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Limb-specific differences in flow-mediated dilation: the role of shear rate

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Nishiyama SK, Wray DW, Berkstresser K, Ramaswamy M, Richardson RS. Limb-specific differences in flow-mediated dilation: the role of shear rate. *J Appl Physiol* 103: 843–851, 2007. First published June 7, 2007; doi:10.1152/japplphysiol.00273.2007.—We sought to examine flow-mediated vasodilation (FMD) in both the arm [brachial artery (BA)] and lower leg [popliteal artery (PA)] of 12 young, healthy subjects. Vessel diameter, blood velocity, and calculated shear rate were determined with ultrasound Doppler following a suprasystolic cuff occlusion (5 min) in both the BA and PA and an additional reduced occlusion period (30–120 s) in the BA to more closely equate the shear stimulus observed in the PA. The BA revealed a smaller diameter and larger postischemic cumulative blood velocity [area under curve (AUC)] than the PA, a combination that resulted in an elevated postcuff cumulative shear rate (AUC) in the BA (BA: 25,419 ± 2,896 s^{-1}·s, PA 8,089 ± 1,048 s^{-1}·s; $P < 0.05$). Thus, when expressed in traditional terms, there was a tendency for the BA to have a greater FMD than the PA (6.5 ± 1.0 and 4.5 ± 0.8%, respectively; $P = 0.1$). However, when shear rate was experimentally matched (PA: 4.5 ± 0.8%; BA: −0.4 ± 0.4%) or mathematically normalized (PA: 6.8 × 10^{-4} ± 1.6 × 10^{-4}%Δ/s^{-1}·s; BA: 2.5 × 10^{-4} ± 0.4 × 10^{-4}%Δ/s^{-1}·s), the PA revealed a greater FMD per unit of shear rate than the BA ($P < 0.05$). These data highlight the importance of assessing the shear stimulus to which each vessel is exposed and reveal limb-specific differences in flow-mediated dilation.

ultrasound Doppler; brachial and popliteal arteries; vasodilation

VASCULAR ENDOTHELIAL FUNCTION is essential for the maintenance of vessel wall health and vasomotor control in both conduit and resistance vessels. Endothelial dysfunction in both the coronary and peripheral circulation has been closely linked to pathologies such as hypertension, hyperlipidemia, coronary artery disease, peripheral artery disease, and subsequent manifestations of atherosclerosis (11, 21, 30). Flow-mediated vasodilation (FMD) has emerged as a broadly applicable, noninvasive clinical tool to study endothelium-dependent peripheral artery vasomotion (5, 10, 34). Predominantly employed in the brachial artery (BA), FMD testing has been widely used as an index of global vascular health and coronary vasoreactivity (1, 33, 34).

However, there is some evidence to suggest that the inference regarding systemic endothelial function derived from conventional FMD testing may be problematic. Recent studies have revealed some vascular adaptations to be limb dependent, with evidence that vascular dysfunction may occur in the lower extremities without significant changes in vessels of the upper extremities (2, 18, 32). The onset of such limb-specific vascular pathologies could be attributed to the large hydrostatic and transmural forces experienced in the legs during upright posture. Indeed, there is some evidence that this intrinsic physiological stressor contributes to attenuated vasoreactivity (9, 16, 31) and arterial stiffening compared with the upper extremities (3, 7). Recent studies have also revealed limb-specific responses to pharmacological (20, 24) and physiological (29) interventions, with the legs apparently less responsive to a variety of stimuli.

In studies employing FMD, an elevated shear stimulus may lead to a greater vasodilatory response, and recent research acknowledging this stimulus-response relationship has further defined the utility of the FMD test to evaluate vascular health (15, 27, 34). Specifically, there is growing accord that blood velocity and diameter measurements across multiple time points must be evaluated to accurately quantify the shear-vasodilation relationship (17). Although others have applied the mathematical normalization of FMD for shear rate (7, 22, 38), to our knowledge no previous studies have implemented an experimental normalization of the shear rate stimuli between vessels that are similarly located relative to their primary muscle bed in the upper and lower extremities. Additionally, with the partial exception of a recent study that focused on women and aging (22), very little is known regarding the limb-specific nature of conduit vessel FMD.

Consequently, we applied endothelium-dependant FMD testing with varying shear stimuli in the arm and leg to comprehensively evaluate vascular reactivity and endothelial function in each limb. We hypothesized that *1*) following 5 min of ischemic cuff occlusion, the arm (BA) would exhibit a larger FMD than the leg [popliteal artery (PA)] when expressed in traditional terms [percent change (%Δ)]; and *2*) conversely, based on preliminary observations by our group, experimental and mathematical normalization for shear rate would reveal a greater sensitivity (FMD) to a given stimulus level in the leg (PA) than the arm (BA).

METHODS

Subjects and General Procedures

Subjects. Twelve young (27 ± 2 yr) healthy men (height: 178 ± 2 cm, weight: 76 ± 4 kg) participated in this study. All subjects were nonsmokers, normotensive (<140/90 mmHg), and free of overt cardiovascular disease. Subjects were excluded from participation if they were taking any medications that would alter vascular responsiveness. The current study was reviewed and approved by the Institutional Review Board committee of the University of California, San Diego, and informed consent was obtained according to the University of California, San Diego, Human Subjects Protection Program require-

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ments. Following the completion of health histories and physical examinations on all subjects, each reported to the laboratory in a fasted state (>4 h postprandial) and had refrained from caffeine and exercise before the studies (>12 h). Studies were performed with initial arm and leg FMD protocols (5-min occlusion) in random order, followed at least 30 min later by the reduced occlusion time protocol in the arm that allowed shear rate to be matched with that experienced by the leg. All studies were performed in a thermoneutral environment.

