Point: Flow-mediated dilation does reflect nitric oxide-mediated endothelial function

Before you embark on this debate with my redoubtable Canadian friends, you may find it advisable to take something to minimize your cardiovascular risk. I recommend the sensible precaution of a bottle of excellent Australian Shiraz. Pour yourself a generous measure and take a few sips.

Now, if your glass appears half full, you might agree that the abundance of flow-mediated dilatation (FMD) studies in the cardiovascular literature in the past decade is attributable to the seductive idea that it provides a functional bioassay for in vivo endothelium-derived nitric oxide (NO) bioactivity in humans. Impaired NO-mediated endothelial function has gained acceptance as a sentinel atherogenic event (4, 11, 21), so a simple and cheap noninvasive marker of NO “dys”-function may provide a “barometer” of cardiovascular disease risk, which could be used to predict individuals at highest risk of future cardiovascular events (2, 27, 30) or those with less stable advanced lesions (4). It might also be possible to optimize medication and risk factor advice to target directly measured vascular health, rather than surrogates such as blood pressure, lipid levels, or inflammatory markers. Risk reduction is, after all, ultimately dependent on changes in the artery wall and a direct measure of arterial “fitness” would provide a powerful tool. Of course, if your glass seems half empty, it is possible that every physician and scientist with access to an ultrasound machine and a delusion of grandeur is riding the latest research bandwagon. So is the mercury rising or falling on the FMD technique and, crucially, is it NO dependent?

It is true that when Celermajer and colleagues (6) introduced the idea of using cuff occlusion to examine endothelial function by inducing arterial shear stress in 1992, important assumptions were made. It was known at the time that human conduit arteries dilated in response to increased blood flow (1, 18, 23, 24), that in animals this response was dependent on an intact endothelium (20, 25), that shear stress-sensitive ion channels existed in endothelial cells (7, 14, 19), that the physiological stimulus to NO [endothelium-derived relaxing factor (EDRF) at that time] production in animals was shear stress (22) and that infusion of NO antagonists [e.g., Nω-monomethyl-L-arginine (L-NMMA)] decreased FMD in situ (8, 12). Vallance et al. (26) had also established that NO was released basally in humans and in response to pharmacological stimulation. From these atmospherics, it was inferred that the dilator response after cuff deflation was likely to be endothelium-dependent and probably NO (EDRF) mediated. Thus, although physiological studies demonstrating NO dependency of the FMD technique had not been performed in humans before the introduction and adoption of the technique, it was a clever idea predicated on sound indicative evidence.

Joannides and colleagues (13) published the first study involving L-NMMA infusion to block NO production after cuff occlusion in humans. They imaged the radial artery for diameter and flow at rest and after 3 min of ischemia induced by a wrist cuff placed distal to the ultrasound probe. L-NMMA, infused into the brachial artery upstream, converted the radial artery FMD response (3.6%) to a constriction (−2.8%). This abolition of FMD by NO blockade occurred in the absence of changes in peak radial artery flow, although L-NMMA decreased the duration of hyperemia, raising the possibility of a confounding nonspecific vasoconstrictor-mediated decrease in radial artery shear stress. This possibility was subsequently ruled out by Lieberman et al. (15), who studied the effect of upper arm cuff occlusion (5 min) on brachial artery FMD in the presence of L-NMMA infused above the ultrasound probe. L-NMMA decreased brachial artery FMD from 21 to 7%, indicating a potent effect of NO inhibition on FMD. Importantly, these investigators also measured the effect of a NO-independent vasoconstrictor (phenylephrine) on FMD. Despite having an equipotent effect on reactive hyperemic forearm blood flow as L-NMMA, phenylephrine did not alter brachial artery FMD, confirming the NO dependence of the FMD response. This study reported relatively high FMD data (−21%), possibly due to scanning of the artery below the antecubital fossa, where it is smaller, and the fact that the scanned artery was within the ischemic territory (vide infra). Nonetheless, the authors provided strong evidence that FMD is an endothelium-dependent process, mediated by NO.

