Effects of Nitroglycerin Induced Vasodilation on Elastic versus Muscular Artery Stiffness in Older Veterans

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Abstract

Vascular smooth muscle tone may play an important role in the physiology of increased arterial stiffness that occurs with aging. This study evaluated the impact of smooth muscle tone on arterial stiffness in older individuals following nitroglycerin induced vasodilation in elastic and muscular arteries. 40 older Veterans (60 years old), without known cardiovascular disease, were included in this study. 20 were hypertensive (70.8±6.6 years, 10 female) and 20 were normotensive controls (72.0±9.3 years, 8 female). Nitroglycerin (NTG) induced changes in arterial stiffness were measured locally with vascular ultrasound in the carotid and brachial arteries, and regionally by carotid-femoral pulse wave velocity (cfPWV) by tonometry. With NTG, both hypertensive and normotensive control Veterans showed increased carotid PWV (6.4±1.3m/s to 7.2±1.4m/s, 0.8±1.1m/s, p=0.007) and cfPWV (8.6±1.9m/s to 9.5±2.4m/s,　0.9±2.3 m/s, p=0.020) but did not change brachial PWV (11.2±2.4m/s to 11.1±2.2m/s,　−0.2±2.5m/s, p=0.72). The carotid artery dilated more in control participants than hypertensive Veterans (　0.54±0.19mm vs 0.42±0.12mm, p=0.022). Brachial artery dilation was similar, (　0.55±0.26mm vs 0.51±0.20mm, p=0.46). In older Veterans, without known cardiovascular disease, NTG induced vasodilation increased elastic artery stiffness and did not change muscular artery stiffness. Increased central arterial stiffness and reduction in the arterial stiffness gradient could offset some of the benefits of lowering blood pressure in older patients who are prescribed vasodilators as an antihypertensive therapy. Elastic artery stiffening with vasodilation warrants further investigation as it may be important for antihypertensive medication selection and influence CVD development.

Graphical Abstract

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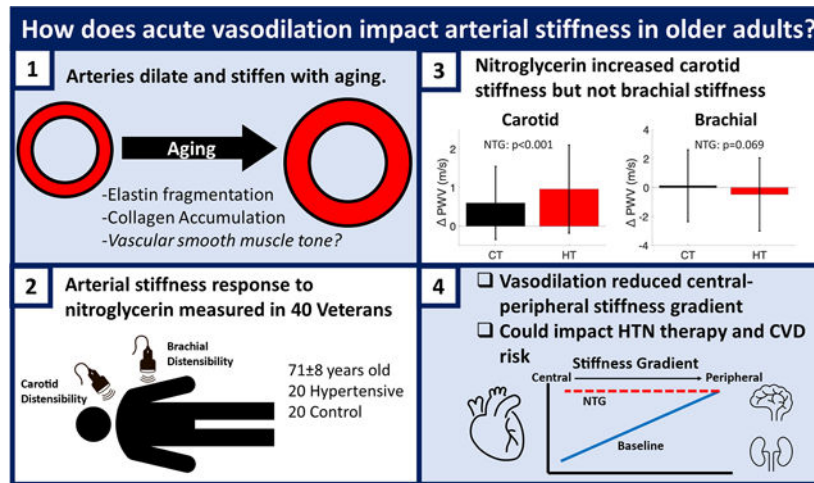
Introduction

Studies of the biomechanics of the central elastic arteries like the aorta and carotid artery have predominately focused on passive contributions to arterial stiffness such as the extracellular matrix (ECM) and neglected active contributions to arterial stiffness from vascular smooth muscle (VSM)[1]. VSM is known to contribute significantly to the mechanical behavior of peripheral, muscular arteries. It has traditionally been believed that VSM and changes in VSM tone are unimportant to central, elastic artery stiffness[1,2] due to their higher concentration of elastin[3,4] and unique functional role to provide compliance and the Windkessel effect[5,6]. However, there is growing evidence that vascular smooth muscle has an important role in elastic artery mechanics. Animal studies show that vasoactive drugs can induce contractile responses in the aorta[7] and the carotid artery[7,8]. While data in humans is more limited, and confounded by blood pressure, both nitroglycerin administration and lower limb venous occlusion in hypertensive adults have been found to invert the expected relationship of decreasing blood pressure leading to an decrease in arterial stiffness[9,10]. Together, these results indicate that VSM contraction may play a more important role in elastic artery function than traditionally thought.

In a recent retrospective analysis of several clinical studies, nitroglycerin (NTG) induced vasodilation was found to both increase and decrease arterial stiffness[11] depending on the ratio of passive-to-active stiffness along the continuum of young-to-old, healthy-to-diseased, and elastic-to-muscular arteries studied. Giudici and Spronck proposed that the results of this retrospective analysis represent an important step towards understanding the role of VSM in large artery mechanics and function[12]. In particular, decreased VSM tone in the carotid artery of hypertensive individuals bore striking resemblance to the effects of 10 years of aging[13,14], the carotid artery dilated and stiffened. These results suggest that VSM tone may be a key component of arterial aging and have different impacts on elastic versus

muscular arteries. We performed the present study to test the responses of vasodilation and changes in VSM tone on arterial stiffness in the brachial and carotid arteries in older Veterans. NTG induced vasodilation was used because NTG is both quick acting and can safely be administered. We hypothesized that NTG induced vasodilation would increase elastic artery stiffness and decrease muscular artery stiffness.

Methods and Materials

This study was reviewed and approved by the University of Wisconsin and Madison Veterans Hospital Institutional Review Board. All participants gave written, informed consent to participate in this study.