Arm FMD protocol. Subjects lay supine, and a pneumatic cuff was positioned on the upper right arm proximal to the elbow, distal to the placement of the ultrasound Doppler probe on the BA (28). After a 20-min rest period, baseline measurements were made, and the arm cuff was then inflated to suprasystolic pressure (>250 mmHg) for 5 min. Full occlusion was documented by the loss of ultrasound spectra in vessels at the wrist, distal to cuff.

Leg FMD protocol. Subjects lay supine on a gurney, modified to allow dorsal ultrasound Doppler access to the popliteal artery. A pneumatic cuff was positioned on the lower right leg below the knee, distal to the placement of the ultrasound Doppler probe on the PA. As with the arm, after a 20-min rest period and baseline measurements, the leg cuff was inflated to suprasystolic pressure (>250 mmHg) for 5 min. Again, full occlusion was documented by the loss of ultrasound spectra in vessels at the ankle, distal to the cuff.

Arm FMD, experimentally matched shear rate, protocol. As with the arm FMD protocol (5 min), subjects lay supine, and a pneumatic cuff was positioned on the upper right arm proximal to the elbow, distal to the placement of the ultrasound Doppler probe on the BA. After a 30-min rest period, baseline measurements were made, and the arm cuff was then inflated to suprasystolic pressure (>250 mmHg) for a period of 30 to ≤120 s to appropriately match the reactive hyperemia and subsequent shear rate observed in the leg FMD study (5-min occlusion). With data for each subject's arm and leg 5-min cuff occlusion FMD response and many sets of pilot data already in hand, the cuff duration for this protocol was appropriately estimated and then instigated, with good precision, in a single trial for each subject.

Additional validation experiments. In a subset of the present subjects ($n = 6$), two additional experiments were performed aimed at determining whether the reduced cuff duration (1 min) used in the present study may have influenced the results or whether the resultant shear rates were the major factor: *1*) a comparison of the vasodilation resulting from the similarly elevated shear rates following a conventional 5-min cuff occlusion and a 1-min cuff occlusion with ischemic handgrip exercise superimposed, and *2*) the vasodilatory effect of a similar shear rate stimulus achieved by varied cuff duration combined with varying the mass of occluded tissue. To accomplish the first aim (additional *experiment I*), two trials were performed: *1*) subjects lay supine and a pneumatic cuff was positioned on the upper right arm proximal to the elbow and inflated to suprasystolic pressure (>250 mmHg) for 5 min (BA FMD protocol); and *2*) with the same positioning of the cuff for 1 min, light-intensity intermittent handgrip exercise (20% of maximal voluntary contraction, 0.5 Hz) was superimposed during the cuff occlusion period. To accomplish the second aim (*experiment II*), three trials were performed with the subjects laying supine, and a pneumatic cuff positioned and inflated to suprasystolic pressure (>250 mmHg) on either *1*) the upper right arm proximal to the elbow for 1 min, *2*) the upper forearm distal to the elbow for 3 min, and/or *3*) the wrist and inflated for 5 min, all of which were distal to the placement of the ultrasound Doppler probe on the BA. In both additional experiments, all trials were performed in random order and were preceded by at least a 30-min rest period.

Measurements

Ultrasound Doppler. The ultrasound system (Logiq 7, GE Medical Systems, Milwaukee, WI) was equipped with two linear array transducers operating at an imaging frequency of 7–8 and 10 MHz, with either selected to optimize Doppler imaging according to vessel depth. Vessel diameter was measured at a perpendicular angle along the central axis of the scanned area, where the best spatial resolution was achieved. The BA of the right arm was insonated approximately midway between the antecubital and axillary regions, medial to the biceps brachii muscle. The PA of the right leg was insonated 4–5 cm proximal to the pneumatic cuff in the popliteal fossa at the back of the knee, where it could be optimally visualized.

The blood velocity profile was obtained using the same transducers with a Doppler frequency of 4.0–5.0 MHz, operated in the high-pulse repetition-frequency mode (2–25 kHz) with a sample depth of 1.5–3.5 cm. Care was taken to avoid aliasing by using scale adjustments, especially after cuff release. In duplex mode, real-time ultrasound imaging and the pulse-wave velocity profile were viewed simultaneously. 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 centered, verified by real-time ultrasound visualization of the vessel. Ultrasound images and Doppler velocity waveforms were measured at rest for a 20-s period and again at −4–16, 25–45, 55–75, and 85–105 s post-cuff release. At all sample points, arterial diameter and angle-corrected, time-averaged, and intensity-weighted mean blood velocity (V_{mean}) values were calculated using commercially available software (Logiq 7, GE).

Shear rate. Shear stress has been identified as a mechanism that stimulates the vascular endothelium and results in subsequent vasodilation (25). However, because blood viscosity was not measured, shear rate was calculated by using the following equation (7, 38, 39): Shear rate (s^{-1}) = $4 \cdot V_{mean}$ (cm/s)/vessel diameter (cm). Cumulative shear rate was expressed using the area under the curve (AUC; $s^{-1} \cdot s$) for shear rate over time (23, 27), integrated with the use of commercially available software (SigmaPlot 8.0, Systat Software, Point Richmond, CA). Cumulative shear rate AUC values were integrated using the trapezoidal rule and calculated as follows: $\Sigma\{y_i[x_{(i+1)} - x_i] + (\frac{1}{2})[y_{(i+1)} - y_i][x_{(i+1)} - x_i]\}$ (x is time, y is shear rate, x_i is initial time point, y_i is initial blood velocity). To mathematically normalize vasodilation for shear rate, FMD was divided by cumulative shear rate (%Δ diameter/$s^{-1} \cdot s$) (27).

Blood pressure and heart rate. Arterial blood pressure was measured using automated radial tonometry (Medwave Vasotrac APM205A; BioPac Systems, Goleta, CA), with one measurement every 8–10 s. Heart rate was recorded from a standard three-lead ECG, an integral part of the Doppler system (Logiq 7, GE).

