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## DOES BRACHIAL ARTERY FMD PROVIDE A BIOASSAY FOR NITRIC OXIDE?

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### Abstract

This study sought to better define the role of nitric oxide (NO) in brachial artery flow-mediated vasodilation (FMD) in young, healthy humans. Brachial artery blood velocity and diameter were determined (ultrasound Doppler) in eight volunteers ( $26 \pm 1$  yrs) before and after 5-min forearm circulatory occlusion with and without intra-arterial infusion of the endothelial nitric oxide synthase (eNOS) inhibitor L-NMMA (0.48 mg/dl/min). Control (CON) and L-NMMA trials were performed with the occlusion cuff placed in the traditional distal position, as well as proximal to the measurement site. FMD was significantly reduced, but not abolished, by L-NMMA in the distal cuff trial ( $8.9 \pm 1.3$  to  $6.0 \pm 0.7\%$ , CON vs. L-NMMA,  $P = 0.02$ ), with no effect of L-NMMA on FMD with proximal cuff placement ( $10.6 \pm 1.2$  to  $12.4 \pm 1.7\%$ , CON vs. L-NMMA,  $P = 0.39$ ). When the reduction in shear stimulus following L-NMMA was taken into account, no drug difference was observed for either distal ( $0.26 \pm 0.02$  to  $0.23 \pm 0.03$ , CON vs. L-NMMA,  $P = 0.40$ ) or proximal ( $0.23 \pm 0.08$  to  $0.23 \pm 0.03$ , CON vs. L-NMMA,  $P = 0.89$ ) FMD trials. These findings challenge the assertion that NO is obligatory for brachial artery FMD, and call into question the sensitivity of this procedure for non-invasive determination of NO bioavailability in young, healthy humans.

### Keywords

Endothelium; Vasodilation; Blood Flow; Ultrasound Doppler

### INTRODUCTION

In 1992, Celermajer *et al.*<sup>1</sup> introduced the method of measuring changes in conduit artery diameter following a period of circulatory occlusion as a non-invasive approach for *in vivo* determination of endothelium-dependent vasodilation in humans. In the two decades that have followed, the application of flow-mediated vasodilation (FMD) testing in clinical research has become widespread, a progression that is exemplified by adoption of this methodology into large-scale clinical trials, including recent phases of the Framingham

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Heart Study <sup>2,3</sup>. The FMD test is particularly appealing due, at least in part, to a previous report revealing a correlation between vasodilatory capacity of the peripheral and coronary arteries <sup>4</sup>, thus presenting FMD as a potential surrogate measure of coronary endothelial health. Further, it has been clearly demonstrated that FMD provides independent predictive information for future cardiovascular events beyond traditional risk factors <sup>5</sup>. FMD testing has thus emerged as a straight-forward, non-invasive approach for determining vascular function in health and disease.

Perhaps one of the most attractive aspects of FMD testing is that it has been purported to provide an assay of vascular endothelial nitric oxide (NO) bioavailability. The presence of this endothelially-derived vasodilator has become synonymous with vascular health <sup>6</sup>, and thus the prospect of quantifying NO in a non-invasive manner makes FMD testing particularly appealing for both risk assessment and patient prognosis <sup>7</sup>. The ability of endothelial nitric oxide synthase (eNOS) inhibition via *N*<sup>G</sup>-monomethyl-L-arginine (L-NMMA) to completely abolish radial <sup>8,9</sup> and brachial <sup>10</sup> artery FMD provided initial evidence that FMD is governed primarily through the release of NO. However, in the face of significant technical and methodologic refinements in the field, recent studies have begun to challenge the existing dogma regarding the NO-dependent nature of the FMD response. Indeed, recent studies in the radial artery have identified only minimal (0–20%) reductions in FMD after L-NMMA administration <sup>11,12</sup>, leaving considerable uncertainty as to whether this test can still be relied upon to provide an index of NO bioavailability. Whether findings from these recent studies in the radial artery may be extended to the brachial artery, the vessel most often used for FMD testing, has not been determined.

In addition to this renewed controversy concerning the role of NO in traditional FMD assessment, there is also evidence that location of the occlusion cuff can impact the mechanisms responsible for vasodilation <sup>13</sup>. Indeed, while placement of the cuff proximal to the imaged artery often results in greater vasodilation, this technique exposes the conduit artery to the ischemic milieu during occlusion, and results in a vasodilation that is only partially NO-dependent <sup>10</sup>. Despite this finding, FMD testing with proximal cuff placement remains commonplace in the clinical literature <sup>14–16</sup>, raising some concern with respect to whether findings from studies utilizing this approach have, in fact, provided a true assessment of NO bioavailability.

