Age and flow-mediated dilation: a comparison of dilatory responsiveness in the brachial and popliteal arteries

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Parker, Beth A., Samuel J. Ridout, and David N. Proctor. Age and flow-mediated dilation: a comparison of dilatory responsiveness in the brachial and popliteal arteries. Am J Physiol Heart Circ Physiol 291: H3043–H3049, 2006. First published July 21, 2006; doi:10.1152/ajpheart.00190.2006.—Previous investigations of age-associated changes in flow-mediated vasodilation (FMD) in women have been limited to the upper extremity and have not accounted for possible age differences in the stimulus for dilation. The purpose of the present study was to compare age differences in brachial and popliteal FMD and its stimulus (changes in shear rate following occlusion). Ultrasonic-derived diameters and Doppler flow velocities of the brachial and popliteal arteries were measured in 14 young (20- to 30-yr-old) and 14 older (60- to 79-yr-old) healthy women at rest and during and after 5 min of distal cuff occlusion. Resting diameters were similar (both P > 0.39) in both age groups. Peak shear rate did not differ with age in either artery: ~1,300–1,400 and ~400–500 s⁻¹ in brachial and popliteal arteries, respectively. FMD (percent change above diameter measured during occlusion) was ~50–60% lower (P < 0.05) in the brachial (15.8 ± 0.8% vs. 8.1 ± 1.5%) and popliteal (4.6 ± 0.7% vs. 1.8 ± 0.4%) arteries of the older women. The normalized response of the brachial and popliteal arteries (%FMD per unit change in shear rate) was also reduced with age (55% and 53%, respectively) but did not exhibit limb specificity. Additionally, endothelium-independent dilatation, as assessed by administration of nitroglycerin, was similarly blunted (by 45–65%) in brachial and popliteal arteries of older women. These results suggest that 1) brachial and popliteal artery FMD (after 5 min of distal occlusion) are similarly reduced with age, and 2) when normalized to the change in shear stimulus, both arteries are equally responsive to 5 min of distal cuff occlusion in women, and 3) the age-associated decline in FMD may be attributable in part to diminished smooth muscle responsiveness. 

Vascular responsiveness; endothelial function; limb-specific dilation

THE VASODILATOR RESPONSE of conduit vessels to an increase in fluid shear stress, termed flow-mediated dilation (FMD), declines with normal aging in the brachial artery (7, 10, 19). However, the influence of age on lower extremity conduit artery FMD has not been well characterized. Lower extremity arteries are particularly susceptible to atherosclerosis, and peripheral vascular disease of the lower extremity is highly correlated with the prevalence of coronary disease (6, 15). To this end, it has been shown that popliteal artery FMD is reduced or abolished in subjects with hyperlipidemia and coronary disease compared with normal controls (2, 39). Moreover, Sanada et al. (36) found impaired leg (but not forearm) blood flow responses to reactive hyperemia in 57 male and female patients with peripheral artery disease, suggesting that diminished vascular reactivity of the leg may be a better indicator of peripheral artery disease than blunted forearm vascular reactivity. Unfortunately, the extent to which these clinical findings reflect normal aging vs. the changes associated with the progression of vascular disease is not known.

Wray et al. (42) recently published findings in men suggesting that 1) the age-associated attenuation in brachial FMD is abolished when the dilatory response is normalized to the shear stimulus and 2) femoral FMD is augmented with age. However, inasmuch as prior research has demonstrated significant sex differences in brachial FMD responses in young (23) and older (7) humans, the extent to which the findings of Wray et al. can be extended to women, for whom aging is accompanied by a rapid reduction in circulating sex hormones, is unclear. Thus the primary aim of the present study was to determine whether the age-related reduction in brachial artery FMD is also observed in the popliteal artery in healthy women, when dilation is normalized to the shear stress to account for baseline differences in conduit diameter (26, 43). Additionally, we sought to compare vasodilator responsiveness to an increase in fluid shear stress in the arm vs. leg of young and older subjects, because previous work has also been conducted solely in men (27, 28, 42, 43). On the basis of the known relation between age and brachial artery FMD, as well as existing literature that has shown a reduction of popliteal artery FMD in clinical populations of patients with coronary disease (2), we hypothesized that popliteal artery FMD would be reduced in older subjects compared with young controls. Furthermore, given our previous findings concerning brachial and femoral artery hyperemic responses to acetylcholine (28), we hypothesized that the vascular dilator responsiveness of the popliteal artery would be blunted compared with that of the brachial artery in young and older women.