Data Processing

Ultrasound Doppler. V_{mean} for the 20 s before cuff inflation (rest) was averaged across the first and last 10 s of the recorded clip. To improve acuity of the initially dynamic blood velocity profile after cuff release, the first 16 s of V_{mean} was evaluated in four consecutive 4-s segments. Because of technical limitations on cuff release (an often unavoidable need to move the sample volume to stay in the vessel), accurate measurements of the time segment 0–4 s were not always possible and so have been dropped from the analyses. As the dynamic nature of the hyperemic response subsided in the remaining 20-s segments (25–45 s post-cuff release), V_{mean} was assessed in two consecutive 10-s segments. As with resting measurements, V_{mean} was then averaged across the first and last 10-s segments for 55–75 s, and 85–105 s post-cuff release. All ultrasound vessel diameter measurements were evaluated during end diastole (corresponding to an R wave documented by a simultaneous ECG signal). In each segment, at least two diameter measurements of the near-to-far wall adventitia-media interface were obtained within close proximity (~0.05 cm) and then averaged. For all FMD trials, the post-cuff release artery diameters represent the final recording segment (85–105 s post-cuff release).

In this study, all ultrasound Doppler measurements and analyses were performed by a single sonographer who demonstrated equal or better reproducibility compared with previously published manual measurements of vessel diameter (36). [specifically, repeated analysis of a single image for vessel diameter across time, coefficient of variation = 9% (10 rereads); repeated analysis of a single subject's diameter across time (i.e., probe replacement and verification of site with landmarks etc.), coefficient of variation = 19%].

Statistics. Statistics were performed using commercially available software (SigmaStat 3.10, Systat Software). Repeated-measures ANOVA and paired *t*-tests were used to identify significant changes in measured variables within and between the BA and PA and during additional validation studies, with the Bonferroni test used for post hoc analysis when a significant main effect was found. All group data are expressed as means ± SE. Statistical significance was established at $P < 0.05$.

RESULTS

Resting Vessel Diameters, Blood Velocities, and Shear Stress

Good quality ultrasound images were attainable at rest, during, and following cuff release (Fig. 1). BA diameter was significantly smaller than the PA (0.47 ± 0.01 and 0.7 ± 0.02