Study Participants

This analysis included 40 Veteran participants (20 normotensive controls and 20 with a diagnosis of hypertension) from the ongoing Functional Arterial STiffness in Veterans (FAST-Vets) study at the Madison Veterans Affairs Hospital (MVAH). Ambulatory, community-dwelling, hypertensive, older Veterans (>60 years old) were recruited from the MVAH and surrounding clinics. Inclusion criteria were: male and female participants over the age 60 years from VA VISN 12, willing to undergo exercise treadmill stress testing, administration of sublingual NTG, 24-hour blood pressure monitoring, have tonometric evaluation of arterial stiffness, and undergo a brachial and carotid artery ultrasound as well as a non-invasive assessment of blood pressure and Cardiac Output (CO) using the Cheetah Starling System (Cheetah Medical, Inc. Newton Center, MA). Exclusion criteria were: known cardiovascular disease, secondary hypertension, chronic kidney disease, changing antihypertensive medication in the last month, active cancer (other than untreated, non-metastatic prostate cancer and non-melanoma skin cancer), hypoxemic pulmonary disease, active rheumatologic diseases (i.e., systemic lupus erythematosus, rheumatoid arthritis, etc.), human immunodeficiency virus, or illness with any infectious etiology or fever >38°C or hospitalization for any reason within the prior 4 weeks. Participants who were unable to walk on a treadmill were also excluded. For safety purposes, participants with baseline resting systolic BP < 110mmHg and mean arterial pressure <90mmHg or heart rate > 90 beats per minute were not administered nitroglycerin and were excluded from this analysis. A diagnosis of hypertension was assessed based on two or more office systolic BP measurements >140 mmHg, a mean home BP reading of >135 mmHg for over 7 days, or active use of antihypertensive medications.

Study Protocol

All studies were performed at the University of Wisconsin Atherosclerosis Imaging Research Program, which is a nationally recognized core ultrasound reading, training, and scanning laboratory. Visits began between 7AM and 11AM and participants withheld medications until after the study visit. For eight hours prior to the visit participants were asked to fast and refrain from smoking, caffeine, and sildenafil. On visit day 1 participants completed a maximal exercise treadmill stress test (data not shown). In between visit days 1 and 2 participants were fitted with a 24 hour ambulatory blood pressure monitor (ABPM) (SpaceLabs Healthcare, Snoqualmie, WA, USA) and were asked to wear the ABPM until

they returned for their study visit the following day. 24 hour ABP was calculated as the mean of all the BP readings during both day and night.

On visit day 2 participants rested in a supine position in a temperature-controlled room for 10 minutes and serial baseline oscillometric brachial blood pressure (Cheetah Starling Fluid Management System, Baxter Healthcare, Deerfield, IL, USA) and radial artery tonometry (Atcor Sphygmacor, Atcor Medical, Sydney, Australia) were performed. Central systolic blood pressure was calculated from radial tonometry using a validated transfer function. Brachial artery flow mediated dilation (FMD) was performed following a forearm occlusion protocol (5 minutes occlusion period at 250mmHg) obtaining images of the right brachial artery 60 and 90 seconds post cuff release. FMD was calculated as the percent change from baseline brachial diameter to the maximum post cuff release diameter. Following a 20-minute wash out period post flow mediated dilation, baseline arterial stiffness measurements were performed in duplicate. Participants were then administered 400 micrograms sublingual NTG. Arterial stiffness measurements were repeated every 1-minute following NTG administration for at least 10 minutes or until the participant's heart rate and blood pressure had returned to baseline (Figure 1).

Arterial Stiffness Measurements

Carotid-to-femoral pulse wave velocity (cfPWV) was measured using an AtCor SphymoCor Px tonometry system (Atcor Medical, Sydney, Australia) which has a Millar micromanometer (Millar, SPT-301B, Houston, TX, USA). Transit distance to calculate cfPWV was measured as the carotid- suprasternal notch (SSN) distance subtracted from the SSN– femoral distance as recommended by AHA's arterial stiffness scientific statement[15]. Vascular ultrasound was performed with a Philips CX-50 (Philips Ultrasound, Bothell, WA) ultrasound system with a 14L5 transducer by trained and registered sonographers from the University of Wisconsin Atherosclerosis Imaging Research Program. The brachial artery of the right arm was imaged 2–7 cm above the elbow and scanned in longitudinal sections with the focus set to the depth of the near wall. The brachial artery was imaged at baseline, 3 minutes post NTG, and 5 minutes post NTG. Longitudinal views of the distal 1cm of the right common carotid artery (CCA) were obtained with the imaging depth set at 4 cm, placing the artery between 2–3 cm deep on the screen, adjusting the focus and gain preferably stacking the jugular vein over the CCA to improve near and far wall resolution. B-mode ultrasound 5 beat DICOM clips were analyzed using Medical Imaging Applications Tools Carotid Analyzer (MIA Inc, Coralville, IA) software to measure far wall intima-media thickness (IMT) and artery lumen diameters over the cardiac cycles.

All baseline arterial stiffness measurements were performed in duplicate and averaged. The post NTG carotid and brachial artery stiffness measures were calculated from the two time points with the largest diastolic diameters which represent the time point with the maximal vasodilatory effect on the carotid or brachial artery. The post NTG carotid-femoral stiffness was calculated from the two time points with the greatest change in cfPWV from baseline. Blood pressure and heart rate were recorded from the measurement time point immediately prior to performing each arterial stiffness measurement. Because arterial

stiffness measurements were performed at slightly different times the blood pressures and heart rates are also slightly different.

Arterial Stiffness Calculation and Modeling

Peterson's Elastic Modulus (PEM) of the carotid and brachial arteries were calculated[16]:

$$PEM = \frac{\Delta p Dd^2}{\left(Ds^2 - Dd^2\right)}$$

#(1)

where Ds represents the internal arterial diameter at peak systole, Dd represents the internal diameter at end-diastole, and Δp represents the brachial blood pressure difference between the systolic and diastolic measurements (pulse pressure). Local carotid and brachial pulse wave velocity (c/b PWV) was calculated from the Bramwell-Hill equation

$$PWV = \sqrt{\frac{PEM}{\rho}}$$

#(2)

where ρ is the density of blood (1050 kg/m^3). Young's elastic modulus (YEM) was then calculated from the Moens-Korteweg equation

$$YEM = \rho PWV^2 \frac{Dd}{IMT}$$

#(3)

where IMT is the far wall artery wall intima-media thickness.

