, therapies that may improve endothelial function such as statins have also been shown to decrease arterial stiffness and reduce blood pressure in subjects with isolated systolic hypertension.

**Conclusions**

To date most studies that have assessed endothelial function in cardiovascular disease states have focused on peripheral resistance and coronary and brachial conduit vessels, and have not involved many elderly subjects. Although small, they demonstrated abnormalities in these vascular beds, and also that such abnormalities are predictive of cardiovascular events. However, there remains considerable controversy over the various methodologies used to assess endothelial function and the mechanical properties of conduit blood vessels. This review does not discuss this controversy, because this has been covered at length by others. However, it is important because such methodological differences could be responsible for some of the discrepancies in the literature.

Importantly, perhaps partly caused by lack of reliable clinically applicable methodologies, few studies have assessed the effect of NO on the large central arteries and those vessels more proximal to the brachial artery, and more work is needed in this emerging field. Age-related stiffening of these vessels results in unfavorable changes in ventricular–
vacular coupling, characterized by increased PWV and early wave reflection, which manifest clinically as a wide peripheral pulse pressure and isolated systolic hypertension in the elderly. Recent evidence linking NO to the functional regulation of stiffness in these large arteries provides a novel therapeutic target for drugs that will slow or reverse the effects of premature vascular aging seen in many cardiovascular disease states. Before such potential can be fully realized, the focus must be shifted away from assessing endothelial function in the peripheral arteries and more toward a better understanding of the effects of NO and other important endothelium-derived vascular mediators on large arterial structure and function and their effects on ventricular–vascular coupling in health and disease.

References


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