In 2001, Doshi et al. (10) specifically investigated the issue of cuff placement on NO dependency of the FMD response. A 5-min cuff occlusion at the wrist, distal to the ultrasound probe placed on the brachial artery, was associated with an ~7% FMD response that was abolished (0.14%) by L-NMMA infusion. In response to 5 min of occlusion induced by a cuff placed on the arm above the ultrasound probe, the ~12% FMD response was only partially decreased by L-NMMA (7.5%). These data therefore suggested that, whereas NO contributed to FMD under both protocols, placement of the cuff was important; dilation of arteries that have been within the ischemic territory is affected by dilators other than NO and may also be complicated by myogenic responses as a result of the pressure fall inside the artery during occlusion. This important study has resulted in general acceptance of the principle that FMD studies should involve cuff occlusion below the antecubital fossa, with proximal brachial artery imaging.

A final study that deserves mention is that by Mullen et al. (17), which used clever experimental approaches to determine whether characteristics of the flow stimulus modified the mechanisms involved in conduit artery dilatation. Brachial artery infusion of L-NMMA decreased the radial artery diameter response to 5 min of distal wrist cuff occlusion from ~5.3 to 0.7%. The L-NMMA infusion had no effect on either the peak or prolonged flow response after cuff deflation. Conversely, after 15 min of wrist cuff inflation, FMD was 9.6% but L-NMMA had no impact on radial artery dilation (9.6 vs. 9.5%). It was also demonstrated that gradual and stepwise increases in blood velocity through the radial artery, induced by stimuli such as hand warming, substantially increased proximal radial artery diameter in a manner that was not L-NMMA sensitive. These elegant physiological studies indicated that different shear stress stimuli, including different periods of ischemia, induce conduit artery dilation that is dependent on different vasodilator mechanisms. Importantly though, it provided further evidence that the widely used FMD...
approach in humans, involving response to a 5-min occlusion induced by a cuff placed downstream from the imaged artery, is almost entirely abolished by L-NMMA and this is not due to a nonspecific vasocostrictor effect of L-NMMA.

In 2002, an important collaborative guideline was published aimed at standardizing technical approaches to the increasingly popular FMD method. The studies detailed above reinforce these guidelines and the use of FMD in humans because they indicate that in response to a period of ~5 min of cuff occlusion in the upper limb, where the cuff is placed below the imaging site, FMD is essentially abolished by NO blockade. Under these circumstances, there can be little argument that FMD does reflect NO-mediated endothelial function in humans. It is therefore entirely consistent that FMD measured in this way is impaired by traditional atherosclerotic risk factors (4, 5, 17, 18), predicts future cardiovascular events (3, 30), and is improved by cardioprotective interventions (16, 28), including, for instance, the consumption of wine (29). Cheers!

REFERENCES


Danny Green
School of Human Movement and Exercise Science
The University of Western Australia
Counterpoint: Flow-mediated dilation does not reflect nitric oxide-mediated endothelial function

The phenomenon of flow-mediated dilation (FMD) relevant to this debate describes the vasodilation in a conduit vessel in response to elevations in flow-associated shear stress. Nitric oxide (NO) is thought to play a key role in vascular health due to its established vasoprotective characteristics (for review, see Ref. 8). Because NO is one of the substances produced by the vascular endothelium in response to elevations in flow-associated shear stress (2), considerable clinical interest has focused on noninvasive assessment of FMD for evaluating NO-specific endothelial function in humans.

Clermayer et. al (4) in 1992 were the first to examine FMD induced by elevated forearm conduit vessel blood flow velocity after ischemia-induced downstream vasodilation [reactive hyperemia (RH)] to assess FMD in groups at risk for atherosclerosis and observed a blunted response. Since then, numerous studies assessing FMD in various pathologies and in response to various therapeutic interventions have been conducted. A blunting of the FMD response relative to healthy controls is taken to represent endothelial dysfunction. With regard to NO, Joannides et al. (17) in 1995 were the first to examine the NO contribution to RH-induced FMD in humans and found that NO blockade completely abolished FMD. Their concluding statement was “...the present investigation demonstrates that NO is essential for flow mediated dilatation of human radial arteries in vivo. Thus this test can be used as a reliable noninvasive estimate of the capacity of human endothelial cells to release NO...” The common clinical view now, as stated in the recent technique report by the International Brachial Artery Reactivity Task Force in 2002 (6), is that vascular endothelial NO production accounts for FMD, and the primary citation supporting this is the above-mentioned Joannides et al. (17) paper.