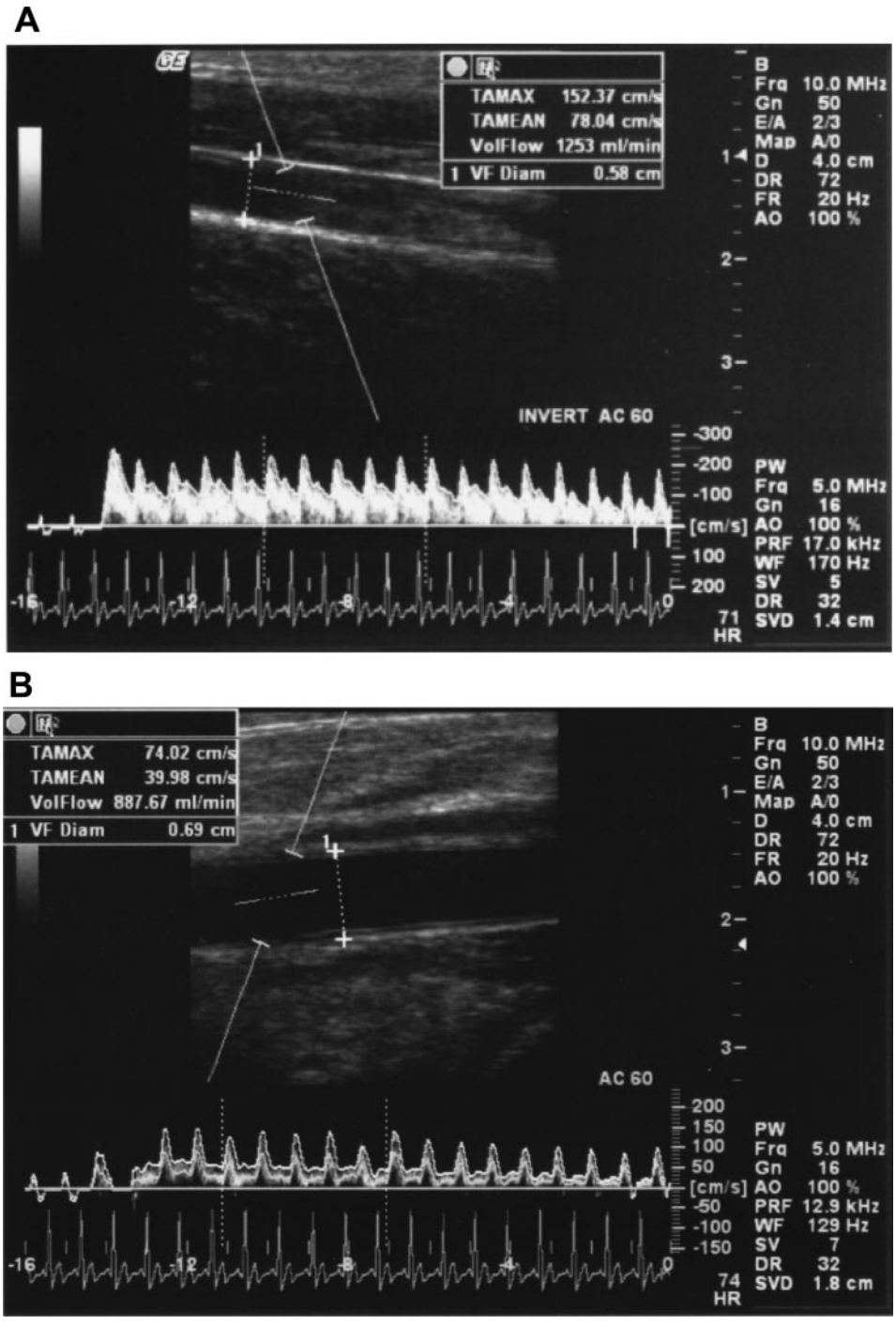

Fig. 1. Ultrasound Doppler screen captures illustrating typical images and blood velocity spectra in the brachial (*A*) and popliteal (*B*) arteries following cuff release. Note first 2–3 cardiac cycles of the Doppler signal are during cuff occlusion, followed by cuff release and subsequent hyperemia. $n = 1$. [TAMAX is V_{peak} (cm/s), TAMEAN is V_{mean} (cm/s), VolFlow is blood flow (ml/min), VFDiam is arterial diameter (cm), AC is Angle Correct, B is B-mode, PW is pulse wave mode, Frq is frequency (MHz), Gn is gain (dB), E/A is Edge enhance/frame average, Map is image mapping, D is depth (cm), DR is dynamic range, FR is line density, AO is auto optimize, PRF is pulse repetition frequency (kHz), WF is wall filter, SV is sample volume length (cm), SVD is sample volume depth (cm)].

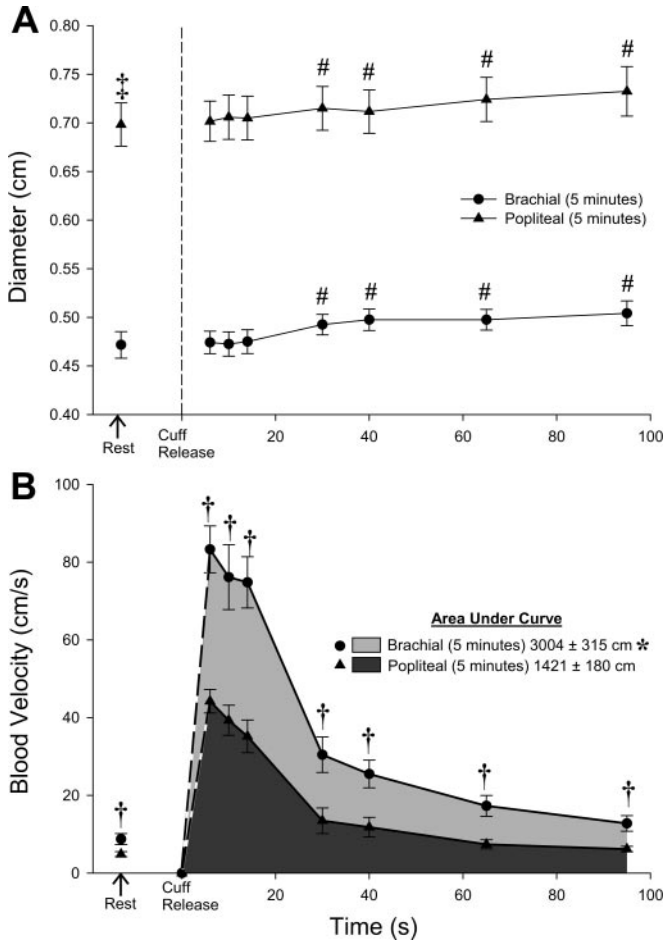

Fig. 2. Diameter (*A*) and velocity (*B*) profiles at rest and following 5 min of cuff occlusion in the brachial and popliteal arteries. Values are means ± SE; $n = 12$. ‡The brachial artery was larger than the popliteal artery at rest, and this difference was maintained following cuff release, $P < 0.05$. #Significantly different artery diameter from rest, $P < 0.05$. †Both at rest and following cuff release, brachial artery mean blood velocity was significantly larger than the popliteal artery, $P < 0.05$. *Cumulative brachial artery mean blood velocity (area under the curve) was significantly larger than the popliteal artery, $P < 0.05$.

cm, respectively; $P < 0.05$; Fig. 2*A*). Resting blood velocity in the BA was significantly larger than in the PA (8.7 ± 1.4 and 5.5 ± 0.6 cm/s, respectively; $P < 0.05$; Fig. 2*B*). Therefore, calculated resting shear rate was significantly larger in the BA than the PA (76.0 ± 13.1 and 28.5 ± 4.15 s^{-1}, respectively; $P < 0.05$; Fig. 3*A*).

Post-Cuff Release Shear Stress

In the 5-min cuff-occlusion protocols, shear rate AUC was significantly greater in the BA than in the PA following cuff release (25,419 ± 2,896 and 8,089 ± 1,048 s^{-1}·s, respectively; $P < 0.05$; Fig. 3*A*). Therefore, average cuff duration during experimental shear rate-matching protocols in the arm was significantly reduced from 5 min to 60 ± 9 s. Both individually and therefore on average, excellent matching of shear rate was achieved (5-min cuff PA cuff occlusion: 8,089 ± 1,048 s^{-1}·s, BA reduced cuff duration: 8,104 ± 1,016 s^{-1}·s; Fig. 3*B*).

FMD

Following 5 min of cuff occlusion, FMD expressed in traditional terms revealed a tendency for a larger FMD in the BA (6.5 ± 1.0%) than the PA (4.5 ± 0.8%), but this did not achieve statistical significance ($P = 0.1$; Fig. 4*A*). In contrast, when shear rate was experimentally matched, the PA revealed a much greater FMD than the BA (4.5 ± 0.8% and −0.4 ± 0.4% respectively; $P < 0.05$; Fig. 4*B*). Mathematical normalization of FMD with shear rate (AUC) confirmed this difference with the PA again revealing a significantly greater dilation for a given shear rate than the BA ($6.8 \times 10^{-4} \pm 1.6 \times 10^{-4}$%Δ/$s^{-1}$·s and $2.5 \times 10^{-4} \pm 0.4 \times 10^{-4}$%Δ/$s^{-1}$·s, respectively; $P < 0.05$; Fig. 4*C*).