To control for changes in blood pressure with NTG administration, a participant-specific exponential model was used to describe the blood pressure dependence of arterial mechanics[14,17]. The mathematical equations are included as a supplement. Using the non-linear model, carotid artery and brachial artery stiffness metrics were calculated at a common 120/80 mmHg reference pressure pre- and post-NTG administration (Figure 2). The blood pressure dependence of cfPWV measurements were similarly adjusted using an exponential model. Guideline statements recommend adjusting cfPWV to MAP[15] but because the waveform features used to calculate cfPWV occur at DBP it may be more appropriate to adjust cfPWV to DBP. We adjusted to both a DBP of 80mmHg and a MAP of 100mmHg.

Lastly, we used a recently developed arterial mechanics model based on pressure-diameter relationships that incorporates the contributions to arterial stiffness from both the passive ECM and active VSM components of the arterial wall[11]. This model is advantageous because it's simple enough to use with limited data available in clinical studies but has physiologically relevant parameters which include passive stiffness, active stiffness and

active smooth muscle tone. The model was used to analyze the effects of nitroglycerin-induced vasodilation on carotid and brachial artery mechanics (Supplemental material).

Statistical Analysis

Continuous variables are presented as mean and standard deviation. Categorical variables are presented as number and percentage. Baseline characteristics for controls and hypertensive participants were compared using independent sample t-tests for continuous variables and $\chi 2$ tests for categorical variables. The arterial stiffness response to NTG was analyzed with a repeated-measures linear model with NTG administration as a within subjects factor and hypertension status as a between subjects factor. Additional repeated measured models were performed for sex, age, smoking status, and diabetes mellitus status). Results from modeling the active and passive contributions to arterial mechanics were compared using the Mann-Whitney U test due to non-normal distributions of some variables.

Results

Participant Characteristics

Participant characteristics are shown in Table 1. Controls and hypertensive Veterans participating in this study were similarly matched for age (72.0±9.3 vs 70.8±6.6 year, p=0.64), sex (40% female vs 50% female, p=0.53), and smoking status. 85% of hypertensive participants were using antihypertensive medications and self-reported an average of 14.7±14.3 years since hypertension diagnosis (range: 2–60 years). From 24-hour ambulatory blood pressure monitoring (ABPM), both SBP and DBP were not significantly different between hypertensive and control participants. 53% of control and 50% of hypertensive participants had well controlled 24-hour ABP (<125/75) according to 2017 AHA/ACC guidelines.

Brachial Artery Stiffness

Brachial artery flow mediated dilation was not significantly different between control and hypertensive groups (4.14±2.48% vs 2.66±2.16%, p=0.054). For 6 control participants brachial artery ultrasound images were of insufficient quality to perform stiffness measurements. Brachial artery stiffness results are shown in Table 2, Figure 3, and individual participant data are presented in the data supplement (Figure S3). For both control and hypertensive groups nitroglycerin administration significantly decreased brachial blood pressure, increased heart rate, increased brachial artery diameter, and decreased brachial artery IMT (Table 2). Brachial artery PEM was similar between control and hypertensive groups at baseline (139±58vs 144±56kPa) and following nitroglycerin administration (141±48vs 133±49kPa). Nitroglycerin administration did not have a significant effect on brachial artery stiffness assessed by PEM, bPWV, and YEM (Table 2). After adjusting brachial artery stiffness parameters to a standard 120/80mmHg blood pressure, nitroglycerin administration significantly increased $YEM_{120/80}$ (p=0.016) and had no effect on $PEM_{120/80}$, or $bPWV_{120/80}$. For brachial artery stiffness parameters there were no significant group differences or nitroglycerin-group interactions. No statistically significant effects were found for sex, age, smoking status, or diabetes mellitus status (Table S1–S4).

Carotid Artery Stiffness

Carotid artery stiffness results are shown in Table 3, Figure 3, and individual participant data are presented in the data supplement (Figure S2). For both control and hypertensive groups, nitroglycerin administration significantly decreased blood pressure, increased heart rate, increased carotid artery diameter, and decreased carotid artery IMT (Table 3). Carotid artery PEM was lower in control participants compared to hypertensive participants at baseline (38.5±15.3vs 51.1±18.8 kPa) and following nitroglycerin administration (47.3±19.6 vs 65.9±21.5 kPa). Nitroglycerin administration significantly increased carotid artery stiffness assessed by PEM, cPWV, and YEM before and after adjusting carotid artery stiffness parameters to a standard 120/80mmHg blood pressure (Table 3). Hypertensive participants had significantly elevated (p<0.05) PEM, cPWV, YEM, $PEM_{120/80}$, and $cPWV_{120/80}$ compared to control participants. No significant nitroglycerin-group interactions were observed for carotid artery stiffness parameters. The only statistically significant nitroglycerin-group interaction for the carotid artery was that control participants had a greater increase in diastolic diameter compared to hypertensive participants following nitroglycerin administration (: 0.54±0.19mm vs 0.42±0.12mm, p=0.022). No statistically significant effects were found for sex, age, smoking status, or diabetes mellitus status (Table S1–S4).

Carotid-Femoral Region Stiffness

Carotid-femoral region stiffness results are shown in Table 4, Figure 3, and individual participant data are presented in the data supplement (Figure S4). For both the control and hypertensive participants nitroglycerin administration significantly decreased SBP (: −20±14 mmHg, p<0.001), DBP (: −7±8 mmHg, p<0.001), and MAP (: −11±9 mmHg, p<0.001) (Table 4. cfPWV was significantly lower in control participants compared to hypertensive participants at baseline (7.6±1.7 vs 9.6±1.8 m/s) and following nitroglycerin administration (8.7±2.0 vs 10.2±2.6 m/s). Nitroglyerin administration significantly increased cfPWV in both groups (: 0.9±2.3 m/s, p=0.020). Hypertensive participants had significantly elevated (p<0.05) cfPWV, $cfPWV_{80}$, and $cfPWV_{100}$ compared to control participants. No significant nitroglycerin-group interactions were observed for carotid-femoral region stiffness parameters. No statistically significant effects were found for sex, age, smoking status, or diabetes mellitus status (Table S1–S4).

