Impact of Age and Body Position on the Contribution of Nitric Oxide to Femoral Artery Shear Rate
Implications for Atherosclerosis


Abstract—Reduced shear stress and augmented oscillatory shear rate are associated with the proatherogenic phenotype observed with aging. To date, mechanisms contributing to the age-related alterations in shear rate in humans have only been examined in the conduit vessels of the arm. Therefore, this study sought to examine the contribution of nitric oxide (NO) bioavailability to age-related alterations in shear rate and the impact of common body positions (supine and seated) in the atherosclerotic-prone conduit artery of the leg. Inhibition of NO synthase (NOS) was accomplished by intrarterial infusion of \( \text{NO}^\text{G}-\text{monomethyl-L-arginine (L-NMMA)} \), and common femoral artery diameter and blood velocity were measured by Doppler ultrasound in healthy young (n=8, 24±1 years) and old (n=8, 75±3 years) men. Old subjects exhibited reduced mean shear rate in the supine (18±3 s\(^{-1}\)) and seated positions (17±3 s\(^{-1}\)) compared with young subjects (supine; 42±6 s\(^{-1}\); seated: 32±4 s\(^{-1}\)). This reduced mean shear in the old was driven by attenuated antegrade shear as there were no differences in retrograde shear. Inhibition of NOS reduced antegrade shear in the young such that age-related differences were abolished. In contrast, NOS-induced reductions in retrograde shear rate were similar between groups. The seated position reduced mean shear rate in the young to that normally observed in old. Overall, this study reveals that age-related reductions in mean shear rate, assessed in the atherosclerotic-prone vasculature of the leg, are largely explained by reductions in antegrade shear as a result of reduced NO bioavailability in the elderly. (Hypertension. 2014;63:1019-1025.) ● Online Data Supplement

Key Words: aging ■ atherosclerosis ■ nitric oxide ■ regional blood flow

Peripheral conduit arteries exhibit an oscillatory pattern of blood flow, consisting of large forward (antegrade) flow during systole followed by smaller back flow (retrograde) and subsequent forward flow during diastole.\(^1,2\) Both the magnitude and direction of blood flow and subsequent shear stress alter endothelial cell phenotype and function, influencing the development of atherosclerosis.\(^3,4\) In cell culture and isolated blood vessels, elevated mean shear rate elicits an antiatherogenic phenotype, whereas a proatherogenic phenotype is characterized by augmented retrograde shear and low mean shear stress.\(^5,14\) In humans, enhanced antegrade shear stress improved endothelial function,\(^15\) whereas attenuation of antegrade shear induced endothelial dysfunction.\(^16\) Conversely, elevations in retrograde shear stress evoked endothelial cell apoptosis\(^17\) and endothelial dysfunction.\(^18\) This array of findings highlight the profound and highly specific effects that disturbances in shear stress can have on endothelial cell phenotype, endothelial function, and the propensity for atherosclerosis.

Aging augments the development and progression of atherosclerosis and is associated with reduced nitric oxide (NO) bioavailability, impaired endothelial function,\(^19,22\) and altered shear rate.\(^23-25\) Interestingly, despite systemic reductions in NO bioavailability and endothelial function with aging, the distribution of atherosclerosis varies within the vasculature such that the conduit arteries of the legs display a greater predisposition for atherosclerotic lesions than such vessels in the arms.\(^26-28\) The underlying mechanism(s) responsible for this limb difference in the propensity for atherosclerosis is not entirely clear, but may be a consequence of low mean shear rate coupled with elevated blood pressure in the legs during periods of upright posture. The impact of these differences may not be trivial as mean shear rate in the legs is 2- to 3-fold lower than the arms.\(^23-25,29,30\) and the average American spends nearly two thirds of the day in an upright posture.\(^31,32\)

Recent mechanistic investigations in the arm, focusing primarily on retrograde shear rate as the culprit of age-related increases in atherosclerosis and vascular dysfunction, revealed important roles of NO bioavailability\(^24\) and \( \alpha \)-adrenergic vasocostriction.\(^23\) Importantly, the occurrence and severity of atherosclerosis is greater in the legs than the arms, thus limiting...
the ability to generalize these previous findings. Beyond a single descriptive report of reduced mean shear rate in the femoral artery of healthy older men, a mechanistic examination of the factors contributing to the age-associated alterations in shear rate in this atherosclerotic-prone conduit artery of the leg is lacking.