METHODS

Subjects. The study was completed by 14 young (20- to 30-yr-old) and 14 older (63- to 79-yr-old) women. All subjects were nonobese [body mass index (BMI) ≤30], were nonsmokers, and had clinically normal blood chemistry (i.e., hemoglobin 11.6–14.8 g/dl, total cholesterol ≤240 mg/dl, and LDL cholesterol ≤150 mg/dl) and resting supine ankle-brachial index ratings (0.90–1.30). All subjects were normotensive (resting blood pressure ≤140/90 mmHg) and were neither extremely sedentary nor extremely fit [i.e., cycle ergometer peak O₂ uptake (VO₂ peak) 20–80% of age-predicted norms (1)]. Subjects were free of overt chronic diseases, as evaluated by medical history questionnaire, a physical examination, and resting ECG. Additionally, no subjects were taking medications with significant hemodynamic effects, including oral contraceptives (young) or hor-

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Values are means ± SE (n = 14). Peak O₂ uptake (V\textsubscript{O₂ peak}) data were collected in only 6 of the 14 young subjects because of the necessity of completing testing before graduation. BMI, body mass index. *Significantly (P < 0.05) different from young.
Table 2.  Brachial and popliteal diameters and shear rates

<table>
<thead>
<tr>
<th>Diameter, mm</th>
<th>Shear Rate, s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Young</td>
</tr>
<tr>
<td>Brachial Rest</td>
<td>2.96±0.14</td>
</tr>
<tr>
<td>Occlusion</td>
<td>2.91±0.13</td>
</tr>
<tr>
<td>Peak</td>
<td>3.37±0.14*</td>
</tr>
<tr>
<td>Popliteal Rest</td>
<td>5.55±0.18</td>
</tr>
<tr>
<td>Occlusion</td>
<td>5.66±0.18</td>
</tr>
<tr>
<td>Peak</td>
<td>5.92±0.18*</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 14). Peak shear rates were obtained, on average, in the first 10–15 s after occlusion, although velocity was monitored for 30 s after cuff release. Peak diameters were obtained, on average, 60–75 s after occlusion, although diameter was monitored for 3 min after cuff release. *Significantly (P < 0.05) different from rest and occlusion. †Significantly (P < 0.05) different from rest. There were no significant age group differences for any of the conditions.

demonstrated that diameter does not change appreciably in the first 10–12 s of cuff release (when peak blood velocity and shear rate are observed) and the Aspen ultrasound machine is not able to measure high-resolution diameters and velocity simultaneously. Finally, FMD was normalized to the absolute change in shear rate from resting to peak conditions or from occlusion to peak conditions.

Statistical analysis. Statistical analyses were performed using Minitab software (State College, PA). Values are means ± SE. Significance was set at P < 0.05. One-way ANOVA and Tukey’s post hoc analysis were used to compare differences between young and older groups. A paired two-sample t-test was used to determine differences between resting, occlusion, and peak diameters in each subject group, as well as differences between FMD in the popliteal and brachial artery of each subject, and an independent two-sample t-test was used to compare FMD responses between young and older subjects. Finally, the variation incorporated into the relation between normalized brachial or popliteal artery FMD and age by independent variables (i.e., baseline characteristics; Table 1) was investigated by analysis of covariance (ANCOVA). In addition, the influence of these variables was analyzed by comparison of subsets of FMD of young and older subjects matched for each of these variables.

RESULTS

Subject characteristics. Blood pressure (systolic and diastolic), total and LDL cholesterol, BMI, and body fat were significantly higher and \( \dot{V}O_2 \text{peak} \) was lower in older than in young subjects (Table 1).

Baseline, occlusion, and peak diameters and shear rate. There were no age group differences in resting, occlusion, or peak shear rates or diameters (Table 2), although peak shear rate in the popliteal artery after occlusion was marginally lower (P = 0.11) in older subjects. A slight, but statistically significant (P < 0.01), dilation was observed during calf occlusion in older subjects that was present, but not statistically significant, in younger subjects (P = 0.095) and was not observed in the brachial artery of young or older subjects.

