Why is flow-mediated dilation dependent on arterial size? Assessment of the shear stimulus using phase-contrast magnetic resonance imaging

Harry A. Silber, Pamela Ouyang, David A. Bluemke, Sandeep Gupta, Thomas K. Foo, and Joao A. C. Lima.

Methods

FMD is greater in small arteries, but it is unclear whether this reflects better inherent endothelial function or merely a property of the measurement process itself. We have previously shown that FMD is greater in small arteries, at least in part, because the shear stimulus during postocclusion hyperemia is greater in small arteries (32). However, it is unknown why the shear stimulus is greater in small arteries. Shear rate is dependent on flow and radius according to Poiseuille’s Law, where shear rate is proportional to flow divided by radius cubed (27). Whether postischemic hyperemic flow is related to arterial size has not been evaluated. Therefore, the second objective of this study was to evaluate the relationship between postischemic systolic flow and arterial size in the brachial and femoral arteries. In pursuing this objective, we also tested the applicability of the Poiseuille relation during transient postischemic hyperemia.

Endothelial function; vasodilation; blood flow

ENDOTHELIAL FUNCTION IS ASSESSED NONINVASIVELY BY MEASURING FLOW-MEDIATED DILATION (FMD) USING ULTRASOUND (3, 11, 34). However, FMD is strongly dependent on arterial size (11, 18, 20, 31), which contributes to a wide range of FMD values in normal subjects (9, 10, 13). The reasons for the inverse dependence of FMD on baseline diameter are poorly understood. Nor is it understood why this phenomenon is observed in the femoral arteries as well as in the brachial arteries (11). Consequently, it is unknown whether greater FMD in small conduit arteries indicates that endothelial function is better in small arteries than in large arteries.

The primary hemodynamic determinant of FMD is wall shear stress (8, 14, 22). Using phase-contrast MRI (PCMRI), we have previously shown that FMD in the brachial artery is proportional to the systolic shear stress that occurs during postischemic hyperemia (32). This relationship has not been investigated in other arterial territories in humans. Accordingly, the first objective of this study was to determine whether the FMD response is proportional to the shear stress stimulus in the femoral artery as well as the brachial artery in subjects without cardiovascular risk factors.

FMD is greater in small arteries, but it is unclear whether this reflects better inherent endothelial function or merely a property of the measurement process itself. We have previously shown that FMD is greater in small arteries, at least in part, because the shear stimulus during postocclusion hyperemia is greater in small arteries (32). However, it is unknown why the shear stimulus is greater in small arteries. Shear rate is dependent on flow and radius according to Poiseuille’s Law, where shear rate is proportional to flow divided by radius cubed (27). Whether postischemic hyperemic flow is related to arterial size has not been evaluated. Therefore, the second objective of this study was to evaluate the relationship between postischemic systolic flow and arterial size in the brachial and femoral arteries. In pursuing this objective, we also tested the applicability of the Poiseuille relation during transient postischemic hyperemia.

Methods

Subjects. Twenty-four healthy subjects (9 men and 15 women), ages 21–41 yr, with no cardiovascular risk factors including hypertension, diabetes, hyperlipidemia, smoking, obesity, or cardiac disease in a first-degree relative were studied. No subject was acutely ill or was on any vasoactive medication. The study protocol was approved by the Institutional Review Board at the Johns Hopkins School of Medicine. All subjects gave written, informed consent.

Study protocol. Subjects abstained from eating or drinking, except water, for at least 6 h before the study. Baseline blood pressure was recorded. MRI was performed using a 1.5-T scanner (CV/i, General Electric Medical Systems, Milwaukee, WI) equipped with cardiac gradient coils (40 mT/m, 120 T·m−1·s−1). Electrocardiographic leads were placed on the thorax. To study the brachial artery, a 3-in. receiver coil was placed on the medial aspect of the upper arm. A sphygmomanometer cuff was placed on the forearm, extending to just above the elbow. To study the femoral artery, a dual cardiac-phased array receiving coil was placed anterior and posterior to the lower thigh. A sphygmomanometer cuff was placed on the lower thigh. For each artery, the limb was positioned to place the artery parallel to the magnet bore. Phase-contrast images were obtained at baseline. The acquisition parameters were as follows:

- **Brachial artery:**
  - **Slice thickness:** 0.75 mm
  - **Matrix size:** 192 × 256
  - **FOV:** 14 cm
  - **Phase-encoding direction:** Right-left
  - **Image acquisition:** 30 frames at 1.75-s intervals
- **Femoral artery:**
  - **Slice thickness:** 1.0 mm
  - **Matrix size:** 188 × 256
  - **FOV:** 18 cm
  - **Phase-encoding direction:** Right-left
  - **Image acquisition:** 30 frames at 1.75-s intervals

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
cuff was then inflated at least 20 mmHg above the subject’s measured systolic blood pressure for 5 min and then released. Images using the same fixed cross-sectional axial prescription as at baseline were obtained immediately after and then at 1 min after cuff release. Serum values of glucose, hematocrit, and fasting lipid panel were obtained after the scanning portion of the study. To assess the reproducibility of measurements, the study was repeated in each of six subjects during a repeat session.

**Imaging protocol.** Coronal and axial scout images of each artery were obtained to locate the artery and to verify that the artery was parallel to the magnet bore. Phase-contrast scans were gated to the electrocardiogram signal. A single imaging plane perpendicular to the artery of interest was prescribed. The imaging parameters at baseline and at 1 min after cuff release were matrix size: 256 × 128; field-of-view: 10 × 10 cm (femoral) or 8 × 8 cm (brachial); slice thickness: 3 mm; flip angle: 25°; bandwidth: 31.2 kHz; repetition time: 11.43 (femoral) or 13.23 ms (brachial); echo time: 5.25 (femoral) or 5.56 ms (brachial); maximum encoded velocity value: 60 cm/s along the superior/inferior axis, 8 views per segment; first-order flow compensation: 20 view-shared phases, no phase-wrap, and no magnitude weighting. A setting of 16 number of excitations was used at baseline and 2 number of excitations at 1 min after cuff release. During hyperemia immediately after cuff release, scan duration was limited to ~25 s using 8 or 10 views/segment, and the maximum encoded velocity value prescribed was 120 cm/s. Resulting temporal resolution for all scans was between 180 and 260 ms.

**Data analysis.** The cardiac phase closest to peak systole was identified using commercially available software (Flow, Medis, The Netherlands). The artery lumen at the vessel wall was outlined with a region-of-interest tool. A plot of spatially averaged velocity vs. cardiac phase was created. The phase yielding the peak spatially averaged velocity was used for further analysis. Image data were imported into a spreadsheet-based (Excel, Microsoft, Mountain View, CA) program created in our laboratory. An approach modified from one applied to the carotid artery by Oyre et al. (28) was used to calculate shear stress and radius in the femoral and brachial arteries. In our approach, the limits of the arterial diameter were estimated in two orthogonal axes. An initial estimate of the center of the cross section was calculated from those limits. The cross section was divided into 12 sectors around the estimated center. For each sector, outer radius of the velocity profile was estimated. A ring segment of datapoints with radius ranging from slightly less than the initially estimated outer radius to ~1 mm inward toward the estimated center was used. The velocity pixels in the ring segment of the sector were fit by least-squares method to a parabola with the assumption that blood flow velocity at the lumen wall is zero. Shear rate was calculated as the slope of the velocity profile at the lumen-wall interface. Radius was calculated as the distance from the center of the velocity parabola to the point where the parabola crosses zero velocity. The calculated lumen radius and shear values were averaged over the 12 sectors in the arterial cross section. This approach provides subpixel precision in calculating lumen radius and was shown to be accurate compared with glass tubes of known manufactured diameter (28). Furthermore, the approach is not constrained by the geometry of the lumen perimeter, i.e., the arterial cross section does not have to be perfectly circular. Systolic flow was measured directly by summing all of the velocity pixels in the arterial cross section. Calculated shear rate was obtained from measured flow and radius using the Poiseuille equation:

\[
\text{shear rate} = \frac{4 \times \text{flow}}{\pi \times (\text{radius}^3)}
\]

Calculated shear rate was compared with measured shear rate to test the applicability of the Poiseuille equation to the systolic velocity profile during hyperemia. Systolic shear stress was calculated by multiplying systolic shear rate by blood viscosity. Blood is a non-Newtonian fluid, meaning its viscosity varies at different shear rates. After all the shear rates were calculated, a viscosity value was used that corresponded with the observed range of shear rates (7). Arterial size was also expressed as diameter to follow the convention of most ultrasound-based FMD studies. FMD was expressed as the percent change in diameter from baseline to 1 min after release of arterial occlusion.