Considering these recent ambiguities in the literature, and in the face of the increasing inclusion of FMD testing as a primary outcome measure in clinical trials <sup>17,18</sup>, further study of the relationship between brachial artery FMD and NO is required. Thus, using up-to-date methods for FMD testing <sup>19</sup>, the present study was undertaken to critically examine the role of NO in brachial artery FMD in young, healthy humans. Based upon the preponderance of prior data and inferences in the literature, we hypothesized that: 1) With conventional (distal) cuff placement, eNOS inhibition with L-NMMA would ablate the brachial artery FMD response; and 2) With proximal cuff placement, L-NMMA would diminish, but not abolish, brachial artery FMD.

## METHODS

### Subjects and General Procedures

Eight young, healthy volunteers participated in the current study. All subjects were non-smokers, normotensive (<140/90 mmHg), and free of overt cardiovascular disease, as determined by health history questionnaire and physical examination. Females were studied within the first five days of their menstrual cycle, as FMD measurements of the brachial artery have been documented to fluctuate with menstrual phase <sup>20</sup>. Protocol approval and written informed consent were obtained according to the University of Utah and Salt Lake

City Veteran Affairs Medical Center Institutional Review Board requirements. All data collection took place with subjects supine, in a thermoneutral environment.

## Protocol

Subjects reported to the laboratory at 0800 in the fasted state, having abstained from exercise and caffeine for the previous 12 hours. Using sterile technique, an arterial catheter (Arrow, 18G, 20cm) was placed in the brachial artery approximately 10 cm distal to the axilla and advanced 6–8 cm in the retrograde direction. The catheter was placed in this region of the upper arm to ensure that L-NMMA entered the artery “upstream” (~15 cm) of the ultrasound Doppler sample volume, allowing the assessment of NOS blockade on brachial artery diameter and blood velocity. The experimental protocol consisted of four separate FMD tests; a control trial with cuff “above” (proximal to) the ultrasound probe, a control trial with cuff “below” (distal to) the ultrasound probe, a L-NMMA trial with proximal cuff placement, and a L-NMMA trial with distal cuff placement. The protocol was balanced for cuff placement, but ordered such that the control trials were always performed prior to L-NMMA infusion due to the long-lasting effects of the drug, with 60 min between control and L-NMMA trials.

## Measurements

Details of the FMD procedure have been described previously<sup>21</sup> and were performed in accordance with recently published guidelines<sup>19</sup>. Briefly, a blood pressure cuff was placed on the right arm, either proximal or distal to the ultrasound Doppler probe on the brachial artery. Simultaneous measurements of brachial artery blood velocity and vessel diameter were performed using a linear array transducer operating in duplex mode, with an imaging frequency of 14 MHz and Doppler frequency of 5 MHz (Logic 7, GE Medical Systems, Milwaukee, WI). All measurements were obtained with the probe appropriately positioned to maintain an insonation angle of 60° or less. The sample volume was maximized according to vessel size and was centered within the vessel on the basis of real-time ultrasound visualization. The brachial artery was insonated approximately midway between the antecubital and axillary regions, and measurements of diameter and blood velocity ( $V_{\text{mean}}$ ) were obtained continuously at rest and for 2 minutes after cuff deflation. End-diastolic, ECG R-wave-gated images were collected via video output from the Logic 7 for off-line analysis of brachial artery vasodilation using automated edge-detection software (Medical Imaging Applications, Coralville, IA). Heart rate was monitored from a standard three-lead ECG. Arterial blood pressure measurements were collected continually from within the brachial artery, with the pressure transducer placed at the level of the catheter (Transpac IV, Abbot Laboratories).

## L-NMMA

Upper and lower arm volumes were determined anthropometrically, and then used for the calculation of drug dosing, as described previously<sup>22</sup>. L-NMMA (Bachem, Germany) was infused intra-arterially at 0.48 mg/dl (2.9–4.4 ml/min infusion rate) for 10 min before and continuously during FMD testing.

## Analyses

FMD was quantified as the maximal change in brachial artery diameter after cuff release, expressed both in absolute units (i.e. mm) and as a percentage increase (%) above baseline. Shear rate was calculated according to the equation: shear rate ( $s^{-1}$ )= $V_{\text{mean}} \cdot 8 /$  vessel diameter. Blood flow was calculated as: Blood flow (mL · min)= $(V_{\text{mean}} \cdot \pi \cdot [\text{vessel diameter}/2]^2 \cdot 60)$ . For both shear rate and blood flow, cumulative area under the curve values were integrated with the trapezoidal rule and calculated as follows:

$$\sum \{y_i[x_{(i+1)}-x_i]+(1/2)[y_{(i+1)}-y_i][x_{(i+1)}-x_i]\}.$$

10,15

Normalized FMD was calculated by dividing FMD (percentage) by the cumulative shear rate area under the curve at the time of peak brachial artery vasodilation, multiplied by 1000. Mean arterial pressure (MAP) was calculated using the time integral of the directly measured arterial waveform.