Given the established associations between endothelial dysfunction, shear rate, and atherosclerosis, this study sought to determine whether reduced NO bioavailability accounts for the age-related proatherogenic shear rate pattern in the leg. We directly tested the following hypotheses: (1) inhibition of NO synthase (NOS) would abolish differences in shear rate between young and old, revealing a critical role of NO in the recognized age-related differences in shear rate, and (2) NOS inhibition would not alter shear rate patterns in the old subjects because of preexisting age-associated reductions in NO bioavailability. Additionally, to evaluate the effect of posture on shear rate patterns and to provide a thorough understanding of the potential role of shear stress and NO in the preferential development of atherosclerosis in the leg, we studied the impact of 2 common body positions (supine and seated) on femoral artery shear rate in these healthy young and old subjects.

Methods
Eight healthy young (24±1 years) and 8 healthy older men (75±3 years) volunteered to participate in this research study. Subjects were not taking any prescription medications and were free of overt cardiovascular disease. Protocol approval and written informed consent were obtained according to the University of Utah and Salt Lake City Veterans Affairs Medical Center (VAMC) Institutional Review Board, in accordance with the principles outlined in the Declaration of Helsinki. All data collection took place at the Salt Lake City VAMC’s Geriatric Research, Education, and Clinical Center in the Utah Vascular Research Laboratory.

Experimental Protocol
Subjects reported to the laboratory between 0700 and 0800 hours after an overnight fast. On arrival at the laboratory, body mass and height were recorded, and the right femoral artery was catheterized (18-gauge central venous catheter, Arrow International, Reading, PA) using the Seldinger technique. After a 30-minute rest period, subjects were positioned in either the seated or supine position. Subjects then rested in either the seated or supine position for an additional 20 minutes, while instrumentation was completed to ensure stable blood flow and central hemodynamics. Body position was counterbalanced. Because of lasting effects of L-NMMA, the control (saline) trials were always performed before L-NMMA infusion.

L-NMMA Infusion
Thigh volume was determined anthropometrically and then used for the calculation of drug dosing. L-NMMA (Bachem, Switzerland) was diluted from 250 mg lyophilized powder in normal saline to a concentration of 5 mg/mL. L-NMMA was infused at a priming dose of 0.48 mg/dL thigh volume for 5 minutes before measurements. After the loading, dose L-NMMA was infused at a maintenance dose of 0.24 mg/dL thigh volume, which was then maintained for the duration of the study. All measurements were obtained 2 minutes after starting the maintenance dose and were collected during 60 s. During control trials, normal saline was infused intra-arterially at the same rate and duration, as described for L-NMMA.

Measurements
Femoral artery blood velocity, vessel diameter, and intima-media thickening were measured by Doppler ultrasound (Logic 7, General Electric Medical Systems, Milwaukee, WI). Please refer to the online-only Data Supplement for complete description of the measurements.

Data and Statistical Analysis
Ultrasound images and Doppler velocity spectra were recorded continuously during infusions. During each 60-s ultrasound Doppler segment, $V_{mean}$ was averaged across 5 intervals of 12 s each, which were matched with intima-to-intima femoral artery diameter measurements evaluated during diastole. Statistics were performed with the use of commercially available software (SigmaPlot 11.0, Systat Software, Point Richmond, CA). A 2-way repeated-measures analysis of variance (ANOVA) was used to identify significant changes in measured variables within and between conditions and groups. After a significant main effect or interaction, pairwise comparisons were made using Fischer least significant difference. Significance was set at an α level of 0.05, and data are presented as means±SE.