Passive versus Active Arterial Mechanics

A two-term exponential model was applied to nitroglycerin vasodilation data from each participant to determine the passive ECM and active VSM contributions to arterial mechanics of the carotid and brachial arteries. Carotid artery data for one control participant and two hypertensive participant were excluded from analysis of active stiffness and basal smooth muscle tone due to residual error after parameter fitting (Supplemental Material). Modeling results are shown in Figure 4. For the carotid artery, hypertensive participants had greater passive stiffness compared to control participants (10.8±2.7 vs 8.5±3.4, p=0.003, dimensionless) while active stiffness was not significantly different (5.7±3.6 vs 4.5±2.8, p=0.44, dimensionless). For the brachial artery, hypertensive and control participants had similar passive and active stiffness (Figure 4). Carotid artery basal smooth muscle tone

(parameter k in model) was lower in hypertensive participants (0.052± 0.013 vs 0.062 ± 0.017, p=0.019, dimensionless). Brachial artery basal smooth muscle tone was similar between groups (0.115± 0.038 vs 0.117 ± 0.052 p=0.96, dimensionless). In the brachial artery 86.7±14.1% of baseline SBP was supported by active elements of the artery wall compared to 43.9±13.0% in the carotid artery. For both the carotid artery and the brachial artery, the percentage of baseline SBP supported by active muscular elements of the artery wall was not significantly different in hypertensive participants compared to control participants (Figure 4).

Discussion

This study assessed the effects of nitroglycerin induced vasodilation on the stiffness of elastic and muscular arteries in older Veterans. The major finding was that the stiffness of central, elastic arteries significantly increased following sublingual nitroglycerin administration. Based on prior studies examining overall CVD risk associated with arterial stiffness parameters, this is a clinically significant observation that equates to an approximate increase of 15% in CVD risk[18]. In contrast to the carotid artery and carotid-femoral region, nitroglycerin did not change the stiffness of the muscular brachial artery.

In this study vasodilator administration decreased brachial BP by approximately 10 mmHg, but also had the negative effect of paradoxically increasing elastic artery stiffness. This result was confirmed with two independent measures of elastic artery stiffness, carotid ultrasound and cfPWV. Vasodilator administration also did not change muscular artery stiffness, which would reduce the central-to-peripheral arterial stiffness gradient and increase pulsatile energy transmitted to the microvasculature[19]. Awareness of the increase in central arterial stiffness and reduction in the arterial stiffness gradient, despite a decrease in BP, is paramount because it could offset some of the benefits of lowering BP in older patients who are prescribed vasodilators as an antihypertensive therapy. These results emphasize that direct vasodilators should be reserved as 4th-5th line antihypertensive therapy options, particularly in older patients, and prompt the need to test the impact of other hypertensive therapies on active and passive stiffness parameters. Angiotensin converting enzyme inhibitors have a greater effect on decreasing arterial stiffness compared to calcium channel blockers and beta blockers[20] which could be due to different effects on elastic artery smooth muscle tone.

Nitroglycerin induced vasodilation also had different effects on the stiffness of elastic versus muscular arteries, likely due to their different functions and the ratio of active stiffness of vascular smooth muscle cells versus passive stiffness of the ECM. Vasodilation increased carotid artery stiffness by 27% and did not change brachial artery stiffness. Our results in older Veterans show different effects of nitroglycerin on arterial stiffness than previous studies in younger to middle-aged adults. Nitroglycerin decreased elastic artery stiffness in younger control individuals[9,21] and either did not change[9] or increased elastic artery stiffness in younger hypertensive individuals[22]. In younger non-hypertensive individuals, nitroglycerin decreased muscular artery stiffness[21,23,24]. These results cannot solely be due to the straightening of collagen fibers with dilation as then vasodilation would be correlated with increased stiffness. We did not find changes in arterial diameter were correlated with

changes in arterial stiffness (Supplemental Figure S5). This is in contrast to previous results showing that increased radial artery diameter with vasoactive drugs was correlated with decreased brachial-radial PWV in young men[25]. In addition to the differences in age, sex, and the arteries studied, statistical analysis is another potential reason for this difference. Fok et al., analyzed correlations of the average group response to different drugs and doses, which may be different from the correlations of the individual participant response.

We hypothesize that the different stiffness response of elastic versus muscular artery stiffness to vasodilation are due to the ratio of passive and active contributions to arterial stiffness based on a Hill-type partition of passive-elastic and active-contractile forces[11] (Figure 5). When an artery has low passive stiffness compared to active stiffness, decreasing VSM tone will decrease arterial stiffness. In contrast, when an artery has high passive stiffness compared to active stiffness, decreasing VSM tone will increase arterial stiffness. Our results are in line with this hypothesis as the elastic arteries of older individuals likely have higher passive stiffness due to mechanisms such as collagen accumulation and elastin fragmentation[26]. The brachial artery transition from NTG decreasing stiffness in young men to NTG not changing stiffness in older Veterans would indicate that with aging the brachial artery remodels from having stiffer VSM than ECM to having relatively equal VSM and ECM stiffness.

The arterial stiffness response to vasodilation is likely important in the development of CVD. Among individuals suspected of coronary artery disease (CAD), those in whom NTG increased carotid artery stiffness had both greater prevalence and severity of angiographic coronary artery disease compared to those in whom NTG decreased carotid artery stiffness[27]. The older Veterans in this study were free from known CVD, so the abnormal response of vasodilation increasing carotid artery stiffness could be a precursor to CVD development and may represent a novel risk marker. Changes in elastic artery VSM tone could have adaptive or maladaptive effects on pathological arterial remodeling in hypertension. In our analyses, the carotid artery basal smooth muscle tone was lower in hypertensive participants. Increased VSM tone has been shown to reduce arterial stiffness and reduce excess stress on the ECM which promotes adaptive arterial remodeling in the carotid of hypertensive rats[28] and the thoracic aorta remodeling in hypertensive mice[29]. In rodents, increased VSM tone in hypertension is an acute phenomenon, only lasting a handful of weeks. Our data shows the opposite effect (decreased VSM tone) in older Veterans who have had hypertension for years. Decreased elastic artery VSM tone in hypertensive individuals could also partially explain increased carotid artery diameter in hypertension. The average difference in carotid artery diameter versus controls was smaller with vasodilation (0.24 mm) compared to baseline (0.36mm). This suggests that basal VSM tone may be a therapeutic target to promote adaptive arterial remodeling in humans with hypertension.