### Statistics

Statistics were performed with the use of commercially available software (SigmaPlot 11.0, Systat Software, Point Richmond, CA). Two-way repeated-measure analysis of variance was used to identify significant changes in measured variables within and between drug conditions and cuff placement, with the Bonferroni test used for post hoc analysis when a significant main effect was found. All group data are expressed as means  $\pm$  SE. Significance was established at  $P < 0.05$ .

## RESULTS

Subject characteristics are presented in Table 1.

### FMD with Distal Cuff Occlusion

Prior to cuff occlusion, L-NMMA administration resulted in a significant reduction in brachial artery blood flow, while heart rate (HR), mean arterial blood pressure (MAP), brachial artery diameter, and brachial artery shear rate all remained unchanged (Table 2). Following 5-min suprasystolic cuff occlusion, both cumulative blood flow AUC (Table 2) and cumulative shear rate AUC (Figure 1) were significantly reduced following administration of L-NMMA. A modest ( $\approx 33\%$ ) reduction in absolute change in brachial artery diameter (Table 2) and %FMD was observed following L-NMMA; however, when FMD was normalized for the reduced shear rate following L-NMMA, the difference between L-NMMA and control trials was no longer apparent (Figure 1).

### FMD with Proximal Cuff Occlusion

As with the distal cuff trial, L-NMMA administration reduced resting brachial artery blood flow, while HR, MAP, brachial artery diameter, and brachial artery shear rate were similar between control and L-NMMA trials (Table 2). After cuff release, the hyperemic response was greater than with distal cuff occlusion, with augmented peak shear rate and shear rate AUC at peak vasodilation in both control and L-NMMA trials (Table 2). Similar to the distal cuff trial, L-NMMA administration blunted the post-occlusion hyperemic response, with significant reductions in both blood flow AUC (Table 2) and shear rate AUC (Figure 2). FMD was similar between control and L-NMMA trials, whether expressed as absolute change in brachial artery diameter (Table 2), %FMD, or FMD normalized for shear rate (Figure 2).

## DISCUSSION

Using up-to-date measurement techniques, we have identified for the first time that the majority of vasodilation provoked by brachial artery flow-mediated vasodilation (FMD) testing is not attributable to NO. Indeed, with the occlusion cuff placed in the traditional, distal position, inhibition of eNOS via intra-arterial L-NMMA resulted in only a modest

( $\approx 33\%$ ) reduction in FMD. However, L-NMMA administration also reduced the post-occlusion hyperemic response, thereby diminishing the shear stimulus for brachial artery vasodilation. When this effect was taken into account, FMD normalized for shear rate was not different between control and L-NMMA conditions. With the occlusion cuff placed proximal to the ultrasound probe, the FMD response was unchanged by eNOS inhibition, highlighting the NO-independent nature of this variant of the FMD method. Taken together, these findings challenge the assertion that NO is obligatory for brachial artery FMD, and question the role of this procedure in the non-invasive determination of NO bioavailability in young, healthy humans.

### The Role of NO in FMD

In recent years, it has become widely accepted that FMD is a NO-dependent event, and therefore results from FMD testing have been thought to provide a “bioassay” for NO. Just three years after FMD testing was first described, a seminal study from Joannides *et al.*<sup>8</sup> reported complete ablation of flow-dependent dilation in the radial artery following intra-arterial L-NMMA administration, leading to the conclusion that “NO is essential for FMD of large human arteries”. This was followed by additional studies in the radial<sup>9, 23–27</sup> and superficial femoral<sup>28</sup> arteries that collectively reported a reduction or complete absence of FMD in the presence of L-NMMA (Table 3). However, it is noteworthy that even the more recent of these NO-blocking studies did not adhere to what are now standardized procedures for FMD testing<sup>19</sup>, leaving some doubt with respect to interpretation of the findings. In fact, two very recent studies in the radial artery undertaken with strict adherence to current guidelines for FMD testing have challenged this earlier body of work, together identifying a minimal contribution of NO to FMD in young, healthy humans<sup>11, 12</sup>. Thus, the literature concerning the ability of FMD to non-invasively assay NO bioavailability now appears somewhat equivocal (Table 3), and has become an area of increased debate<sup>29–35</sup>.

