Flow-Mediated Dilation of the Radial Artery Is Offset by Flow-Induced Reduction in Transmural Pressure

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Abstract—Flow-mediated dilation of the brachial or radial artery in response to transient hyperaemic flow, the most widely used test of endothelial function, is only manifest after flow decays back to baseline. We examined whether this dissociation of flow and diameter might be explained by a reduction in transmural pressure generated by high flow. Studies were performed in healthy subjects 20 to 55 years of age. Flow-mediated dilation was measured in the radial artery using a standard protocol and after flow interruption at peak hyperemia during brachial artery infusion of saline and the NO synthase inhibitor $N^G$-monomethyl-L-arginine (8 μmol/min). Flow interruption 20 seconds after cuff release (during high flow but no dilatation) produced an immediate increase in radial artery diameter of 5.36±2.12%, inhibited by $N^G$-monomethyl-L-arginine to 1.09±0.67% (n=8; P<0.001). Mean intra-arterial radial blood pressure and, hence, transmural pressure fell after cuff release by a mean of 26±1.8 mm Hg (n=6; P<0.0001) at the time of peak hyperemic flow. Modulation of transmural pressure within the brachial artery by cuff inflation around the artery demonstrated that this fall is sufficient to reduce arterial diameter by an amount similar to flow-mediated dilation. These results suggest that flow-dependent, NO-dependent dilatation is offset by a flow-induced fall in local arterial pressure and, hence, in transmural pressure. Shear related NO release is likely to play a greater role in the short-term regulation of arterial tone than that suggested by flow-mediated dilation. (Hypertension. 2011;57:00-00.)

Key Words: blood flow velocity ■ blood pressure ■ nitric oxide ■ vasodilation ■ vascular endothelium-dependent relaxation
generate hyperaemic flow in the forearm. Radial artery images and velocity waveforms were acquired just before cuff deflation and for 5 minutes after deflation during reactive hyperemia. After 30 minutes recovery, L-NMMA (8 μmol/min) was infused into the brachial artery for 5 minutes and continued, while an identical FMD measurement was performed (ie, an identical sequence of image acquisition before and after cuff inflation for 5 minutes). This dose of L-NMMA has no effect on baseline arterial diameter but markedly blunts the response to FMD. The flow interruption protocol was identical, but the forearm cuff was inflated for a second time 20 seconds after the 5 minutes cuff inflation at the time of maximal hyperaemic flow. In both protocols, the diameter of the radial artery was measured by edge detection software (Brachial Analyzer, Medical Imaging Applications). FMD was calculated as the maximal percentage change, from baseline, in radial artery diameter. Control studies without infusion of L-NMMA were performed in 6 subjects to confirm that measurements of FMD (using either protocol) repeated after 30 minutes were similar.

To investigate the fall in hydrostatic pressure and, hence, change in transmural pressure along the upper limb during hyperaemic flow, a standard FMD protocol was performed without brachial artery infusion of drugs (n=6) but during continuous monitoring of digital artery blood pressure (Finometer, Finapres). In 2 subjects this was repeated during continuous intra-arterial monitoring of radial artery blood pressure. A 22-gauge cannula was inserted into the right radial artery under local anesthesia and connected to a pressure monitor (model 64s, Hewlett Packard), the output of which was digitally recorded. The change in arterial calibre induced by a change in transmural pressure was investigated using a custom-made fluid-filled cuff (n=6). This cuff distributed pressure circumferentially around the arm but had an acoustic window allowing ultrasound imaging of the brachial artery. The cuff was placed around the brachial artery, the brachial artery was scanned in longitudinal section, and end-diastolic images were acquired as above. Pressure in the cuff was then increased to 20 mm Hg with images acquired throughout. The brachial artery was imaged rather than the radial artery, because cuff placement around the forearm does not transmit pressure directly to the radial artery because of the deep course of the radial artery in the mid forearm. To investigate the role of α-adrenergic tone on blood flow and radial artery diameter, we infused phenolamine (10, 30, and 100 μg/min; each dose for 7 minutes; n = 6) into the brachial artery (using the same methodology as described for L-NMMA).

Statistical Analysis

Results are presented as mean±SE. Comparison of responses between those obtained using the standard and flow interruption protocols was by ANOVA for repeated measures. All of the tests were 2 tailed, and P<0.05 was taken as significant. SPSS version 16.0 was used for all of the analyses.