Arterial stiffness is implicated in the pathway from isolated systolic hypertension to heart failure with preserved ejection fraction (HFpEF) in older individuals[30] and development of isolated systolic hypertension through decreased arterial compliance[31]. Abnormal arterial stiffness responses to physiologic and pharmacologic changes can alter left ventricle afterload and exercise capacity and ultimately lead to left ventricular dysfunction[32]. It may

be possible to beneficially alter these negative outcomes by modifying arterial stiffness through VSM tone.

Limitations of this study include the relatively small number of subjects and is limited to an older, albeit less studied, patient population. Peripheral brachial artery BP was used instead of central carotid artery BP to calculate carotid artery stiffness. Nitroglycerin can have a greater impact on central BP compared to peripheral BP[33] although the difference between central and peripheral BP should be lower in older individuals. We did not correct for changes in heart rate, but the increase in heart rate was small (3.5±4.5 BPM). Based on past studies[34], this increase in heart rate would only account for a cfPWV increase of approximately 0.05 m/s while we found cfPWV increased by 0.9 m/s. A washout period for antihypertensive medications prior to the study visit was not used. Even though participants did not take medications the morning of their visit, medications taken the prior day could exert some vasoactive effects and influence results. Finally, no histological data was collected or evaluated.

Perspectives

In older Veterans, without known cardiovascular disease, NTG induced vasodilation increased elastic artery stiffness and did not change muscular artery stiffness. Elastic artery stiffening with vasodilation may be important for antihypertensive medication selection and influence CVD development and should be a focus of future studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

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NTG

	Baseline	1 min	2 min	3 min	4 min	5 min	6 min	...	10 min
BP	X	X	X	X	X	X	X	...	X
PWA	X							...	
cfPWV	X	X	X	X	X	X	X	...	X
CCA US	X	X	X	X	X	X	X	...	X
BA US	X			X		X		...	

Figure 1:

Diagram of the timeline for baseline and post-administration of 400 mcg sublingual nitroglycerin (NTG). After NTG administration, participants were monitored for at least 10 minutes or until blood pressure and heart rate returned to baseline. BP – Blood pressure, PWA – pulse wave analysis, cfPWV – carotid-femoral pulse wave velocity, CCA US – common carotid artery ultrasound, BA US – brachial artery ultrasound.
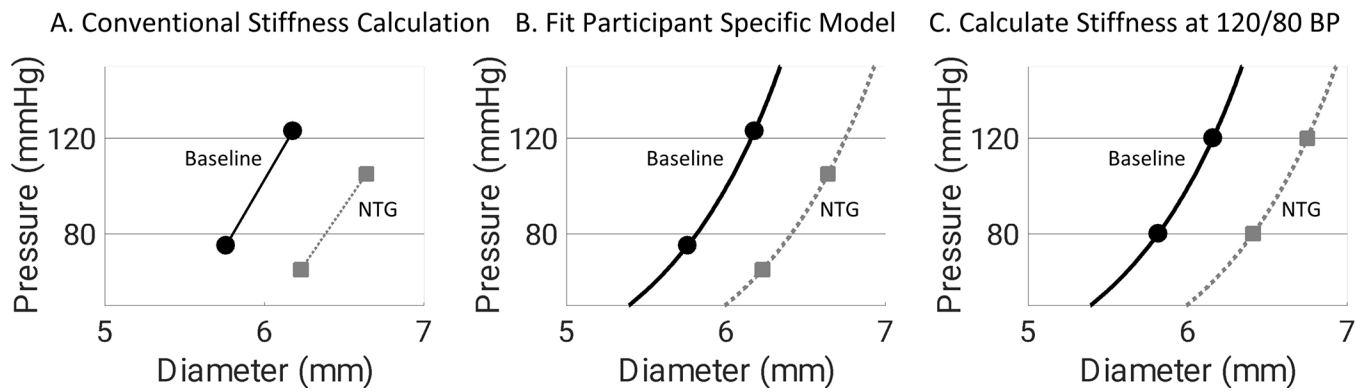
A. Conventional Stiffness Calculation   B. Fit Participant Specific Model   C. Calculate Stiffness at 120/80 BP