Results

Subject Characteristics, Intima-Media Thickening, and Mean Arterial Pressure
Young and old subjects were well matched for height, weight, body mass index, thigh volume, femoral artery diameter, and blood characteristics (Table 1). Absolute intima-media thickening (IMT) and IMT normalized for femoral artery diameter were both greater in the old than in the young (P<0.01; Table 1). Resting mean arterial pressure (MAP) was not different between young and old in either the supine or seated position (Table 2). MAP was increased in the seated position compared with the supine position in both groups, reflecting an increase in hydrostatic pressure. NOS inhibition evoked a small, but significant elevation in MAP in all conditions (Table 2) except in the young in the seated position (P=0.12).

Leg Blood Flow and Leg Vascular Conductance
Resting leg blood flow (LBF) and leg vascular conductance were, on average, ~20% to 25% lower in the old compared with the young, independent of body position; however, none

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Subject Stature, Intima-Media Thickening, and Blood Characteristics</th>
</tr>
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<tbody>
<tr>
<td>Variables</td>
<td>Young</td>
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<tr>
<td>Age, y</td>
<td>24±1</td>
</tr>
<tr>
<td>Height, cm</td>
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<tr>
<td>Weight, kg</td>
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<td>Body mass index, kg/m²</td>
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<td>Thigh volume, dl</td>
<td>72±4</td>
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<tr>
<td>Femoral artery diameter, cm</td>
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<tr>
<td>Intima-media thickening, cm</td>
<td>0.052±0.002</td>
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<tr>
<td>Intima-media thickening normalized for femoral artery diameter</td>
<td>0.058±0.003</td>
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<tr>
<td>Glucose, mg/dL</td>
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<tr>
<td>Cholesterol, mg/dL</td>
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<td>Triglycerides, mg/dL</td>
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<tr>
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</tr>
<tr>
<td>Low-density lipoprotein, mg/dL</td>
<td>101±13</td>
</tr>
</tbody>
</table>

Values are means±SEM.
*P<0.05, significant difference between young and old.
of these differences reached statistical significance (Figure 1 and Table 2). Although LBF was not significantly lower in the old, this group demonstrated augmented retrograde LBF while seated ($P=0.02$) and tended to exhibit elevated retrograde LBF while supine ($P=0.06$; Table 2). NOS inhibition reduced LBF in both groups (Figure 1), and the magnitude of this reduction was greater in the supine compared with the seated position (Young, supine: $−162±21$, seated, $−94±10$ mL·min$^{-1}$, $ΔP=0.06$; Old, supine: $−146±29$, seated, $−68±13$ mL·min$^{-1}$, $ΔP=0.05$).

Table 2. Hemodynamics in the Supine and Seated Positions Under Control and L-NMMA Conditions in Young and Old Subjects

| Variables | Supine | | \hline | | Young | Old | Control | L-NMMA | Control | L-NMMA \hline Antegrade BF (ml/min) | 436±28 | 341±39* | 398±36 | 355±25 | 336±36† | 274±27* | 320±18† | 322±23* | \hline Retrograde BF (ml/min) | 93±7 | 159±17* | 131±18 | 234±34* | 54±6† | 87±11* | 103±17† | 173±27* | \hline MAP (mmHg) | 90±3 | 97±3* | 93±2 | 100±2* | 114±2† | 118±4† | 111±2† | 115±3† | \hline LVC (ml/min per mmHg) | 3.8±0.3 | 1.9±0.2* | 2.9±0.6 | 1.2±0.4* | 2.5±0.3† | 1.5±0.2* | 2.0±0.2 | 1.3±0.2* | \hline

Values are means±SEM. BF indicates blood flow; L-NMMA, NG-monomethyl-L-arginine; LVC, leg vascular conductance; and MAP, mean arterial pressure.