The present study is of significance inasmuch as it extends findings from the recent radial artery studies to the brachial artery, the conduit vessel most often utilized for FMD testing. With this approach, we have demonstrated, for the first time, that NO is not obligatory for brachial artery FMD, which is in contrast to the work of Doshi *et al.*<sup>10</sup>. In the only other study that has examined the effect of L-NMMA on brachial artery FMD, these authors reported complete ablation of brachial artery FMD following L-NMMA, leading to the conclusion that FMD is mediated exclusively by NO. There are at least two key differences between the former and current studies that may explain these divergent results. First, the use of wrist occlusion in the previous study provoked ischemia only in the hand, a significantly smaller volume of tissue than the present study, where the entire lower arm was occluded, which has become the standardized approach<sup>19</sup>. Indeed, while wrist occlusion evoked a 6-fold increase in peak brachial artery blood flow, this hyperemic response is substantially less than the 11-fold increase observed in the present study (Table 2, Figure 1) and may preclude a direct comparison between studies. In addition, the study from Doshi *et al.*<sup>10</sup> did not examine the time integral for the hyperemic stimulus, reporting instead a singular, peak value for blood flow that did not differ between saline and L-NMMA trials. This is an important distinction, as the preferred method of quantifying the stimulus for FMD has evolved substantially in recent years and now involves examining accumulated shear rate up to the time of peak vasodilation, a numerical value which may subsequently be used to “normalize” FMD to the shear stimulus on an individual basis<sup>10, 19, 36</sup>. Our group has previously documented the importance of the stimulus-response relationship for proper interpretation of FMD testing in the elderly, where a diminished hyperemic response following cuff occlusion appears to partially explain the age-related decrement in FMD<sup>37</sup>. The relevance of FMD normalization is further illustrated in the present study, where the modest but statistically significant reduction in FMD following L-NMMA is abolished when

FMD is viewed in the context of the hyperemic stimulus (Figure 1). Thus, using state-of-the-art methodology and accepted standardized techniques, findings from the present study challenge the overall conclusion from this previous study<sup>10</sup>, identifying instead only a minimal role of NO for brachial artery FMD in young, healthy humans.

### Proximal Versus Distal Cuff Placement

One of the most influential methodological factors in FMD testing is the anatomic location of the occlusion cuff relative to the measurement site. Present guidelines for FMD testing recommend placement of the occlusion cuff distal to the region of the brachial artery where measurements are performed, since proximal cuff placement exposes the vessel to an ischemic environment that may provoke vasodilation which is not endothelium-dependent<sup>13</sup>. Despite this, many clinical studies in a variety of patient populations continue to adopt this proximal cuff approach<sup>14-16</sup>, publishing FMD findings with inference to NO bioavailability. Thus, one additional aim of the present study was to further examine the role of NO on brachial artery FMD with the occlusion cuff placed proximal to the measurement site.

As expected, brachial artery vasodilation was less affected by eNOS inhibition in the proximal cuff trial; in fact, there were no significant differences in either standard or normalized FMD between control or L-NMMA conditions (Figure 2). This is in contrast with previous studies<sup>10, 38</sup> that have identified a small but significant role for NO in the FMD response with proximal cuff placement. Thus, the present results with proximal cuff placement may be viewed as challenging this previous work, and lend further support to the NO-independent nature of vasodilation for the brachial artery FMD test in young, healthy humans.

### Alternative Approaches for the Assessment of NO-dependent Vasodilation

Considering the ongoing clinical interest in endothelial function testing for risk assessment and cardiovascular disease prognosis<sup>39</sup>, our group has recently begun to investigate additional experimental paradigms for non-invasive evaluation of NO bioavailability. In keeping with the established principle that NO is released in response to vascular wall shear stress<sup>40</sup>, we have utilized small muscle mass (rhythmic handgrip) exercise to increase limb blood flow, and examined whether conduit artery vasodilation in response to this sustained increase in shear occurs via an NO-dependent mechanism. In young, healthy adults, we observed a 70% reduction in brachial artery vasodilation during handgrip exercise in the presence of L-NMMA<sup>22</sup>, implicating NO as a major contributor to shear-induced vasodilation with this exercise modality. Similarly, we have recently developed a passive exercise paradigm that provokes a substantial rise in leg blood flow<sup>41-44</sup>, and subsequently identified an 80% reduction in vasodilation in the passively moved limb following intra-arterial L-NMMA administration<sup>45</sup>. Each of these experimental approaches is easily performed, provoke a robust rise in shear rate, and appear much more NO-dependent than conventional FMD testing, especially in light of the current findings. It thus appears that these alternative paradigms may represent a preferable approach for non-invasive, *in vivo* assessment of NO bioavailability in humans.