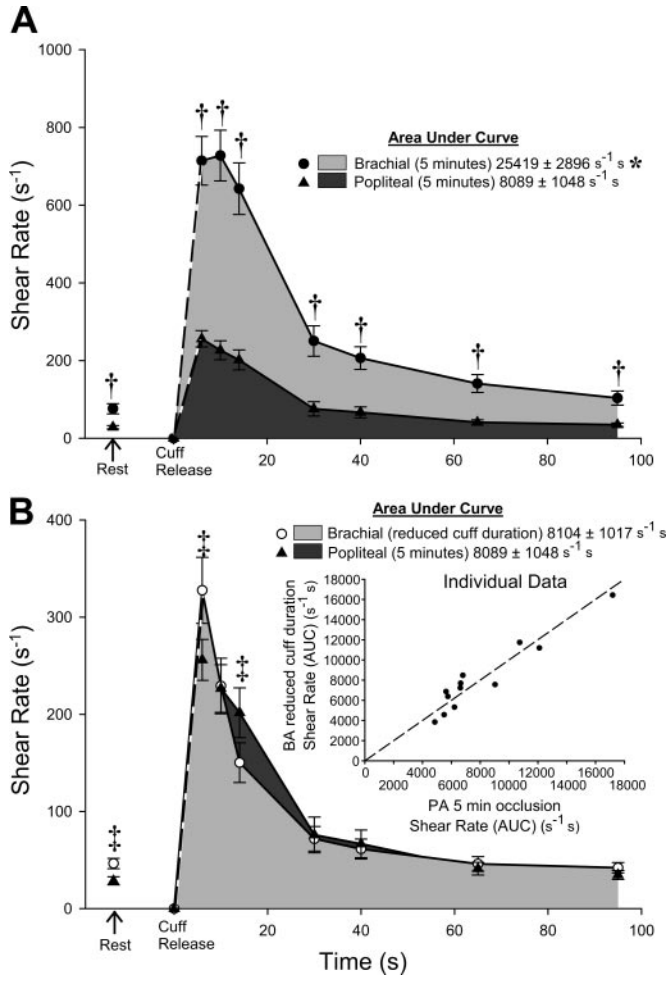

Fig. 3. Shear rate at rest and following 5 min of cuff-occlusion in the brachial artery and the popliteal artery (PA) (*A*) and at rest following reduced cuff duration in the brachial artery (*B*). Values are means ± SE; $n = 12$. †Brachial artery shear rate at rest and following cuff release was significantly larger than the popliteal artery, $P < 0.05$. *Following cuff release, cumulative brachial artery shear rate [area under the curve (AUC)] was significantly larger than the popliteal artery, $P < 0.05$. ‡Significant difference between brachial and popliteal artery shear rate, $P < 0.05$. By experimental design in the matched studies, cumulative shear rate (area under the curve) was very similar in the 2 vessels. *Inset* in *B*: documentation of efficacy of experimentally matching for shear rate yielding a good fit with the identity line.

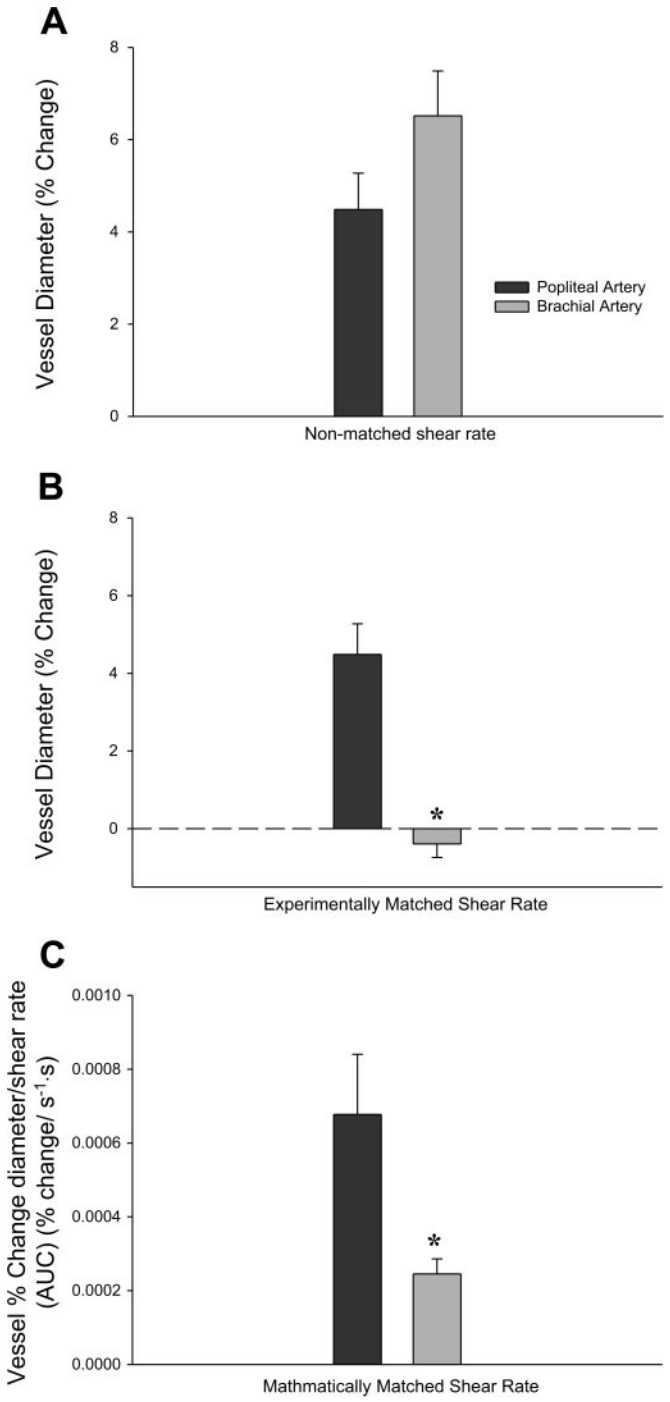

Fig. 4. Brachial and popliteal flow-mediated dilation following conventional 5-min cuff occlusion (*A*), experimentally matched shear rates attained by reduced cuff duration in the brachial artery (*B*), and mathematically normalized for shear rate following conventional 5-min cuff occlusion (*C*). Values are means ± SE; $n = 12$. *Flow-mediated dilation in response to experimentally and mathematically normalized shear rate were significantly greater in the popliteal than the brachial artery, $P < 0.05$.