**Statistical analysis.** Results are expressed as means ± SD. A paired t-test was used to compare measured parameters before and after cuff release and to compare parameters between brachial and femoral artery. Linear regression analysis was used to assess the relationships between variables. A P value of <0.05 was considered significant. A Bland-Altman plot was used to compare hyperemic systolic shear rate that was directly measured and hyperemic systolic shear rate that was calculated using the Poiseuille equation (6). To assess reproducibility of radius, shear rate, and FMD measurements in the six subjects who underwent repeat scans, within-subject standard deviation was calculated.

**RESULTS.** Subject lipids, hematocrit, and glucose are shown in Table 1. Measurements of systolic shear rate, systolic shear stress, arterial lumen diameter, and FMD are shown in Table 2. Figure 1 shows a PCMRI image (velocity-encoded image), a surface plot of velocity across the arterial cross section, and a plot of velocity vs. radius for one sector of a typical femoral artery and a typical brachial artery. The velocity vs. radius plot includes the parabola segment that is the best fit for the segment of the velocity profile near the arterial wall. Figure 2 shows velocity vs. radius and best-fit parabola for one sector at baseline, during hyperemia, and at 1 min after cuff release for a typical femoral artery and for a typical brachial artery.

**FMD and systolic shear stress.** For the range of shear rates measured, 20–1,580 s⁻¹, blood viscosity varies between 0.0032 and 0.0036 N·s/m² (16). Therefore, a viscosity value of 0.0034 N·s/m² was used to calculate systolic shear stress. From baseline to hyperemia, there was an increase in femoral artery systolic shear stress (1.3 ± 0.3 to 2.8 ± 0.7 N/m²; P < 0.0001) and in brachial artery systolic shear stress (1.2 ± 0.4 to 3.8 ± 0.8 N/m²; P < 0.0001). From baseline to 1 min postrelease, there was an increase in femoral artery lumen diameter (7.03 ± 0.81 to 7.26 ± 0.76 mm; P < 0.0001) and in brachial artery lumen diameter (4.26 ± 0.73 to 4.47 ± 0.76 mm; P < 0.0001). FMD was proportional to hyperemic systolic shear stress in the femoral artery (F = 0.004, r = 0.57), in the brachial artery (F = 0.02, r = 0.49), and when the femoral and brachial arteries were analyzed together (F < 0.0001, r = 0.60; Fig. 3). FMD was also proportional to the

Table 1. Subject characteristics and measurements

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>24 (15F/9M)</td>
</tr>
<tr>
<td>Age, yr</td>
<td>27 ± 6</td>
</tr>
<tr>
<td>BMI</td>
<td>22 ± 3</td>
</tr>
<tr>
<td>TChol, mg/dl</td>
<td>171 ± 32</td>
</tr>
<tr>
<td>HDL, mg/dl</td>
<td>62 ± 13</td>
</tr>
<tr>
<td>Trig, mg/dl</td>
<td>79 ± 32</td>
</tr>
<tr>
<td>LDL, mg/dl</td>
<td>93 ± 27</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>82 ± 6</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>42 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SD. F, females; M, males; BMI, body mass index; TChol, total cholesterol; HDL, high-density lipoprotein; Trig, triglycerides; LDL, low-density lipoprotein.
Table 2. Arterial measurements

<table>
<thead>
<tr>
<th></th>
<th>Brachial</th>
<th>Femoral</th>
<th>Both Arteries</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>24</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>SSS Base, N/m²</td>
<td>1.2±.4</td>
<td>1.3±.3</td>
<td>1.3±.3</td>
</tr>
<tr>
<td>SSS Hyp, N/m²</td>
<td>3.8±.8*</td>
<td>2.8±.7+†</td>
<td>3.3±.9*</td>
</tr>
<tr>
<td>D Base, mm</td>
<td>4.26±.73</td>
<td>7.03±.81†</td>
<td>5.64±1.60†</td>
</tr>
<tr>
<td>D 1 min, mm</td>
<td>4.47±.76*</td>
<td>7.26±.76+†</td>
<td>5.87±1.60*</td>
</tr>
<tr>
<td>FMD, % ΔD</td>
<td>5.2±4.1</td>
<td>3.5±3.0‡</td>
<td>4.3±3.7</td>
</tr>
</tbody>
</table>

Values are means ± SD. SSS indicates systolic shear stress; Base, baseline value; Hyp, hyperemia immediately after cuff release; D, diameter; 1 min, 1 min after cuff release; FMD, flow-mediated dilation; % ΔD, percent change in diameter. *P < 0.0001 vs. baseline. †P < 0.0001 vs. brachial. ‡P = 0.03 vs. brachial.

change in systolic shear stress from baseline to hyperemia in the brachial and femoral arteries (P = 0.0002, r = 0.51).