Age-associated differences in percent dilation. To investigate the effect of the slight dilation during calf occlusion in the older subjects, we calculated \%D\text{rest} or \%D\text{occlu}. After 5 min of forearm occlusion, \%D\text{rest} was reduced (P = 0.03) in older vs. young women: 14.6 ± 1.9% vs. 8.9 ± 1.5%. After 5 min of calf occlusion, \%D\text{rest} was also reduced (P = 0.04) in older vs. young women: 6.7 ± 0.9% vs. 4.0 ± 0.8%. After forearm occlusion, \%D\text{occlu} was reduced (P < 0.01) in older women: 8.1 ± 1.5% vs. 15.8 ± 0.8% for young women. After calf occlusion, \%D\text{occlu} was also reduced (P = 0.01) in older women: 1.8 ± 0.4% vs. 4.6 ± 0.7% for young women. There was a significant difference (P < 0.01) between brachial and popliteal artery dilation (relative to rest or dilation) in young and older individuals.

Age-associated differences in brachial and popliteal artery normalized FMD. There was a significant difference between normalized FMD (percent change in diameter versus unit change in shear rate) from occlusion to peak conditions for young and older subjects in brachial (0.013 ± 0.002 and 0.006 ± 0.001 for young and older, respectively, P < 0.01) and popliteal (0.011 ± 0.002 and 0.005 ± 0.001 for young and older, respectively, P = 0.01) arteries (Fig. 1). However, in young (P = 0.21) and older (P = 0.76) subjects, there was no significant difference between the normalized FMD responses in brachial and popliteal arteries.

Endothelium-independent dilation. After NTG administration, the dilatory response in brachial (32.1 ± 1.7 and 17.8 ± 2.5 for young and older, respectively, P < 0.01) and popliteal (8.6 ± 0.9 and 3.0 ± 0.7 for young and older, respectively, P < 0.01) arteries in older women was reduced by 45% and 65%, respectively (Figs. 2 and 3). Normalization of the FMD response to the NTG response abolished age group differences in brachial (0.49 ± 0.14 and 0.45 ± 0.18 for young and older, respectively, P = 0.86) and popliteal (0.53 ± 0.16 and 0.60 ± 0.17 for young and older, respectively, P = 0.79) arteries (Fig. 4).

Influence of independent variables. Because of previous work demonstrating the inverse relation between individual characteristics such as blood pressure (44), cholesterol (total and LDL) (8), and BMI (3) on brachial artery FMD, we sought to determine whether our observations of age-related changes in FMD were mediated by independent variables. Investigation of the relation between normalized brachial or popliteal FMD, age, and independent variables by ANCOVA yielded no significant results (P > 0.05) for the influence of total cholesterol, LDL cholesterol, percent body fat, BMI, systolic blood pressure, diastolic blood pressure, hematocrit, forearm muscle mass, or calf muscle mass. Matching a subset of six to eight...
young and older subjects for each of these variables did not change the magnitude or significance of the age-associated attenuation in FMD, except in the case of the blood pressure-matched subjects. In older subjects matched to younger subjects for systolic pressure, brachial and popliteal artery FMD were attenuated only 30% and 25%, respectively. Similarly, in subjects matched for diastolic pressure, brachial and popliteal artery FMD were reduced only 35% and 45%, respectively, with age.

**DISCUSSION**

The major finding of this research is that FMD is diminished to a similar extent (50–60%) in brachial and popliteal arteries of healthy older women. Additionally, the relation between the increase in shear rate due to 5 min of distal cuff occlusion and conduit artery dilation appears to be attenuated in older women but, contrary to our hypothesis, is not significantly different between the brachial and the popliteal artery in young or older women.

**Reductions in conduit artery FMD with age.** In the present study, when we measured brachial artery FMD as the conventional %D_{rest} (9), the dilatory response to 5 min of distal occlusion was reduced by ∼40% in the older women, which is comparable to that reported previously in the literature (7, 19, 35). Popliteal artery FMD, measured in the same manner, was reduced in older women by ∼30%, which is less than has been reported in the literature comparing patients with hyperlipidemia (39) and coronary disease (2) with healthy controls; however, these studies evoked FMD through 5 min of proximal occlusion.