$^*P<0.05$, significant difference from control.

$^†P<0.05$, significant difference from control.

$^‡P<0.05$, significant difference from young.

Shear Rate: Impact of Aging, NOS Inhibition, and Body Position

**Mean Shear Rate**

As illustrated in Figure 2A, old subjects exhibited a lower mean shear rate compared with the young subjects, and this was evident in both the supine and seated positions. The magnitude of reduction in mean shear rate because of NOS inhibition was not different between young and old subjects (Table 3). The seated position resulted in a lower mean shear rate in the young but not in the old subjects. The NOS-induced reduction in mean shear rate in the young was less when seated than when supine (Table 3).

**Antegrade Shear Rate**

As illustrated in Figure 2B, antegrade shear rate was lower in the old subjects in the supine but not in the seated position when compared with the young. NOS inhibition attenuated antegrade shear in the young in both positions. In contrast, NOS inhibition had no impact on antegrade shear in the old subjects (Figure 2B and Table 3). The seated position resulted in a lower antegrade shear in the young but had no effect in the old.

**Retrograde Shear Rate**

As depicted in Figure 2C, there were no age-related differences in retrograde shear rate. Moreover, the magnitude of the NOS-induced increase in retrograde shear rate was similar between the young and old subjects (Table 3). The seated position resulted in a reduction in retrograde shear rate in the young but not old subjects.

**Oscillatory Shear Index**

As illustrated in Figure 2D, the old subjects exhibited elevated oscillatory shear in the seated position compared with the young subjects. In the supine position, oscillatory shear tended to be elevated in the old subjects ($P=0.12$). NOS inhibition elicited similar increases in oscillatory shear in the young and old subjects (Table 3). The impact of position was unremarkable with respect to oscillatory shear.

Discussion

Alterations in shear stress, aging, and atherosclerosis are linked such that reductions in mean shear stress increase the propensity for atherogenesis with age. However, the mechanisms involved in age-associated alterations in shear rate and the relative importance of antegrade compared with retrograde shear rate in the atherogenic process are unclear. Thus, this study sought to determine whether aging alters the contribution of NO to leg shear rate patterns, as assessed in the common femoral artery, in the often assumed supine and seated positions. To our knowledge, this is the first mechanistic evaluation of age-related differences in shear rate patterns in the atherosclerotic-prone vasculature of the leg. The primary novel findings of this study are that age-related alterations in shear rate in the leg are characterized by reduced mean shear rate, driven primarily by diminished antegrade shear. The inhibition of NOS elicited reductions in antegrade shear rate such that age-associated differences were eliminated. In contrast, retrograde shear rate was not different between groups, and the magnitude of the increase in retrograde shear attributable to NOS inhibition was similar between the young and the old revealing an important, albeit age-independent, contribution of NO to retrograde shear rate. Finally, changing body position from supine to seated reduced mean shear rate in young but not in the old subjects. Collectively, these data reveal that posture is an important modulator of shear rate in the young, and that the age-related reduction in NO bioavailability contributes to...
the attenuation of mean shear rate by lowering the antegrade shear rate in the atherosclerotic-prone vasculature of the leg.