Shear rate, flow, and radius. Lumen radius during peak hyperemia immediately after cuff release was unchanged from baseline radius (P = not significant). Systolic flow during peak hyperemia correlated significantly with baseline radius (r = 0.93, P < 0.0001; Fig. 4). The substitution of radius² for flow in the Poiseuille equation explains why hyperemic shear rate is inversely proportional to baseline radius: 1) shear rate is proportional to flow/radius³; 2) shear rate is proportional to radius²/radius³; and 3) shear is proportional to 1/radius. Measured hyperemic systolic shear rate indeed correlated very closely with calculated hyperemic systolic shear rate (r = 0.96, P < 0.0001), confirming the applicability of the Poiseuille model during transient postischemic hyperemia. A Bland-Altman plot comparing measured and calculated hyperemic systolic shear rate (Fig. 5) shows that directly measured shear rate was usually greater than calculated shear rate. Measured systolic shear rate was confirmed to be inversely proportional to lumen radius (r = 0.72, P < 0.0001; Fig. 6). As expected, the results also confirmed that FMD is inversely related to baseline lumen radius (r = 0.35, P = 0.02).

Reproducibility. The average time between the first scan and the repeated scan was 137 ± 68 days (range: 66–255 days). For repeated scans, the standard deviations for within-subject measurements in the femoral artery and brachial artery, respectively, were 0.12 and 0.11 mm for baseline radius, 0.15 and 0.09 mm for radius at 1 min, 39 and 62 s⁻¹ for baseline shear rate, 143 and 146 s⁻¹ for hyperemic shear rate, and 2.1 and 3.4% for FMD.

DISCUSSION

The main findings of this study are that 1) the FMD response is proportional to the hyperemic systolic shear stress stimulus in both the femoral artery and the brachial artery; 2) the Poiseuille model of flow is applicable during postischemic hyperemia in the femoral and brachial arteries: systolic shear rate is proportional to systolic flow divided by lumen radius³; and 3) hyperemic systolic flow is proportional to baseline radius², which, when substituted for flow in the Poiseuille equation, explains why hyperemic systolic shear rate is inversely proportional to radius. The results explain a frequently noted but poorly understood phenomenon: why FMD is strongly and inversely dependent on baseline arterial size in both the brachial and femoral arteries.

This study explains that the hyperemic systolic shear stimulus for FMD is inversely proportional to baseline arterial radius due to the dependence of hyperemic systolic flow on 1/radius². Previous studies in the common femoral (29, 30) and coronary arteries (21) suggest that baseline conduit arterial size is determined by the mass of the bed being perfused. Postischemic conduit flow is controlled by the downstream arterioles, which depend on the muscle mass being supplied. Therefore, the inverse dependence of FMD on baseline arterial

![Fig. 1. For a typical femoral (top) and brachial (bottom) artery, a baseline velocity-encoded phase-contrast magnetic resonance image of the cross section during systole is shown, followed by a surface plot of the velocity profile, followed by a plot of systolic velocity vs. radius for 1 of 12 sectors around the arterial circumference.](http://ajpheart.physiology.org/)

![Image 209x55 to 326x165](http://ajpheart.physiology.org/)

![Image 209x190 to 326x307](http://ajpheart.physiology.org/)
size does not necessarily reflect better conduit artery endothelial function. Instead, it reflects how FMD is induced.

It is possible that the close relationship between hyperemic shear and FMD is also partly due to a common vascular mediator. In fact, it has been shown that postocclusion hyperemia in the forearm is dependent, in part, on nitric oxide (25). Therefore, disease states or risk factors that impair brachial artery endothelial function could also impair forearm flow after occlusion release. Indeed, a recent publication did demonstrate an association between reduced postischemic forearm flow and various cardiovascular risk factors (26).

Poiseuille’s Law refers to steady-state flow in a long, straight, rigid tube. Its applicability to transient postischemic hyperemia has not been evaluated. In our study, directly measured hyperemic systolic shear rate correlated closely with calculated hyperemic systolic shear rate. This confirms the applicability of the Poiseuille equation during transient postischemic hyperemia. However, the directly measured value was usually greater. This reflects the fact that the systolic velocity profile during pulsatile flow in distensible arteries is somewhat blunted, which leads to a shear rate calculated using a fully developed paraboloid model to underestimate the true value (19, 28, 32, 33). The method of directly measuring shear rate used in this study accounts for a skewed, blunted velocity profile in a cross section that does not have to be precisely circular.