Additional Validation Experiments

Experiment I. Shear rate AUC was similar between the 5-min cuff and the 1-min cuff with handgrip exercise (19,842 ± 1,708 s^{-1}·s and 23,282 ± 2,115 s^{-1}·s, respectively; $P > 0.05$; Fig. 5, *Ia*). FMD expressed in traditional terms revealed a similar vasodilation (5-min cuff: 7.4 ± 0.6%, 1-min cuff with handgrip exercise: 7.6 ± 0.9%; $P > 0.05$; Fig. 5, *Ib*). The mathematical normalization of FMD with shear rate (AUC) revealed no difference between the 5-min cuff and 1-min cuff with handgrip exercise ($3.8 \times 10^{-4} \pm 0.3 \times 10^{-4}$%Δ/$s^{-1}$·s and $3.3 \times 10^{-4} \pm 0.4 \times 10^{-4}$%Δ/$s^{-1}$·s, respectively; $P > 0.05$; Fig. 5, *Ic*). For comparison with the present method, the data collected in the additional *experiment II* using this method (1-min resting cuff occlusion) (see below) was included and was significantly different from the 5 min cuff and 1 min cuff with handgrip exercise in terms of shear rate, and vasodilation both with and without normalization ($P < 0.05$; Fig. 5, *Ia*, *Ib*, and *Ic*).

Experiment II. Shear rate AUC was similar and relatively low in all three trials with varied cuff duration and tissue mass occluded (1-min cuff proximal to elbow: 10,860 ± 1,928 s^{-1}·s, 3-min cuff at the forearm: 12,065 ± 1,250 s^{-1}·s, 5-min cuff at the wrist: 12,088 ± 2,355 s^{-1}·s; $P > 0.05$; Fig. 5, *IIa*). As a result, FMD expressed in traditional terms revealed a slight, but statistically insignificant, vasodilation in all three trials (1-min cuff proximal to elbow: 0.7 ± 0.4%, 3-min cuff at the forearm: 0.7 ± 0.4%, 5-min cuff at the wrist: 0.7 ± 0.4%; $P > 0.05$; Fig. 5, *IIb*). Similarly, mathematical normalization of the FMD with shear rate (AUC) revealed no difference between all three trails (1-min cuff proximal to elbow: $0.4 \times 10^{-4} \pm 0.3 \times 10^{-4}$%, 3-min cuff at the forearm: $0.6 \times 10^{-4} \pm 0.4 \times 10^{-4}$%Δ/$s^{-1}$·s, 5 min cuff at the wrist: $0.4 \times 10^{-4} \pm 0.3 \times 10^{-4}$%Δ/$s^{-1}$·s; $P > 0.05$; Fig. 5, *IIc*).

DISCUSSION

In the present study, arm (BA) and leg (PA) FMD were compared following a 5-min cuff occlusion, followed by experimental and mathematical matching of the shear stimulus between limbs. After the 5-min cuff occlusion, there was a tendency for the BA to have a greater FMD than the PA when expressed in traditional terms. In contrast, when the limb FMD responses were matched for shear stimuli, the PA revealed significantly greater shear stress sensitivity than the BA. Thus, in two vessels with locations in the upper and lower extremities, there appears to be a limb-specific sensitivity to shear rate and subsequent endothelium-dependent vasodilation that favors the leg.

Limb Vascular Heterogeneity

There is accumulating evidence to suggest that vascular function in the arms and legs of humans is not uniform. At rest, evidence of a larger blood flow response to physiological vasodilators has been observed in the arms (20), whereas a greater sympathetically mediated vasoconstrictor response has been recognized in the legs (24). Teleologically, some of these limb vascular differences may be the result of gravity-induced structural and functional adaptations due to the vertical stance of humans (3, 7, 9, 16, 31). It is tempting to speculate that the greater endothelium-dependent vascular reactivity observed in the PA may be an essential adaptation in response to the recognized "vascular stiffening" in the lower extremities (9).

Recently, our group (39) reported that during exercise conduit vessel dilation for a given shear rate is reduced in the upper leg (common, deep, and superficial femoral arteries)