**Contribution of NO to Shear Rate: Limb-Specific Differences**

Mean shear rate in the legs is 2- to 3-fold lower than in the arms as a result of a greater vessel diameter, reduction in antegrade shear rate, and an elevation in retrograde shear rate.23,24,29,30 This diminished mean shear rate in the vasculature of the legs has been proposed to reflect the heightened propensity for atherosclerosis in the legs compared with the arms.30 Despite these limb-specific differences and the greater contribution of antegrade shear rate to overall mean shear rate, the age-related increase in atherosclerosis in the leg has been largely attributed to the deleterious and proatherogenic impact of augmented retrograde shear rate.24,25,37 In the current study, mean shear rate was reduced with age (Figure 2A) due to a reduction in antegrade shear rate (Figure 2C) and not an elevation in retrograde shear rate as previously reported.25 This discrepancy between the current study and Young et al may be explained by several factors including the substantially smaller femoral artery diameter of the older subjects in the previous study (0.86±0.03 cm) likely due to their inclusion of women and the substantial age differences between the older groups in these 2 studies (15 years). In the current study, the reduction in antegrade shear seems to be largely explained by reduced NO bioavailability as NOS inhibition abolished the age-related differences in antegrade shear rate. Despite this age-dependent role of NO in the regulation of antegrade shear rate, the reduction in mean shear due to NOS inhibition was similar between groups, also highlighting an important NO-independent component of mean shear rate. The underlying reason for differing regulatory pathways for mean and antegrade shear rate is not entirely clear but identifies an important role of NO in the regulation of shear rate patterns in both young and old.

**In vivo animal data support the role of reduced mean shear rate due to diminished antegrade shear rate, in the atherogenic process, as larger and more vulnerable atherosclerotic lesions were evident in regions exposed to reduced mean shear rate compared with regions of elevated oscillatory shear rate.38 Thus, important limb differences in shear rate must be considered when examining mechanisms contributing to the development and progression of atherosclerosis, and one must question the generalizability of previous findings performed in the vasculature of the arm. Indeed, a potentially critical role of reduced antegrade shear rate in the leg must be considered when examining the heightened propensity for atherosclerosis in the lower limbs with age. Additionally, vessel diameter, a key variable in the calculation of shear rate, must also be considered when comparing across limbs or when vessel diameter is dissimilar between groups.

<table>
<thead>
<tr>
<th>Variables, Δ s⁻¹</th>
<th>Supine</th>
<th>Seated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean shear rate</td>
<td>-20.2±3.7</td>
<td>-11.7±3.5</td>
</tr>
<tr>
<td>Antegrade shear rate</td>
<td>-12.5±3.0</td>
<td>-1.3±1.8†</td>
</tr>
<tr>
<td>Retrograde shear rate</td>
<td>7.7±1.5</td>
<td>10.4±4.2</td>
</tr>
<tr>
<td>Oscillatory shear rate</td>
<td>0.14±0.01</td>
<td>0.13±0.02</td>
</tr>
</tbody>
</table>

Values are means±SEM. NOS indicates nitric oxide synthase. *P<0.05, significant difference from supine. †P<0.05, significant difference from young.
changes in mean, retrograde, and oscillatory shear in the old, indicating that the contribution of NO to these shear rates is independent of age in the leg. The NOS-induced changes in shear rate are not explained by elevated MAP during NOS inhibition as the relatively small increases in MAP were similar between groups and within postures (Table 2). Thus, it seems that NO accounts for the age-related differences in antegrade shear and is additionally an important regulator of retrograde shear rate and overall shear rate patterns in the leg of both the young and old.

**Impact of Posture and the Role of NO in Determining Shear Rate in the Leg**

The average American spends nearly two thirds of the day in an upright posture, exposing the vasculature of the legs to elevated blood pressure, which may contribute to the increased propensity for atherosclerosis in the leg. Additionally, a recent report indicates that adults, on average, spend >8 hours per day during waking hours engaged in sedentary behavior, primarily prolonged sitting. Therefore, the examination of alterations in shear rate patterns in the upright seated compared with supine position may provide clinically important information as prolonged sitting is an established risk factor for all-cause mortality. The deleterious effects of sitting have been fundamentally linked to metabolic and, to a lesser extent, vascular dysfunction; however, distinguishing between the effects of physical inactivity from prolonged sitting is inherently difficult as the 2 occur concomitantly. Thus, assessment of shear rates in 2 common postures, seated and supine, is important to fully elucidate the potential role of shear stress in the preferential development of atherosclerosis in the vasculature of the leg.