Figure 2:
Graphical representation of the methods used to control for the blood pressure dependence of carotid artery stiffness for a representative participant. Baseline data are shown as black circles and NTG data is shown as gray squares. For conventional stiffness calculations NTG decreased Peterson's elastic modulus (PEM) (318 to 296 mmHg) but blood pressure also decreased. NTG increased PEM (329 to 369 mmHg) after adjusting stiffness calculation at a constant 120/80mmHg blood pressure.
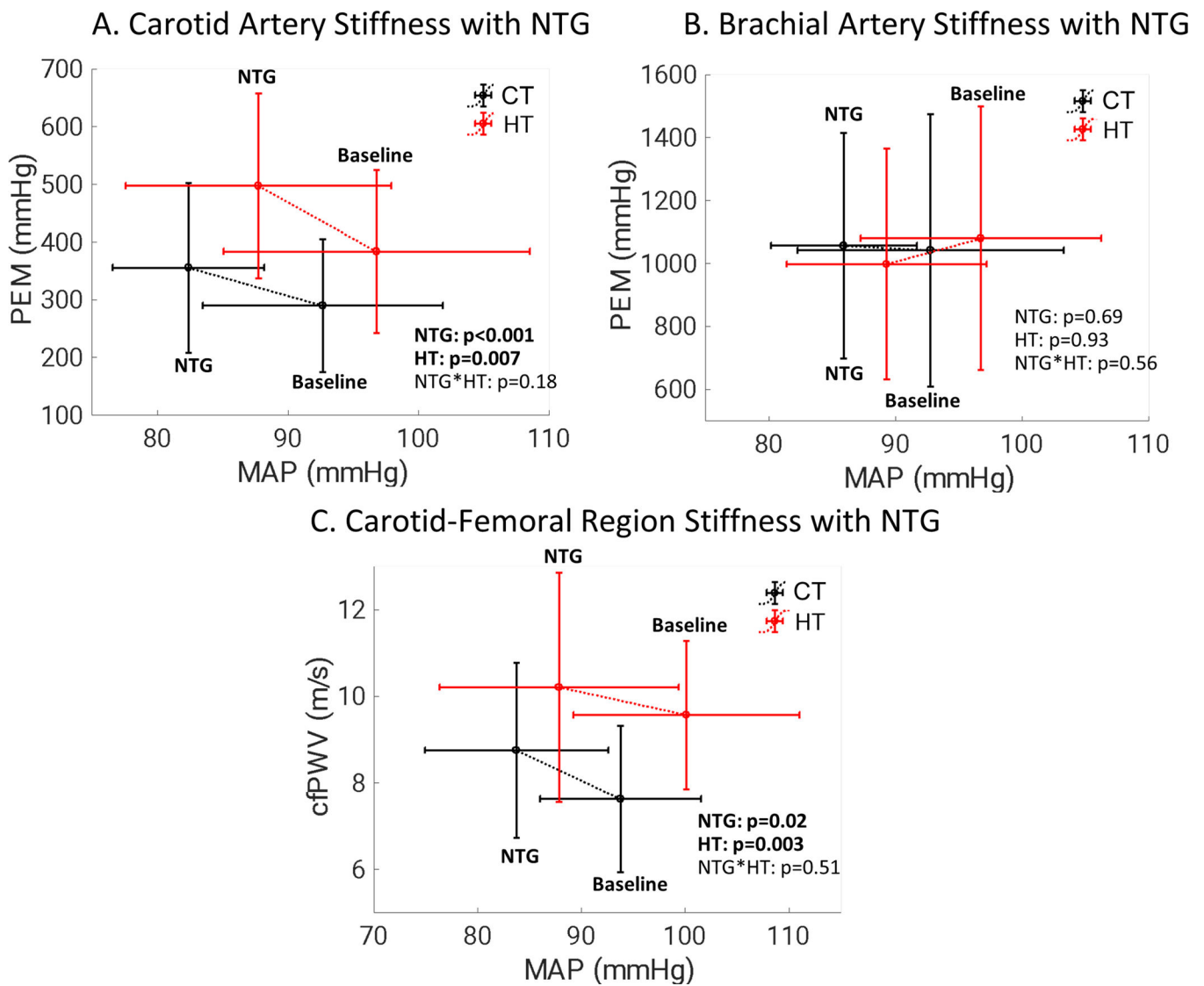
A. Carotid Artery Stiffness with NTG

B. Brachial Artery Stiffness with NTG

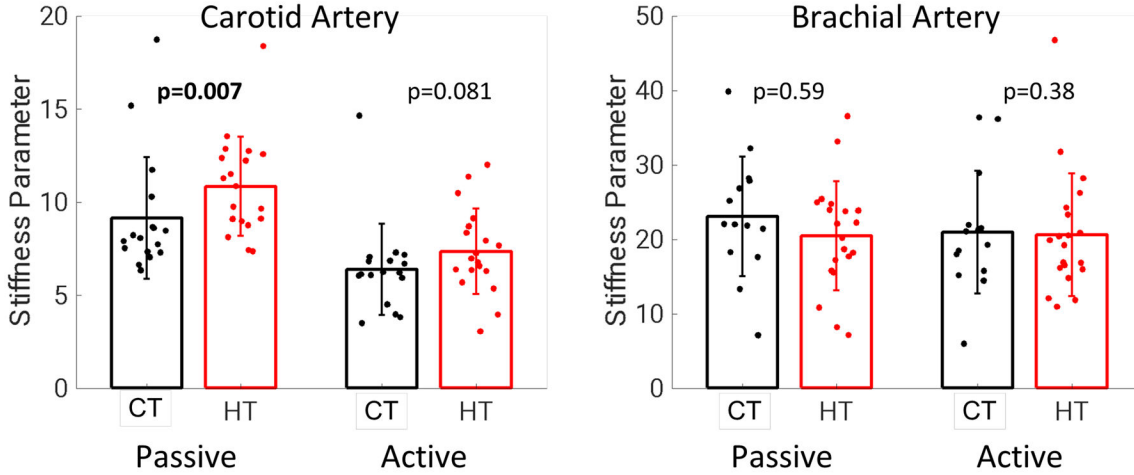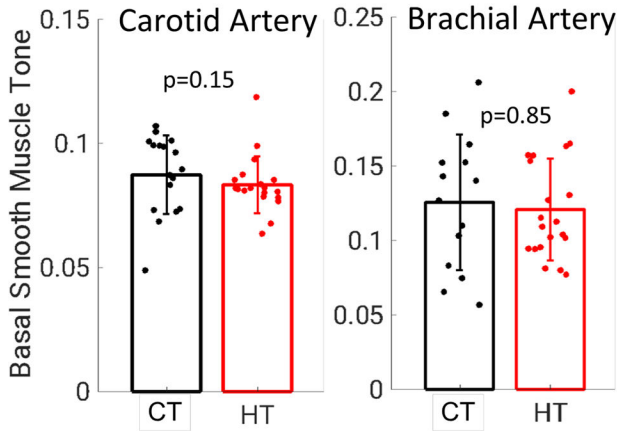## C. Carotid-Femoral Region Stiffness with NTG

Figure 3:

Despite causing approximately 10mmHg decreases in mean arterial pressure (MAP), nitroglycerin (NTG) administration A. increased carotid artery Petersons' elastic modulus (PEM), B. did not change brachial artery PEM, and C. increased carotid-femoral pulse wave velocity (cfPWV). Abbreviations: CT – Control, HT – hypertensive
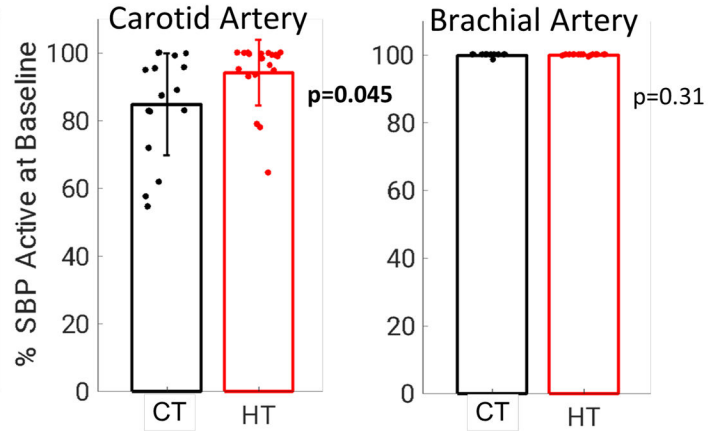
Figure 4:
A. Carotid artery passive stiffness from modeling was elevated in hypertensive participants while carotid artery active stiffness, brachial artery passive stiffness, and brachial artery active stiffness were not different between groups. B. Carotid artery basal smooth muscle tone was lower in hypertensive participants and brachial artery basal smooth muscle tone was similar between groups. C. For the carotid artery, a similar percentage of baseline SBP was supported by active and passive elements of the artery wall. For the brachial artery, the majority of baseline SBP was supported by active components of the artery wall for both groups.
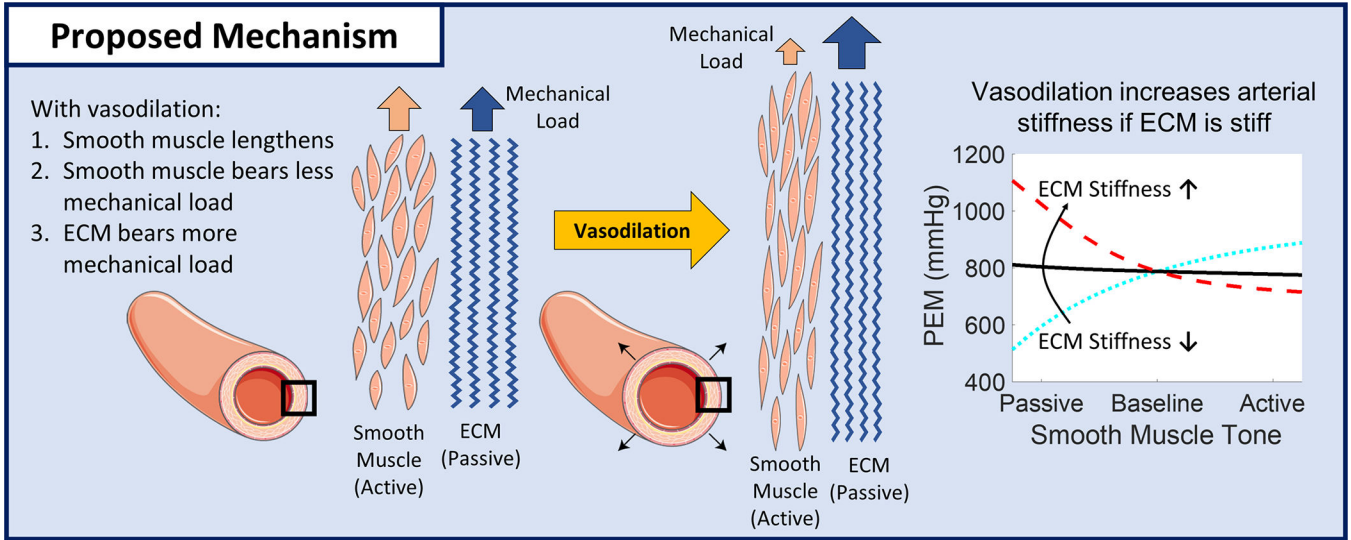
Figure 5:

Proposed mechanism for elastic artery stiffening with vasodilation in older adults. With vasodilation, decreasing smooth muscle tone, vascular smooth muscle supports less mechanical load and the ECM supports more mechanical load. If the ECM is stiffer than the smooth muscle (as is likely the case in older adults), vasodilation shifting load from smooth muscle to the ECM will make the artery stiffer. Figure created with images from Servier Medical Art[35].

Table 1:

Participant Characteristics

	Control (n=20)	Hypertensive (n=20)	p-value
Age (years)	72.0±9.3	70.8±6.6	0.64
BMI (kg/m²)	27.6±4.5	27.0±6.5	0.72
Sex			0.53
Female (n, %)	8 (40%)	10 (50%)	
Race/Ethnicity			1.0
White (n, %)	19 (95%)	19 (95%)	
Black (n, %)	1 (5%)	1 (5%)	
Diabetes Mellitus (n, %)	2 (10%)	6 (30%)	0.11
Smoking Status			0.41
Current (n, %)	2 (10%)	3 (15%)	
Former (n, %)	9 (45%)	12 (60%)	
Never (n, %)	9 (45%)	5 (25%)	
Pack Years	14.7±19.2	16.7±18.7	0.74
Supine Blood Pressure			
SBP (mmHg)	127±13	134±13	0.095
DBP (mmHg)	76±8	80±7	0.084
MAP (mmHg)	93±8	98±8	0.054
cSBP (mmHg)	119±10 (n=19)	121±14	0.77
24hr ABPM	N=19	N=16	
24hr SBP (mmHg)	121±9	125±11	0.19
24hr DBP (mmHg)	69±6	73±7	0.09
24hr BP < 125/75 (n, %)	10 (53%)	8 (50%)	0.88
Antihypertensive Medications (n,%)			--
0		3 (15%)	
1	--	11 (55%)	
2	--	6 (30%)	
Antihypertensive Medication Classes (n,%)			--
ACE Inhibitor	--	2 (10%)	
Angiotensin II Receptor Blocker	--	8 (40%)	
Calcium Channel Blocker	--	5 (25%)	
Beta Blocker	--	3 (15%)	
Thiazide Diuretic	--	3 (15%)	
Unknown	--	2 (10%)	
Self-Reported Years Since HT Diagnosis	--	14.7±14.3	--