During the course of the 5-min calf occlusion, we observed a small, but statistically significant, dilation in the popliteal artery in the older subjects. Popliteal artery diameter also increased, but not statistically significantly, in the younger women. Because the shear stimulus is directly related to the diameter measured immediately before cuff release (16, 20, 29, 37), we also expressed FMD as %D_{occlu}. %D_{occlu} tended to magnify the effect of age in popliteal artery FMD because of the greater dilatory response during occlusion in older subjects. Our only explanation for the differential dilation of the popliteal artery comes from the observation that myogenic dilation in response to sustained decreases in transmural pressure is augmented with age in the brachial artery (24). Nevertheless, we believe that it is worthwhile to present FMD as a function of occlusion diameter, because use of resting diameter would encompass a dilatory change during occlusion that was not caused by the peak shear stimulus. That is, inasmuch as FMD represents the dilatory response to the increase in fluid shear stress after occlusion, the expression of dilation should only represent changes in vessel diameter in direct relation to the shear stimulus, particularly when FMD is being used to detect small (0.1- to 0.3-mm) diameter changes in a large vessel with only 8% dilation. Thus, for our subsequent calculations (normalized FMD, limb comparisons, and ratio to endothelium-independent dilation), we used %D_{occlu} as the representation of FMD. However, use of %D_{rest} to represent FMD yielded the same, significant results.

**Conduit artery dilator response in older women is reduced, despite similar increases in shear rate.** Although there were no age differences in shear rate during rest, occlusion, or peak measurements (Table 2), the dilatory response in popliteal and brachial arteries of older women was reduced compared with that of younger women. Moreover, when the diameter changes in either artery were normalized to the shear stimulus, FMD remained attenuated compared with that of young women by 55% and 53% in the brachial and popliteal artery, respectively (Fig. 1). This analysis suggests that the age-associated reductions in conduit artery FMD are a function of differences in the dilatory response, rather than a diminished shear stimulus, in older limbs. These results differ from the recently published...
data of Wray et al. (43), who found that FMD was preserved (brachial) and augmented (femoral) with age in men; collectively, these findings suggest that the influence of age on FMD of upper and lower extremity arteries may be sex specific.

The relation between shear rate and diameter increases in the brachial and popliteal arteries does not appear to be limb specific. The dilatory response of the popliteal artery to 5 min of distal occlusion (when measured as percent dilation) was significantly less than that of the brachial artery. However, the lower percent dilation in the popliteal artery could reflect a reduced peak shear stimulus (i.e., \( \sim 400\text{--}500 \text{ s}^{-1} \)) compared with that measured after 5 min of brachial artery occlusion (\( \sim 1,300\text{--}1,400 \text{ s}^{-1} \); Table 2). Because the calf comprised a much larger lean tissue mass than the forearm in these women (dual-energy X-ray absorptiometry results from Table 1), the resistance arteriolar dilation induced by 5 min of occlusion may simply not have been as great in the calf as in the forearm. However, when the dilatory response to occlusion was examined with respect to the increased shear rate following cuff release (Fig. 1), normalized FMD did not differ between the arm and the leg in young or older individuals. This suggests that conduit artery responsiveness to an increase in shear stress evoked by 5 min of distal cuff occlusion is not limb specific.

**What underlies the attenuated arm and leg dilator response but preserved shear stimulus in older women?** After forearm and calf occlusion, the dilatory response of the upstream conduit artery was blunted in older women, despite increases in shear rate that were similar to those observed in young women. Interestingly, endothelium-independent dilation was also blunted similarly in older women (Fig. 2), such that accounting for the deficit in smooth muscle function (ratio of FMD to NTG dilation; Fig. 3) abolished age-related dilatory differences in brachial and popliteal arteries. Thus it is certainly possible that alterations in the smooth muscle response to endothelium-derived dilators evoked after 5 min of occlusion underlie blunted FMD. However, because the signaling mechanisms behind FMD have not been systematically investigated in women, the lower limb vasculature, or older humans, we cannot conclude whether the attenuation in FMD is attributable solely to a deficit in smooth muscle signaling/responsiveness or other age-related alterations, such as, diminished NO production and/or bioavailability, a reduction in synthesis or release of alternative and/or additional endothelium-derived dilators, elevation in sympathetic activity (12, 13, 17), or shear-induced release of endothelin (4, 45, 46).

**Why do our findings differ from the existing literature concerning limb vascular heterogeneity?** Previously, limb-specific differences in the dilator response to intra-arterial infusions of endothelium-dependent vasodilators have been documented, such that brachial artery blood flow is increased to a greater extent than femoral artery blood flow after an infusion of acetylcholine, an NO-dependent dilator (28). Recently, Wray et al. (43) found that dilation of the femoral artery (per unit increase in shear rate) during knee extensor exercise was less than dilation of the brachial artery during handgrip exercise. Our report of similar vascular responses between the popliteal and brachial arteries may be attributable to a sex difference (men vs. women), differences in vessel size (the femoral artery is almost twice as large as the popliteal artery and, as such, may be less responsive to increases in shear stress or NO-dependent agonists), and/or differences in the stimuli used to assess limb-specific responses (i.e., infusion of acetylcholine vs. exercise hyperemia vs. tissue ischemia).