Results

Typical changes in arterial blood flow, diameter, and intraradial blood pressure after a standard FMD protocol with intra-arterial blood pressure monitoring are shown in Figure 2. After cuff deflation, flow rapidly rises to a maximum within 9 seconds and then decays to baseline over 1 to 2 minutes. Mean±SE values of baseline and maximal flow were 46±2.5/163±8.6 mL/min, respectively, with corresponding estimates of shear stress of 13.7±0.7 dynes/cm² at baseline and 40.3±2.1 dynes/cm² after cuff deflation. Arterial diameter starts to increase as flow returns to baseline (mean time to maximal increase from cuff deflation of 49±2.7 seconds). The rapid increase in flow was associated with a mean drop in mean arterial blood pressure of 26±1.8 mm Hg (P<0.001, with a similar fall obtained during intra-arterial recordings: 20 and 26 mm Hg; Figure 2). Figure 3 shows typical changes in velocity and arterial diameter over the flow interruption protocol.
in blood flow and diameter after the flow interruption protocol where hyperaemic flow distal to the arterial segment imaged is interrupted. Intra-arterial pressure is not shown, because pressure in the distal radial artery is obliterated by cuff inflation to interrupt flow. Immediately after interruption of hyperaemic flow, the radial artery proximal to the flow-interrupting cuff starts to dilate (mean time to maximal dilation from flow interruption: 8.0 ± 1.2 seconds). The degree of dilation immediately after flow interruption was similar to maximal dilation occurring later in the standard protocol when flow had decayed close to baseline (6.55 ± 0.65% versus 5.36 ± 2.12%, for standard and flow interruption protocols, respectively; P value not significant).

In both protocols, FMD was substantially inhibited by L-NMMA (from 6.55 ± 0.65% to 1.01 ± 0.81% and from 5.36 ± 2.12% to 1.09 ± 0.67% for standard and flow interruption protocols, respectively; each P < 0.001; Figure 4). Cuff inflation around the brachial artery to decrease transmural pressure produced a progressive decrease in arterial diameter with a mean decrease of 7.65 ± 0.98% (P < 0.001) when transmural pressure was decreased by 20 mm Hg. Changes in shear stress during cuff inflation were negligible (<10%) in comparison with those during reactive hyperemia (>200%). Local infusion of phentolamine into the brachial artery produced significant changes in blood flow (from 36 ± 4 to 69 ± 8 mL/min; P < 0.01) and shear stress (from 10.7 ± 1.2 to 20.5 ± 2.4 dynes/cm²; P < 0.01), but there was no significant change in radial artery diameter (mean change in diameter: 1.6 ± 1.1% at the highest dose; P value not significant).

**Discussion**

The time delay of FMD after peak hyperaemic flow such that FMD occurs when flow and shear have returned almost to resting levels could be attributed to a delayed release, in response to an increase in shear stress, of NO or of vasodilatation of vascular smooth muscle to NO. However, rapid release of NO in response to shear stress is seen in isolated cells, and rapid vasodilation to NO occurs in isolated vessels. An alternative possibility is that, during high flow, reduction in vascular tone by shear stress-induced NO-mediated vasodilation is masked by an opposing vasoconstrictor stimulus. Such vasoconstriction could result from a hemodynamic effect leading to a decrease in transmural pressure and/or an increase in vascular smooth muscle tone, such as a shear stress-stimulated release of a vasoconstrictor mediator. The main finding of the present study is that, when peak hyperaemic flow is interrupted, there is an immediate dilation of the artery, which can be blunted by NO synthase inhibition. This suggests that the dilation is because of immediate and sustained release of NO stimulated by the initial high shear stress and immediate cessation, on flow interruption, of an opposing flow-associated constrictor force. Such constriction could arise through shear-stimulated release of a constrictor mediator, such as endothelin, or a

Figure 2. Typical traces showing intra-arterial pressure in the radial artery, radial artery flow velocity, radial artery diameter, and cuff pressure during a standard flow-mediated dilation (FMD) protocol. Reactive hyperemic flow is associated with a drop in mean arterial pressure of approximately 20 mm Hg. Maximal dilation occurs when flow velocity and mean arterial pressure return to baseline values.
transient increase in sympathetic tone. However, the immediate cessation of such a stimulus to vasoconstriction on flow interruption is unlikely. Furthermore, effects of high-dose intra-arterial $\alpha$-adrenergic blockade on radial artery tone are minimal, which makes a transient increase in sympathetic tone unlikely. The simplest explanation for a flow-induced constriction is a reduction in transmural pressure resulting from a drop in the local intra-arterial pressure generated by the high flow. A pressure drop in mean intra-arterial pressure along conduit arteries is an inevitable consequence of flow through the conduit artery (Poiseuille law) but under resting conditions is usually $<3$ mm Hg. During peak hyperaemic flow, however, a substantial drop in pressure across the conduit arteries is expected. In the present investigation, we found that mean intra-arterial radial artery pressure fell by $20$ mm Hg during peak hyperaemia. This would result in a fall in transmural pressure of the same magnitude. To determine whether such a change in transmural pressure could influence radial artery diameter independent of flow, we modulated transmural pressure by inflation of an external cuff around the artery. Decreasing transmural pressure by $20$ mm Hg resulted in a decrease in radial artery diameter of 7.6%, that is, a change of diameter similar to the maximal change after peak hyperaemic flow. This suggests that NO-mediated reduction in vascular tone occurring immediately after an increase in shear stress is offset by a vasoconstrictor force resulting simply from the hydrostatic drop in mean arterial pressure along the brachial and radial arteries and resultant drop in transmural pressure at the radial artery. We cannot exclude an additional “myogenic” type response to change in transmural pressure having an additional influence on arterial tone and diameter. However, because the myogenic response to an increase in transmural pressure is