### Perspectives

It has been clearly demonstrated that FMD provides independent, predictive information for future cardiovascular events beyond traditional risk factors<sup>5</sup>, and based on previous studies identifying the role of NO in FMD testing<sup>9</sup> it has been assumed that the prognostic value of FMD is related to the ability of this test to estimate NO bioavailability. While the current findings challenge the role of NO in the FMD response, it should be noted that this does not contradict the large body of evidence supporting FMD testing as a valid assessment of

disease risk and progression. Rather, it suggests that the FMD response should be viewed as endothelium-dependent vasodilation, but not necessarily NO-dependent vasodilation. This concept is supported by a recent brief review in *Hypertension* which discussed FMD testing and cardiovascular event prediction<sup>5</sup>, and concluded that “FMD derived using a proximal cuff is at least as predictive (of future cardiovascular events) as that derived using distal cuff placement, despite the latter being more NO dependent”.

The robust endothelium-dependent vasodilation observed in the presence of L-NMMA does raise the question of what other, non-NO pathways may contribute to the FMD response. While identifying such vasodilatory mechanisms is beyond the scope of the present study, it seems reasonable to speculate that prostaglandins and/or endothelium-derived hyperpolarizing factor (EDHF) may play an important compensatory role in the FMD response, particularly in the absence of NO. Regarding the former, Parker *et al.*<sup>12</sup> recently examined radial artery FMD during independent and combined inhibition of NO and prostaglandins via intra-arterial L-NMMA and Ketorolac, respectively, in young, healthy adults. While this study identified a small reduction in radial artery FMD following L-NMMA, no additional change was observed in the L-NMMA + Ketorolac trial, suggesting a minimal role for prostaglandins in the FMD response. With respect to EDHF, it has been reported that maximal radial artery dilation during sustained shear stress was substantially reduced when both L-NMMA and tetraethylammonium chloride (an EDHF inhibitor) were infused concomitantly compared to L-NMMA alone, implicating EDHF as an important contributor to FMD in the absence of NO. Whether the EDHF pathway is involved in BA FMD remains unknown, and is certainly deserving of further study.

## Experimental Considerations

As we did not pharmacologically challenge the eNOS blockade, we cannot exclude the possibility that the lack of a drug effect on FMD was due to incomplete inhibition of eNOS. However, the dose of L-NMMA utilized in this study (0.48 mg/dl/min,  $\approx$ 8mg/min) is equivalent to that used previously by our group<sup>22, 45</sup>, and is among the highest intra-arterial doses ever administered in humans, almost 3-fold higher than previous work demonstrating complete abolition of brachial artery FMD in the presence of L-NMMA<sup>10</sup>. This, in combination with our published evidence of a plateau in brachial artery blood flow during L-NMMA dose-response experiments<sup>22</sup>, increase confidence that local eNOS inhibition was accomplished, and that the observed responses are, therefore, not attributable to incomplete eNOS blockade. By design, in the present study the control trials always precede the L-NMMA trials, and thus we acknowledge the possibility that a drug ordering effect may exist.