Unfortunately, the history of FMD research represents a clinically driven desire to evaluate endothelial function that has bypassed careful mechanistic dissection of FMD in vivo in humans. The issue here is whether FMD specifically reflects NO-mediated endothelial function. A careful mechanistic exploration of FMD reveals three critical characteristics that seriously question the current dogma that FMD represents NO-mediated endothelial function: 1) the nature of the shear stimulus affects the vasoregulatory mechanisms evoked, 2) shear stress acts as a stimulus for endothelial release of vasoconstrictors, and 3) elevated sympathetic activation, characteristic of numerous pathologies in which endothelial dysfunction is observed, can blunt the FMD response.

Shear stimulus characteristics determine FMD mechanisms. It is well established that there are a number of signaling pathways activated with an increase in shear stress and at least three vasodilators who’s production increases have been identified (NO, prostacyclin, endothelium-derived hypopolarizing factor) (3). Therefore, a common stimulus (shear stress) exists for the simultaneous activation of a number of vasoregulatory mechanisms.

Mullen et al. (22) investigated the impact of the duration of the shear stress stimulus on the NO dependence of the FMD response in the radial artery. They found that only the FMD in response to a brief shear stress stimulus was reduced by L-NMMA blockade of NO. In contrast, the FMD response to more sustained stimuli caused by release of 15 min ischemia, skin warming, or distal acetylcholine infusion was unaffected, indicating an NO-independent mechanism(s). Of note, similar blunting of FMD with L-NMMA infusion reported by Joannides et al. (17) can be explained by the L-NMMA infusion-induced attenuation of the reactive hyperemia in their study in healthy humans. In other words, blunted FMD with L-NMMA infusion could be due to a blunted stimulus, not necessarily a reduction in NO-mediated dilation (25). Both the identity and dynamics (when the role of NO ends and that of another vasodilator(s) begins) of the mechanism(s) that is responsible for the FMD in response to a more prolonged elevation in shear stress are therefore still unknown.

The relevance of considering shear stress stimulus characteristics beyond the simple 5-min ischemia-induced transient shear elevation is clear when we consider the relevance of this stimulus for FMD in the coronary arteries. Coronary artery FMD is recognized as a critical response for adequate perfusion of cardiac muscle during increased work of the heart (26). The nature of the shear stress stimulus for these vessels is not reflected by the 5 min RH stimulus. Shiode et al. (26) demonstrated that shear stress-induced coronary artery dilation is not dependent on NO. Zeiher et al. (30) demonstrated that impaired coronary artery vasodilation in exercise is associated with myocardial ischemia. This implies that improvements in coronary artery FMD to a sustained shear stress stimulus may attenuate myocardial ischemia. Therefore, FMD in response to sustained shear stress may constitute a critical component of endothelial function for which NO may not be obligatory.

Shear stimulus evokes endothelin release. In addition to release of vasodilators in response to elevations in shear stress, it has been demonstrated that endothelin release can also be stimulated (21, 28, 29). Endothelin is a potent vasoconstrictor. Thus alterations in the responsiveness of endothelin release by the vascular endothelium would be expected to influence FMD. Of particular relevance to this point is the observation by Berger et al. (1) that blockade of endothelin-A receptors improves FMD in patients with chronic heart failure. Indeed, these authors also point out the importance of considering that vascular tone is the net result of interaction between simultaneously active vasoregulatory factors.