Figure 3. Typical traces showing intra-arterial pressure in the radial artery, radial artery flow velocity, radial artery diameter, and cuff pressure during an flow-mediated dilation (FMD) protocol, where flow is interrupted at peak hyperemia. Flow interruption is immediately followed by a dilation of the radial artery.

Figure 4. Effect of N$^\omega$-monomethyl-L-arginine (l-NMMA) on flow-mediated dilation (FMD) measured using a standard protocol and immediately after flow interruption at peak hyperemia. *P<0.001 vs saline control.
vasoconstriction, any such response when transmural pressure decreases would be opposite to that observed.

These findings provide an explanation for the temporal dissociation of FMD with flow/shear and the complex relationship of FMD with shear stress observed in a number of previous investigations. Lack of a clear relationship between FMD and shear stress has been cited as evidence against shear stress being the stimulus for FMD. The present findings are, however, entirely consistent with immediate release of NO in response to shear as observed in isolated cells. When FMD is measured using a standard protocol >30 seconds after cuff release when flow has returned to baseline values, effects of the opposing flow-induced change in transmural pressure and associated vasoconstriction are likely to be minimal, and FMD will represent the dilator response to NO release in response to preceding shear stress. However, it is possible that correction for such flow/pressure-induced vasoconstriction would allow FMD to be standardized both for the magnitude and duration of shear and for the opposing vasoconstrictor force. This work has implications for the physiological regulation of arterial tone and calibre by both transient and sustained alterations in flow. Shear related NO release is likely to play a greater role in the short-term regulation of arterial tone than that suggested by FMD. Previous studies that have investigated the vasodilator response to a sustained increase have concluded that the dilator response to a sustained increase in flow is largely NO independent and that sustained shear does not increase NO. However, the opposing flow/pressure-induced constrictor response, although of lower magnitude than that occurring after reactive hyperemia, will also be sustained, thus masking sustained shear induced NO.

Our study is subject to a number of limitations. Although the reduction in transmural pressure is sufficient to account for a transient flow-induced vasoconstriction opposing FMD, we cannot exclude another cause of transient vasoconstriction. Our findings of a transient flow/pressure-induced vasoconstriction relate to the brachial artery, and there may be differences in different arterial beds.

In conclusion, the initial flow/shear-mediated dilator response to reactive hyperemia is masked by an opposing vasoconstriction caused by a drop in hydrostatic pressure along the upper limb (as a consequence of the high flow) and, hence, fall in transmural distending pressure. These findings explain the temporal dissociation and complex relation of FMD to shear stress. FMD, when measured 30 seconds to 2 minutes after cuff release, when flow has decayed close to baseline values, remains a reliable measure of the NO response to preceding shear.

Perspectives

FMD, dilation of the brachial or radial artery in response to an increase in flow generated by downstream reactive hyperemia, is the most widely used noninvasive test of endothelial function. An increase in flow and, hence, shear stress is thought to activate endothelial NO synthase with the resultant increase in NO causing a shear induced vasodilation. However there is a temporal dissociation between the increase in flow/shear and vasodilation such that vasodilation only occurs when flow decays back to baseline. The role of shear as a stimulus for FMD has thus been questioned. This dissociation likely results from a fall in local intra-arterial pressure and, hence, in transmural pressure distending the artery, which is generated by high flow. Interrupting flow removes this confounding influence and reveals an immediate response to shear stress. These results are consistent with FMD as a response to shear stress. Correction for influences of flow and shear might make the measurement more sensitive to a true change in endothelial cell function. Shear related NO release is likely to play a greater role in the short-term regulation of arterial tone than that suggested by FMD.

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Disclosures

None.

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