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## NOVELTY AND SIGNIFICANCE

### What is New?

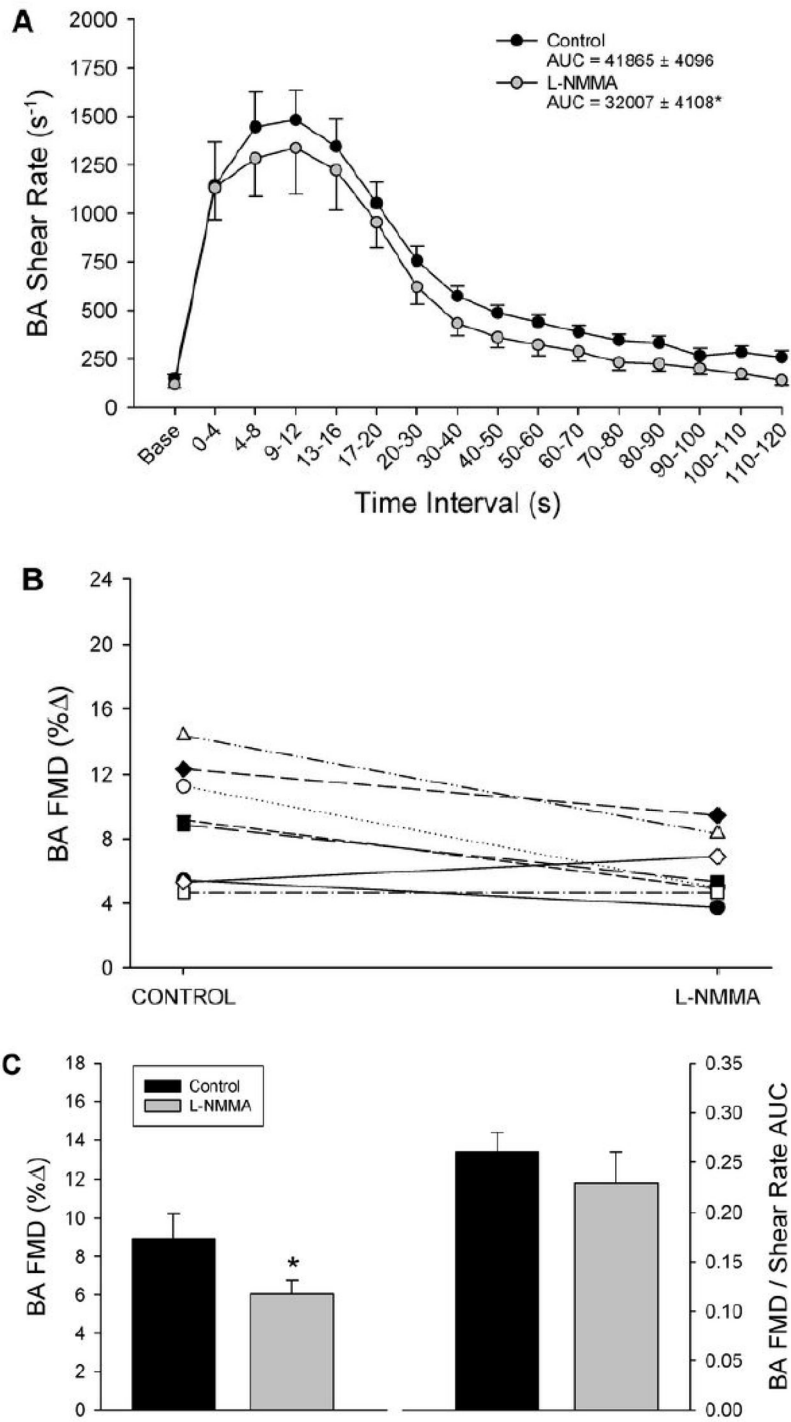
Using up-to-date measurement techniques, we have identified for the first time that the majority of vasodilation provoked by brachial artery flow-mediated vasodilation (FMD) testing is not attributable to nitric oxide (NO).

### What is Relevant?

FMD testing has been purported to provide an assay of vascular endothelial nitric oxide (NO) bioavailability, making it particularly appealing for both risk assessment and patient prognosis. Results from the present study indicate that NO is not obligatory for brachial artery FMD, and question the role of this procedure in the non-invasive determination of NO bioavailability in young, healthy humans.