Abbreviations: BMI – body mass index, SBP – systolic blood pressure, DBP – diastolic blood pressure, MAP – mean arterial pressure, cSBP – central systolic blood pressure, ABPM – ambulatory blood pressure monitor, HT - Hypertension

Table 2:

Brachial Artery Stiffness Response to Nitroglycerin

	Control (n=14)		Hypertensive (n=20)		p-value		
	Baseline	NTG	Baseline	NTG	NTG	HT	NTG*HT
SBP (mmHg)	128±15	117±10	132±14	116±12	<0.001	0.82	0.47
DBP (mmHg)	74±11	71±6	79±9	76±7	0.012	0.071	0.86
MAP (mmHg)	92±10	86±6	97±10	89±10	<0.001	0.19	0.82
HR (BPM)	57±6	60±8	64±9	68±9	<0.001	0.008	0.73
D_{Dia} (mm)	3.68±0.74	4.33±0.66	3.64±0.69	4.16±0.72	<0.001	0.55	0.46
IMT (mm)	0.48±0.08	0.46±0.07	0.50±0.08	0.47±0.10	0.013	0.75	0.94
PEM (kPa)	139±58	141±48	144±56	133±49	0.69	0.93	0.56
bPWV (m/s)	11.2±2.4	11.4±2.1	11.5±2.2	11.0±2.2	0.72	0.93	0.47
YEM (kPa)	1075.9±630	1376±620	1116±561	1330±771	0.068	0.83	0.81
$PEM_{120/80}$ (kPa)	142±70	153±53	137±53	140±50	0.57	0.56	0.71
$bPWV_{120/80}$ (m/s)	11.3±2.7	11.9±2.2	11.2±2.0	11.3±2.2	0.48	0.62	0.55
$YEM_{120/80}$ (kPa)	1122±699	1517±676	1072±559	1381±805	<0.001	0.27	0.86

Abbreviations: NTG – nitroglycerin, HT – hypertension status, SBP – systolic blood pressure, DBP – diastolic blood pressure, MAP – mean arterial pressure, HR – heart rate, D_{dia} – diastolic diameter, IMT – intima-media thickness, PEM – Peterson's elastic modulus, bPWV – brachial pulse wave velocity, YEM – Young's elastic modulus, 120/80 subscript – adjusted to 120/80 blood pressure using participant specific model

Table 3:

Carotid Artery Stiffness Response to Nitroglycerin

	Control (n=20)		Hypertensive (n=20)		p-value		
	Baseline	**NTG**	**Baseline**	**NTG**	**NTG**	**HT**	**NTG*HT**
SBP (mmHg)	128±14	110±12	134±17	117±16	<0.001	0.11	0.81
DBP (mmHg)	75±9	71±6	78±11	76±7	<0.001	0.066	0.51
MAP (mmHg)	93±9	82±6	97±12	88±10	<0.001	0.090	0.65
HR (BPM)	58±8	60±8	64±9	68±9	<0.001	0.022	0.92
D_{Dia} (mm)	5.80±0.73	6.34±0.76	6.16±0.68	6.58±0.68	<0.001	0.19	**0.022**
IMT (mm)	0.73±0.12	0.70±0.14	0.83±0.16	0.78±0.15	0.002	0.051	0.341
PEM (kPa)	38.5±15.3	47.3±19.6	51.1±18.8	65.9±21.5	<0.001	0.007	0.18
cPWV (m/s)	6.0±1.1	6.6±1.3	6.8±1.3	7.8±1.3	<0.001	0.007	0.29
YEM (kPa)	312±139	439±182	378±127	565±180	<0.001	0.036	0.21
$PEM_{120/80}$ (kPa)	39.9±15.9	55.9±22.9	48.4±15.1	70.8±19.2	<0.001	0.030	0.24
$cPWV_{120/80}$ (m/s)	6.1±1.1	7.2±1.4	6.7±1.1	8.1±1.1	<0.001	0.022	0.37
$YEM_{120/80}$ (kPa)	328±144	533±196	365±110	617±172	<0.001	0.16	0.39

Abbreviations: NTG – nitroglycerin, HT – hypertension status, SBP – systolic blood pressure, DBP – diastolic blood pressure, MAP – mean arterial pressure, HR – heart rate, D_{dia} – diastolic diameter, IMT – intima-media thickness, PEM – Peterson's elastic modulus, cPWV – carotid pulse wave velocity, YEM – Young's elastic modulus, 120/80 subscript – adjusted to 120/80 blood pressure using participant specific model

Table 4:

Carotid-Femoral Region Stiffness Response to Nitroglycerin

	Control (n=20)		Hypertensive (n=20)		p-value		
	Baseline	NTG	Baseline	NTG	NTG	HT	NTG*HT
SBP (mmHg)	129±10	110±16	138±18	118±17	<0.001	0.067	0.84
DBP (mmHg)	76±9	69±7	81±8	73±10	<0.001	0.065	0.48
MAP (mmHg)	94±8	84±9	100±11	88±12	<0.001	0.081	0.54
cfPWV (m/s)	7.6±1.7	8.7±2.0	9.6±1.8	10.2±2.6	0.02	0.003	0.51
cfPWV$_{80}$ (m/s)	7.9±1.7	9.9±1.8	9.5±1.8	11.4±2.4	<0.001	0.005	0.74
cfPWV$_{100}$ (m/s)	7.9±1.7	10.3±1.9	9.6±1.7	11.6±2.4	<0.001	0.007	0.68

Abbreviations: NTG – nitroglycerin, HT – hypertension status, SBP – systolic blood pressure, DBP – diastolic blood pressure, MAP – mean arterial pressure, cfPWV, 80 subscript – adjusted to 80mmHg diastolic blood pressure using participant specific model, 100 subscript – adjusted to 100mmHg mean arterial pressure using participant specific model