Based on the findings of this study and work by Newcomer et al in young subjects, posture, independent of physical activity, seems to alter shear rate in healthy young but not old subjects (Figure 2). Specifically, antegrade and mean shear rate were reduced in the young subjects in the seated compared with that in the supine position. Additionally, the seated position reduced the contribution of NO to mean shear rate in the young such that the change in mean shear rate due to NOS inhibition was lower in the seated compared with the supine position. The current findings of a proatherogenic shear rate pattern in the seated compared with supine position may provide an explanation for the improvements in vascular function despite nonuniform alterations in retrograde and oscillatory shear rate. In vivo evidence implicates augmented retrograde and oscillatory shear in impaired vascular function. Thijssen et al demonstrated a dose-dependent reduction in vascular function in response to graded elevations in retrograde shear rate. Conversely, acute increases in antegrade shear improved vascular function despite nonuniform alterations in retrograde shear. Consequently, these authors suggested that the magnitude of antegrade shear is the primary contributor to alterations in flow-mediated dilation and therefore vascular health in humans.

**Contribution of Sympathetic Nervous Activity and α-Adrenergic Vasoconstriction in Determining Shear Rate**

This study reveals that NO clearly contributes to shear rate patterns in the leg of both young and old subjects (Figure 2); however, other mechanisms including heightened sympathetic nervous activity (SNA) and α-adrenergic vasoconstriction may also play a role. Indeed, acute elevations in SNA, evoked by lower body negative pressure, elicited increases in retrograde and oscillatory shear in the arm of the young such that differences between young and old subjects were eliminated. Likewise, in the arm of young subjects, stimulation of α-adrenergic receptors increased retrograde and oscillatory shear while blockade of these receptors, by phentolamine, abolished retrograde and oscillatory shear. In contrast, α-adrenergic blockade reduced the accentuated retrograde and oscillatory shear in the old but did not eliminate these shear pattern characteristics, suggesting multiple factors are involved in the regulation of shear rate in the arm of the old. Altered SNA and α-adrenergic vasoconstriction may also contribute to the differences in leg shear rate observed in the current study; however, direct evidence supporting these mechanisms is lacking. Given the apparent limb-specific differences in the contribution of NO to shear and the heightened propensity of atherosclerosis in the leg with aging, future investigations of non-NO–dependent mechanisms regulating shear rate patterns are warranted.

**Shear Stress, Vascular Function, and Atherogenesis**

Compelling in vitro and in vivo evidence underscores the detrimental impact of altered shear stress on vascular function and the development of atherosclerosis. However, the pattern and direction of shear stress (ie, reduced antegrade, elevated retrograde) associated with a proatherogenic hemodynamic environment is equivocal, likely dependent on the duration of exposure (acute versus chronic) and experimental model used (in vitro versus in vivo). Studies using endothelial cell culture and isolated arteries indicate that exposure to increased retrograde and oscillatory shear, as well as reduced mean shear, contribute to vascular dysfunction at the molecular, cellular, and functional levels. In vivo evidence implicates augmented retrograde and oscillatory shear in impaired vascular function.

Thijssen et al demonstrated a dose-dependent reduction in vascular function in response to graded elevations in retrograde shear rate. Conversely, acute increases in antegrade shear improved vascular function despite nonuniform alterations in retrograde shear. Consequently, these authors suggested that the magnitude of antegrade shear is the primary contributor to alterations in flow-mediated dilation and therefore vascular health in humans.