**Does the peak shear rate accurately portray vascular responsiveness in conduit arteries?** It has recently been noted that the peak shear rate, a parameter commonly used to normalize FMD to the stimulus (11, 25, 34), may not comprise the true nature of the shear stimulus (32, 33). Rather, the full 1-min postocclusion shear rate (assessed as area under the curve) may more closely estimate the stimulus for FMD. We compared the effects of normalizing FMD to the 1-min area-under-the-curve shear rate vs. peak shear rate in eight young and older subjects and found no difference in our results and conclusions concerning limb specificity (Fig. 4) and age group differences when using either normalization technique.

**To what extent do our findings reflect primary aging?** As noted in results, assessment of the influence of independent variables on FMD by ANCOVA did not yield significant results for any variable, possibly because the relation between FMD and cardiovascular risk factors is apparent only in younger adults with few risk factors (41). However, we were able to match six to eight young subjects to six to eight older subjects for each characteristic to examine further whether reduced FMD was modulated by elevated baseline variables, such as blood pressure and cholesterol, in older subjects. In older adults matched to younger adults for systolic and diastolic blood pressure, we found that the reduction in arm and leg FMD was blunted but remained significant. This finding suggests that the influence of age on FMD may be mediated in part through structural alterations of the peripheral vasculature that partially underlie increased blood pressure, such as intimal thickening, increased collagen content, and decreased elastin (21), inasmuch as older subjects demonstrated significantly higher peripheral pulse wave velocity measurements (data not shown). Furthermore, because of the close relation between age, vascular stiffness, regulation of smooth muscle tone, and atherosclerosis, it is impossible to determine in this study whether vascular aging and/or atherosclerosis result in blunted FMD (22).

Finally, given that our study population was restricted to women, it is certainly possible that our results are indicative of a primary or additional effect of reduced sex hormones on FMD in postmenopausal women. A discussion of the manner in which age and sex hormones may independently and jointly affect endothelial and smooth muscle function is beyond the scope of this study. However, we believe that our results are not marginalized by the sex of our study population, inasmuch as the loss of sex hormones is an inherent aspect of the aging process in women.

**Experimental considerations.** A limitation of this study is that our Doppler ultrasound machine samples the peak, or center, blood velocity envelope, rather than an intensity-weighted mean blood velocity, which reflects the different blood velocities across the parabolic curve comprising fluid movement in an artery. In young subjects, the correlation between peak velocity, spatially averaged mean blood velocity, and shear rate is supported by research (38). However, it is not known whether, in an older subject with a stiffer vessel, the relation between peak blood velocity and mean blood velocity is altered. Silber et al. studied femoral and brachial artery blood velocities in young and older men and found that, in all subjects, the ratio of the center velocity to the spatially aver-
Aged mean velocity is smaller than for a fully developed parabola and does not differ among subject groups (unpublished observation). Although use of center velocity to estimate mean blood velocity and shear rate will result in a greater underestimation of either parameter in a large vessel, such as the femoral artery, given that larger arteries have a larger “blunt zone” in the center (40), vessels of more similar size, such as the popliteal and brachial arteries, will not be influenced significantly by this discrepancy (H. A. Silber, personal communication).

In addition, because the subjects experienced difficulty keeping their leg in a position in which the Doppler probe could be maintained at a <60° angle of insonation (the standard range for blood velocity measurements), with diameter still imaged accurately, the angle was standardized to 68° for all subjects for arm and leg measurements. Inasmuch as intrinsic spectral broadening with an increasing angle of insonation leads to consistent overestimation of the true flow velocity (14, 18), it is likely that our velocity measurements overestimate true blood velocity. However, because our values would be systematically overestimated for the entire subject population, we are not attempting to quantify volumetric blood flow, and because the standard angle of insonation of 60° also overestimates peak velocity (18), we do not believe that this issue significantly influences the results of the study.

In conclusion, the present study demonstrates that the well-documented age-associated reduction in brachial artery FMD is also observed in the popliteal artery in women. However, the age-related reduction in normalized FMD is not limb specific; that is, although a given increase in shear rate evokes a smaller percent diameter increase in older women, this relation is similar in brachial and popliteal arteries. Finally, attenuated percent diameter increase in older women, this relation is significant influences the results of the study.

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