Previous studies in humans have shown that baseline shear of the brachial artery predicts FMD (21), even independent of baseline diameter (17), and that the ratio of FMD to postischemic percent shear rate change differs between men and women (24). Using PCMRI, we have previously shown in the brachial artery that FMD is proportional to hyperemic systolic shear stress of normal subjects (32). The present study extends our previous study in several important ways. First, the femoral artery was evaluated in this study as well as the brachial artery, which broadens the range of arterial sizes studied. Second, shear stress and radius were calculated directly using a best-fit parabola near the wall. This approach allows subpixel precision in dimension measurement to be achieved (28). Third, the FMD-systolic shear stress proportionality was demonstrated using an occlusion distal to the site being imaged, despite the fact that FMD after release of a distal occlusion is less than FMD after proximal occlusion (1, 5, 38). This may be important because FMD after distal occlusion is more fully nitric oxide dependent (16).

Fig. 2. Plot of systolic velocity vs. radius for 1 of the 12 sectors around the arterial circumference at baseline, during hyperemia, and at 1 min after occlusion release for a typical femoral artery and for a typical brachial artery.
The standard deviations of artery dimension and FMD measurements repeated on a separate occasion are similar to reported values in some ultrasound studies (15, 18) but are larger than in other ultrasound studies (35, 37). The variability is higher than that recommended by a recent expert consensus on FMD by ultrasound (12). Hence, the within-subject, repeated-measures variability of our present MRI technique limits its usefulness for measuring FMD routinely. Measuring radius in all acquired phases of the cardiac cycle could potentially improve this variability in future studies.

MRI has only recently been applied toward assessing peripheral endothelial function (2, 32, 36). The advantages of using MRI are twofold: 1) a cross-sectional image is obtained and 2) a fixed imaging plane can be obtained repeatedly, reducing operator dependence. An additional advantage of using PCMRI, in which flow velocity is encoded as pixel intensity, is that dimension and shear information can be obtained simultaneously.

Limitations. Accuracy of measurement is limited by the need to optimize the MRI scanning parameters that determine spatial resolution, temporal resolution, signal-to-noise ratio, and duration of image acquisition. For example, increasing temporal resolution typically compromises signal-to-noise ratio and, therefore, accuracy of radius measurement. The instantaneous postischemic systolic shear stress could not be obtained because the duration of the postischemia PCMRI scan was 25 s. However, this may actually be an advantage, because duration of hyperemia is an important component of the stimulus for FMD (13, 23). Therefore, our measure is essentially an integration of the hyperemic shear stimulus acting over a finite time interval.

In conclusion, we have used PCMRI to show why the hyperemic shear stimulus for FMD is greater in small arteries. We showed that postischemic systolic flow is proportional to

Fig. 3. Flow-mediated dilation (FMD) response, expressed as percent change in diameter, is proportional to the hyperemic systolic shear stress stimulus (HSSS) for both the brachial and femoral arteries.

Fig. 4. Hyperemic systolic flow is strongly proportional to baseline radius² for the brachial and femoral arteries.

Fig. 5. Bland-Altman plot comparing directly measured with Poiseuille-calculated hyperemic systolic shear rate. The 2 measures are strongly correlated (r = 0.93, P < 0.0001), but the calculated value usually underestimates the measured value modestly.

Fig. 6. Hyperemic systolic shear rate is inversely proportional to the baseline radius for the brachial and femoral arteries.
radius squared in the range of femoral and brachial artery sizes. We then applied the Poiseuille relationship, which we showed to be applicable in transient postischemic hyperemia. When substituting radius² for flow in the Poiseuille relationship, where shear is proportional to flow/radius², the result was that hyperemic systolic shear rate is proportional to radius²/radius³, or 1/radius. Because hyperemic flow induced by distal ischemia is not a function of conduit artery endothelial function, these findings suggest that the greater FMD in small conduit arteries compared with large arteries does not reflect better inherent endothelial function of small conduit arteries. By enabling the evaluation of the hyperemic shear stimulus for FMD, PCMRI enhances the understanding of basic mechanisms underlying FMD.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the assistance of Ann Munson in coordinating the research participants.

REFERENCES