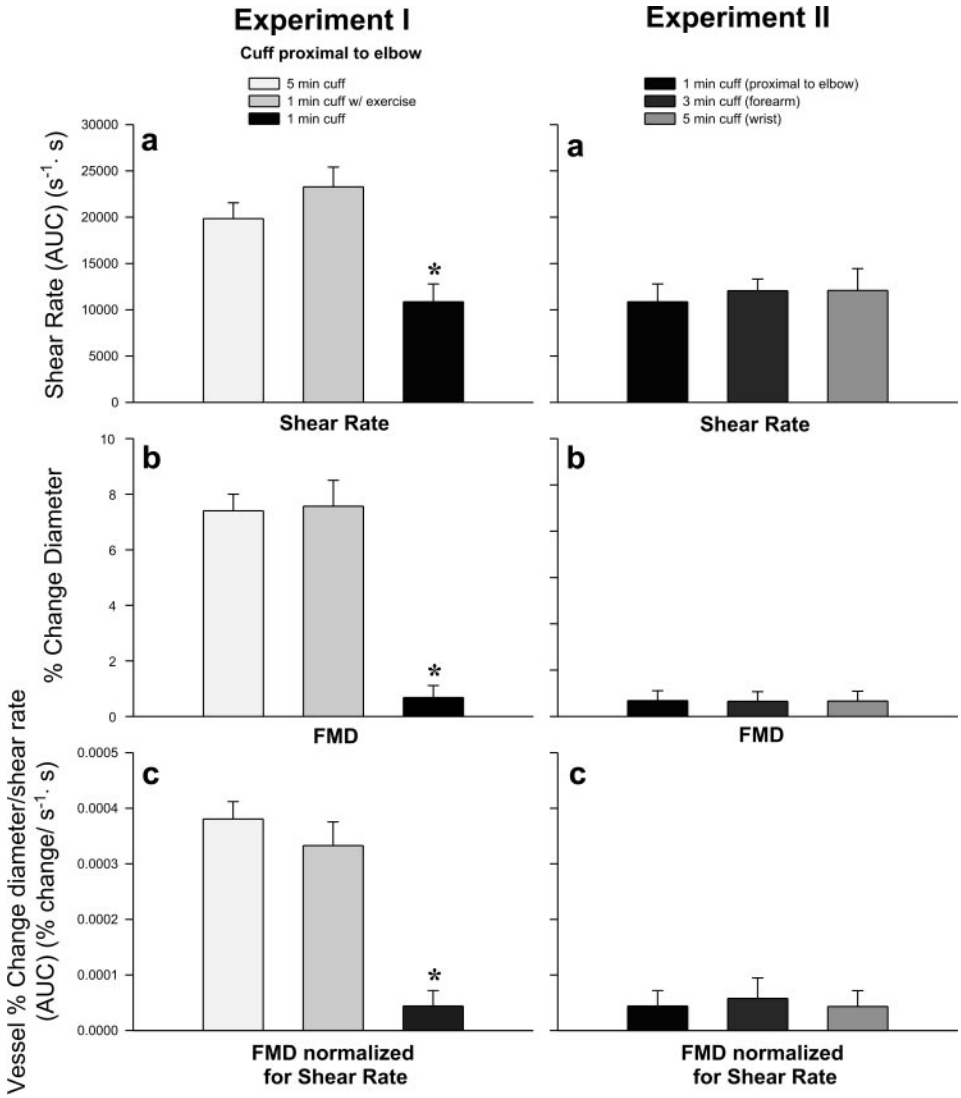

Fig. 5. Effect of similar, elevated shear rate, but varied cuff duration contrasted with short cuff duration and low shear rate (*experiment I*) and similar, elevated shear rate attained by varied cuff duration and mass of tissue occluded (*experiment II*) on brachial artery shear rate (area under the curve) (*Ia* and *IIa*), 5-min cuff flow-mediated dilation (FMD; *Ib* and *IIb*), and mathematically normalized flow-mediated dilation (*Ic* and *IIc*). Values are means ± SE; $n = 6$. *Statistically different from the 5-min cuff and 1-min cuff with exercise, $P < 0.05$.

compared with the arm (BA) of healthy humans. These findings are somewhat at odds with the current data that reveal a greater sensitivity to shear in the PA of the leg, an incongruity that may be explained by methodological and anatomic differences between studies. Although exercise protocols offer physiologically significant data, exercise-induced vasodilation as employed in our laboratory's previous work (39) is a complex process and may involve numerous confounding variables. In contrast, the present study utilized an FMD test rather than exercise, offering a somewhat simplified experimental model to assess endothelium-dependent vasoreactivity (19, 27). In addition, the cross-sectional areas of PA and BA are closer in size than the femoral arteries and are found in more similar anatomic locations within the arterial tree, and they are thus more comparable than previous studies examining vessels of the upper leg (39). However, in the present study, it should also be recognized that differences in shear mediated vasoreactivity could stem from the quite different roles of the muscle beds perfused by the BA and PA. Specifically, the bipedal nature of humans fosters a relatively more active gastrocnemius-soleus complex perfused by the PA, whereas the BA perfuses the relatively less frequently recruited muscles of the forearm (brachioradialus and wrist flexors). This differing use may result in disparate exercise-induced physiological adaptation [e.g., endothelial nitric oxide (NO) synthase upregulation (14)], which may also play a potential role. Although interesting, our present data offer little insight into the validity of this link between the type of muscle bed perfused, exercise adaptation, and vasoreactivity of the conduit artery and further research will be needed to confirm or deny this relationship. In summary, however, the present study extends previous findings from our exercise-based research, representing a more rigorous assessment of vascular reactivity between limbs at rest.

FMD and the Shear Rate Stimulus

The FMD technique, first employed by Celermajer et al. (4), has emerged as a broadly applicable, noninvasive clinical tool to study endothelium-dependent peripheral artery vasomotion (5, 10, 34). In their original work and the present study, a 5-min or less cuff occlusion was employed with the aim of eliciting strictly a NO-dependent endothelial-mediated response (8, 19). However, it was not until recently that the importance of the shear stress stimulus and its nonuniformity across subjects and

studies was recognized, leading to a need to accurately manipulate or correct for this force (17, 27).

Some have characterized shear stress as a peak attained after cuff release and used this variable to normalize FMD for the stimulus (7, 22). However, it has become increasingly apparent that the peak shear rate may not reflect the true nature of the shear stimulus. Therefore, the AUC for shear rate across time ($s^{-1} \cdot s$) has gained favor as the most appropriate approach to quantify the cumulative stimulus contributing to the vasodilatory response (27). In the present study, we have adopted this approach and applied it accordingly, both experimentally and mathematically normalizing for shear rate (Fig. 4, *B* and *C*). However, it should be noted that additional analyses of the present data using alternative approaches (peak shear rate and delta shear rate from rest) yield essentially identical results (data not shown).