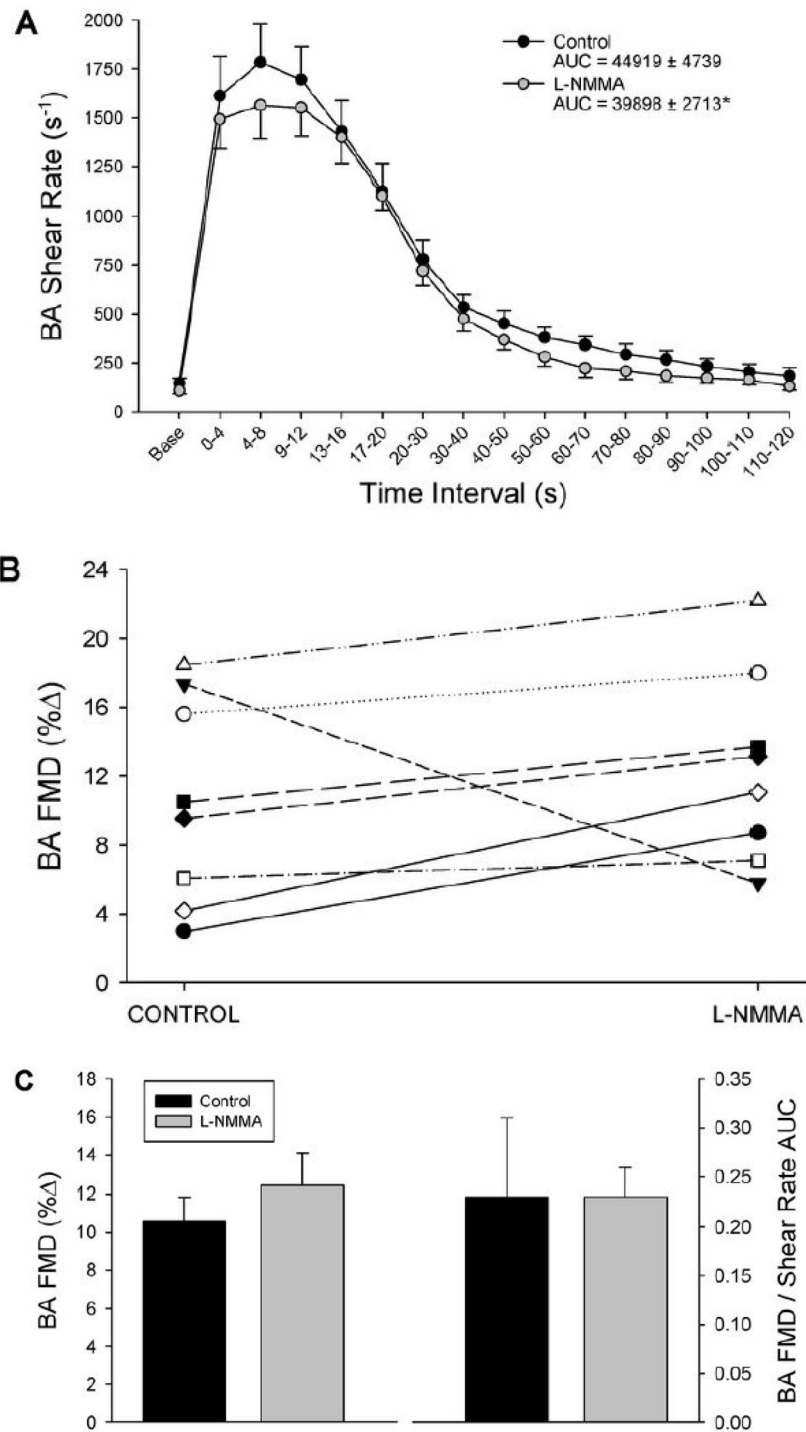
### Summary

Using both proximal and distal cuff placement, we have identified a negligible role of NO in the brachial artery FMD test in young, healthy humans. These findings challenge the convention that FMD testing provides a specific “bioassay” for NO, and emphasize the need for alternative experimental approaches for the non-invasive assessment of NO bioavailability in humans.



**FIGURE 1. Distal cuff FMD responses**

**Panel A:** Brachial artery (BA) shear rate following 5-min of distal cuff was reduced after eNOS inhibition with L-NMMA, as quantified by area-under-curve (AUC) for shear rate across the entire 120-sec post-occlusion time period. **Panel B:** Individual %FMD responses in the control and L-NMMA trials. **Panel C:** %FMD was reduced following L-NMMA (left); however, FMD “normalized” for shear rate AUC at peak dilation was not different between control and L-NMMA conditions (right). \*Significantly different than Control,  $P < 0.05$ .



**FIGURE 2. Proximal cuff FMD responses**

**Panel A:** Brachial artery (BA) shear rate following 5-min of proximal cuff was reduced after eNOS inhibition with L-NMMA, as quantified by area-under-curve (AUC) for shear rate across the entire 120-sec post-occlusion time period. **Panel B:** Individual %FMD responses in the control and L-NMMA trials. **Panel C:** Both %FMD (left) and FMD “normalized” for shear rate AUC at peak dilation (right) were similar between control and L-NMMA conditions. \*Significantly different than Control,  $P < 0.05$ .

**TABLE 1**

Subject characteristics.

<b>Variable, units</b>	<b>Value</b>
Age, yrs	26 ± 1
Sex, male:female	6:2
Height, cm	178 ± 4
Weight, kg	77 ± 5
Body Mass Index, kg/m <sup>2</sup>	24 ± 1
Arm Volume, dL	17 ± 2
Heart Rate, bpm	59 ± 1
Systolic Blood Pressure, mmHg	123 ± 4
Diastolic Blood Pressure, mmHg	72 ± 3
Mean Arterial Pressure, mmHg	90 ± 3
Glucose, mg/dl	87 ± 8
Sodium, mmol/l	140 ± 1
Potassium, mmol/l	3.9 ± 0.1
Chloride, mmol/l	103 ± 1
Calcium, mg/dl	9.1 ± 0.1
Creatinine, mg/dl	1.0 ± 0.06
Cholesterol, mg/dl	164 ± 13
Triglycerides, mg/dl	66 ± 11
HDL, mg/dl	61 ± 6
LDL, mg/dl	100 ± 10

Values are mean ± SE. HDL, High-Density Lipids; LDL, Low-Density Lipids.

TABLE 2

Baseline and FMD responses before and after L-NMMA administration with occlusion cuff placed below and above the measurement site.