Based on these previous in vivo and in vitro experiments, it seems that the detrimental impact of altered shear stress at both the cellular and functional level involves a balance between reduced antegrade and elevated retrograde shear stress. The relative importance of alterations in antegrade and retrograde shear rate and the translation of such changes to the heightened propensity for atherosclerosis in the leg of older individuals are not well understood. With aging, retrograde shear rate seems to be increased by 2 to 3 s−1 in...
the leg, whereas the reduction in antegrade shear rate is several fold higher, up to 20 s$^{-1}$. Clearly the magnitude of change in antegrade shear rate is far greater; however, the impact of large changes in antegrade versus small changes in retrograde shear rate in the atherogenic process is not clear. Based on the current findings, the role of reduced antegrade shear is expected to contribute to the atherogenic process as there were no significant age-related differences in retrograde shear rate. Additionally, under normal physiological conditions (ie, without the acute modulation of shear stress), it could be argued that the reduction in antegrade shear rate leading to the attenuation of mean shear rate in conduit arteries may be largely responsible for the increased propensity for atherosclerosis observed in the lower limbs and with age.26–28 Indeed, in the current study, differences between young and old subjects with respect to antegrade and mean shear rate were 2 to 3 times greater than the differences in retrograde shear, suggesting that the age-related decrease in mean shear seems to be primarily driven by reduced antegrade shear rate (Figure 2).

Perspectives

This study reveals that the attenuated mean shear rate with age in the atherosclerotic-prone vasculature of the leg is driven primarily by reduced antegrade shear rate. Reduced NO bioavailability, as evidenced by NOS inhibition, seems to account for this age-related reduction in antegrade shear rate. Interestingly, although not accounting for age-related differences in retrograde shear, NO also plays an important role in modulating mean and retrograde shear rate across the lifespan. Posture differentially alters shear rate in an age-dependent manner such that in the seated position the young exhibit reductions in shear rate that reflect the shear rate pattern observed in the old. Thus, the seated position may be detrimental for vascular health and promote the development of atherosclerosis.

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Disclosures

None.

References


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Impact of Age and Body Position on the Contribution of Nitric Oxide to Femoral Artery Shear Rate: Implications for Atherosclerosis

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Running Title: Aging, NO, and femoral artery shear rate
**Measurements**

Femoral artery measures were obtained distal to the inguinal ligament and proximal to the deep and superficial femoral bifurcation. The ultrasound system was equipped with a linear transducer operating at an imaging frequency of 10 MHz. Vessel lumen diameter and intima-media thickening (IMT) were determined at a perpendicular angle along the central axis of the scanned area. IMT was measured in duplicate, on the far wall, from the interface between blood and intima and the interface between media and adventitia, and then averaged. ECG R-wave-gated images were collected via video output from the Logic 7 for off-line analysis of IMT using automated edge-detection software (Medical Imaging Applications, Coralville, IA). IMT normalized for vessel lumen diameter was also calculated.

Blood velocity was measured using the same transducer with a frequency of 5 MHz. All blood velocity measurements were obtained with the probe appropriately positioned to maintain an insonation angle of 60° or less. The sample volume was maximized according to vessel size and was centered within the vessel. Arterial diameter was measured, and mean velocity (Vmean) (angle corrected, and intensity-weighted area under the curve) was automatically calculated (Logic 7). Using arterial diameter and Vmean, leg blood flow (LBF) in the femoral artery was calculated as 

\[ \text{LBF} = \text{Vmean} \times \pi \times \left( \frac{\text{diameter}}{2} \right)^2 \times 60 \] 

where blood flow is in milliliters per minute.

Shear rate and oscillatory shear index were calculated according to the following equations; Shear rate = (4 × Vmean/ Arterial Diameter) and Oscillatory shear index = |Retrograde shear|/(|Antegrade shear| + |Retrograde shear|)^2. It should be noted that for the purpose of comparison with previous investigations, Vmean was multiplied by a factor of 4 and not 8, as is commonly used for the calculation of shear rate when sample volume is maximized according to vessel size. Mean arterial blood pressure (MAP) was collected continuously by an indwelling catheter (MAP) connected to a pressure transducer (Transpac IV, Abbot laboratories). The pressure transducer was placed at the level of the catheter to reflect MAP as measured directly in the femoral artery. Leg vascular conductance (LVC) was calculated as LBF/MAP.