The importance of assessing shear rate and then incorporating any differences into the experimental design and/or analysis is highlighted by the finding that the PA exhibited a significantly reduced V_{mean} profile after 5 min of cuff occlusion compared with the BA (Fig. 2*B*). This, combined with a larger diameter (Fig. 2*A*), lead to a much smaller shear rate in the PA compared with the BA (Fig. 3*A*). To experimentally account for this shear rate difference, an additional protocol was performed in which the BA was cuffed for a shorter duration to lessen the ischemic-reperfusion response and therefore reduce BA V_{mean} following cuff release. As a result, shear rates were experimentally matched, and the shear stimulus was thus successfully normalized between the BA and PA (Fig. 3*B*). With this approach, the PA appears much more sensitive than the BA to a given shear rate. However, these findings raise the possibility that the BA has a "shear rate threshold" below which a FMD response cannot be elicited. Nevertheless the fundamental aim of this study was to examine limb-specific shear-mediated vasoreactivity in a scenario in which both the BA and PA received the same shear stimulus, and with this goal achieved the PA responded whereas the BA did not. Therefore, although a simple FMD assessment suggested that the lower extremities tend to have an attenuated vascular function compared with the upper extremities (Fig. 4*A*), when the shear rate stimulus was appropriately incorporated experimentally (Fig. 4*B*) or mathematically (Fig. 4*C*) into the response, the PA of the lower extremities demonstrated greater vascular function than the BA of the upper extremities.

BA and Global Vascular Health

Although limb-specific vascular responses are gaining recognition (26, 37, 39), it has been commonplace for BA vasodilation (%Δ) measured by FMD to be used as an index of global vascular health (6, 35). However, the present data reveal that vascular responses to shear stimuli vary significantly across vessels of differing anatomic origin, and therefore caution must be exercised when the vascular function of a conduit artery of a single limb is determined and the results translated to systemic vascular health.

Sex Differences

In a study that closely parallels the present investigation, Parker et al. (22) recently published findings in young and older women that identified no limb difference in response to a FMD test in the BA and PA. Although the authors did not study men, it was suggested that the failure to identify the hypothesized attenuation in leg vascular function could be attributable to gender. Of note, prior research has demonstrated potential sex differences in BA FMD, with women having a more pronounced shear-mediated arterial vasodilation compared with men (15). However, it is also possible that the lack of limb-specific findings by Parker et al. is partially the consequence of methodological limitations such as a greater than generally accepted insonation angle and lack of intensity-weighted Doppler velocity signal, as recognized by the authors. The present study thus extends these findings with a more accepted approach to the assessment of blood velocity and shear rate, as well as the inclusion of both experimental and mathematical normalization of the shear stimulus. Nevertheless, in consideration of these issues, without data collected by our approach in women we cannot discount the potential for an influence of sex on both limb-specific endothelium-dependent vasoreactivity.

Experimental Considerations

In general, caution should be exercised when directly comparing diameter changes expressed as a percent in arteries of relatively different size, because there is an inherent mathematical bias in favor of the smaller vessel. However, in the present comparison, because the PA has a larger starting diameter (handicapping its %Δ), this mathematical bias cannot explain the improved vascular responsiveness of the PA compared with the BA, but would in fact reduce the chance of this observation.

Although the vasodilatory response to a FMD test has been shown to be predominantly mediated by shear-induced, endothelium-dependent factors (13, 19, 27), in the present study, there was no experimental trial to establish the extent of endothelium-independent vasodilation. The two known studies that have examined this maximal dilatory response (i.e., direct smooth muscle dilation) in the arteries of both the arm and leg have revealed conflicting results (12, 22). It is possible that these disparate findings may to some extent be attributed to the mathematical bias developed by comparing vessels of different diameter, as previously discussed. Also, it is likely that endothelium-independent vasodilation does not differ between limbs, but that is clearly beyond the scope of this work.

It is accepted that a 5-min cuff occlusion results in a predominantly NO-mediated dilation in the conduit vessels supplying the briefly ischemic tissue and that a longer duration of cuff occlusion may result in a multifactoral stimulus (NO is likely to no longer be the only key vasodilator) (19, 27). With this reasoning in mind, it seems intuitive that shorter duration cuffing will not invoke these complications, and the vessel response will therefore remain dependent on NO-mediated dilation stimulated by the same, albeit reduced, shear stress. However, in recognition of this experimental uncertainty, we performed two additional sets of studies on subsets of the subjects who partook in the present investigation ($n = 4$): In the first study, we matched the shear stress stimulus of a standard 5-min cuff occlusion and 1-min cuff occlusion including ischemic handgrip exercise (Fig. 5, *Ia*) and documented the same degree of dilation with a similar shear stress stimulus (Fig. 5, *Ib* and *Ic*). In the second subset of studies, we

demonstrated that matching the post-1-min cuff shear rate, achieved by variations in cuff duration (1, 3, and 5 min) combined with varying the mass of occluded muscle (Fig. 5, *IIa*), yield essentially the same result: no dilation in the BA (Fig. 5, *IIb* and *IIc*). Together, these additional studies confirm that cuff duration of 5 min or less, although an integral constituent of the reactive hyperemia response, is not the sole determinant of the subsequent dilatory response. Rather, the shear stress, a consequence of the cuff duration, is the mediator of the vasodilation.

Conclusion

These findings again highlight the importance of both quantifying and the accounting for the shear stimulus in FMD studies. Without either experimental or mathematical normalization of this important variable in the present study, clear limb-specific differences in endothelium-dependent vasoreactivity would have been left unveiled. Specifically, the present data suggest that the PA in a healthy, young, male population exhibits an enhanced vascular response to a given shear stimulus when contrasted with the BA.

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