Variable, units	CUFF BELOW		CUFF ABOVE	
	Control	L-NMMA	Control	L-NMMA
<b>Baseline</b>				
Heart Rate, beats/min	57 ± 1	58 ± 2	56 ± 1	59 ± 1
Mean Arterial Blood Pressure, mmHg	90 ± 3	94 ± 4	92 ± 4	95 ± 4
Brachial artery diameter, mm	4.2 ± 0.2	4.1 ± 0.2	4.2 ± 0.2	4.1 ± 0.2
Brachial artery blood velocity, cm/s	7.8 ± 0.9	5.9 ± 0.9	7.6 ± 1.3	5.6 ± 0.9
Brachial artery blood flow, ml/min	67 ± 11	46 ± 6*	65 ± 10	47 ± 9*
Brachial artery shear rate, s <sup>-1</sup>	150 ± 18	119 ± 22	146 ± 26	110 ± 18
<b>FMD</b>				
Change in brachial artery diameter, mm	0.36 ± 0.04	0.27 ± 0.04*	0.43 ± 0.07	0.50 ± 0.07
Change in brachial artery diameter, %	8.9 ± 1.3	6.0 ± 0.7*	10.6 ± 1.2	12.4 ± 1.7 <sup>†</sup>
Time to peak vasodilation, s	54 ± 6	51 ± 8	88 ± 5 <sup>†</sup>	78 ± 6 <sup>†</sup>
Blood flow AUC, ml	336 ± 39	237 ± 22*	302 ± 39	258 ± 27*
Peak Shear Rate, s <sup>-1</sup>	1508 ± 166	1387 ± 224	1808 ± 186 <sup>†</sup>	1640 ± 162 <sup>†</sup>
Shear Rate AUC at peak vasodilation	33567 ± 3191	29077 ± 4164	42681 ± 7718 <sup>†</sup>	54052 ± 4295 <sup>†</sup>

AUC, area-under-curve.

\* Significantly different than Control,  $P < 0.05$ ;

<sup>†</sup> Significantly different than Cuff Below,  $P < 0.05$ .

TABLE 3

Summary of studies examining NO-dependency of FMD in young, healthy adults. %FMD is presented as mean  $\pm$  SE.

Reference	Year	Cuff Position	Artery	%FMD Control	%FMD L-NMMA	%
oannides <i>et al.</i> <sup>8</sup>	1995	Distal	Radial	3.6 $\pm$ 0.8	-2.8 $\pm$ 1.7	-177%
Lieberman <i>et al.</i> <sup>38</sup>	1996	Proximal	Brachial	21.1 $\pm$ 3.3	7.0 $\pm$ 1.4	-67%
Doshi <i>et al.</i> <sup>10</sup>	2001	Distal	Brachial	7.3 $\pm$ 2.5	0.2 $\pm$ 2.2	-97%
Doshi <i>et al.</i> <sup>10</sup>	2001	Proximal	Brachial	11.6 $\pm$ 3.2	7.5 $\pm$ 2.3	-35%
Mullen <i>et al.</i> <sup>9</sup>	2001	Distal	Radial	5.3 $\pm$ 1.2	0.7 $\pm$ 0.7	-87%
Bellien <i>et al.</i> <sup>27</sup>	2007	Distal	Radial	10.9 $\pm$ 0.6	9.3 $\pm$ 0.8	-15%
Fischer <i>et al.</i> <sup>24</sup>	2007	Distal	Radial	11.5 $\pm$ 0.9	6.0 $\pm$ 0.7	-48%
Kooijman <i>et al.</i> <sup>28</sup>	2008	Distal	SF	4.2 $\pm$ 0.3	1.0 $\pm$ 0.2	-76%
Seddon <i>et al.</i> <sup>23</sup>	2009	Distal	Radial	6.5 $\pm$ 0.2	1.0 $\pm$ 0.3	-85%
Liumi <i>et al.</i> <sup>26</sup>	2010	Distal	Radial	7.2 $\pm$ 0.9	2.7 $\pm$ 0.7	-63%
Pyke <i>et al.</i> <sup>11</sup>	2010	Distal	Radial	5.6 $\pm$ 1.5	5.6 $\pm$ 1.3	0%
Parker <i>et al.</i> <sup>12</sup>	2011	Distal	Radial	10.0 $\pm$ 1.0	7.6 $\pm$ 1.2	-24%
Wray <i>et al.</i>	2013	Proximal	Brachial	10.6 $\pm$ 1.2	12.4 $\pm$ 1.7	+17%
Wray <i>et al.</i>	2013	Distal	Brachial	8.9 $\pm$ 1.3	6.0 $\pm$ 0.7	-33%

LNMMMA, N<sup>G</sup>-monomethyl-L-arginine; SF, superficial femoral.