Sympathetic activation can account for blunted FMD in numerous pathologies. Emerging evidence of sympathetic modulation of FMD highlights the consequence of a lack of careful dissection of multiple mechanisms that interact to determine FMD. It is well established that hyperactivation of the sympathetic nervous system is implicated in cardiovascular outcomes (5, 9, 27). Many of the pathologies associated with endothelial dysfunction as evidenced by blunted FMD also demonstrate sympathetic hyperactivity. For example blunted FMD and elevated sympathetic tone is characteristic of aging (7, 11), obstructive sleep apnea (16, 23), heart failure (14, 18),
and hypertension (13, 20) to name a few. In addition, non-pathological states associated with elevated sympathetic activation also demonstrate blunted FMD. These include diurnal variations in FMD (24) and mental stress, particularly in persons characterized by high levels of hostility (12).

Recently, Hjimering et al. (15) examined the effect of lower body negative pressure-induced sympathetic elevation in healthy humans. Their results clearly demonstrate that elevations in sympathetic activation result in considerable blunting of FMD. In addition, interventions that have been demonstrated to improve FMD in sleep apnea (continuous positive airway pressure) (16), aging (10), cardiovascular disease (ACE inhibitors) (19) also either reduce sympathetic activity or have sympatheticolytic effects. Taken together, these observations demonstrate that the common assumption that FMD represents NO-mediated endothelial function is particularly misleading in understanding endothelial function and interpreting the impact of therapeutic interventions in conditions where elevated sympathetic activity also exists.

In summary, FMD as an “assay” of NO-mediated endothelial function relies on a shear stress stimulus that has a highly complex signal transduction cascade evoking multiple vasoactive mechanisms. FMD in response to many shear stress profiles is not sensitive to NO blockade. In addition, there is unquestionable evidence that sympathetic activation influences the FMD response, and this activation is characteristic of many of the pathologies and altered states in which blunted FMD is observed. Finally, treatment of these pathologies often involves sympathetic or sympathetic activation-reducing agents/interventions. Thus we believe the current dogma that FMD represents NO-mediated endothelial function is in error.

REFERENCES


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The example of coronary FMD being different to that in the periphery misses the point. Although coronary lesions are focal in nature, atherosclerosis is a systemic disease (5) that can be interrogated via peripheral NO bioassay (1). This is why FMD predicts cardiovascular events (10) and why it may reflect the compound effect of risk factors, including elevated sympathetic nervous system tone, on arterial health. Furthermore, shear stress-mediated upregulation of NO synthase expression and phosphorylation occurs in vivo (5).

Finally, the Hijining paper provides an important reminder that controls need to be instituted before comparing FMD responses, especially between groups. However, this study did not infuse L-NMMA and in no way invalidates others that indicate that FMD is NO dependent (3, 7–9), including those that have isolated improvement in endothelial function to enhanced NO bioavailability (4, 6).

Tschakovsky and Pyke are to be lauded for bringing the issue of stimulus-response specificity to the forefront of the FMD debate. Their conclusion, that “FMD in response to any shear stress stimulus, the widely adopted approach involves quantification of radial or brachial vasodilator response to an ~5-min ischemic stimulus where the cuff is distal to the probe (2, 3). The papers reviewed previously (3, 7–9) indicate that, under these circumstances, FMD is NO mediated and Tschakovsky and Pyke do not directly contradict this. Rather they make the case that stimulus-response specificity exists in FMD responses, where FMD is more broadly defined (e.g., different ischemic periods). I agree. Ultimately, however, the point is that we have a noninvasive technique, which, if performed appropriately, provides a valid index of NO function in vivo.

Although FMD can technically be defined as the response to any shear stress stimulus, the widely adopted approach involves quantification of radial or brachial vasodilator response to an ~5-min ischemic stimulus where the cuff is distal to the probe (2, 3). The papers reviewed previously (3, 7–9) indicate that, under these circumstances, FMD is NO mediated and Tschakovsky and Pyke do not directly contradict this. Rather they make the case that stimulus-response specificity exists in FMD responses, where FMD is more broadly defined (e.g., different ischemic periods). I agree. Ultimately, however, the point is that we have a noninvasive technique, which, if performed appropriately, provides a valid index of NO function in vivo.

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Tschakovsky and Pyke are to be lauded for bringing the issue of stimulus-response specificity to the forefront of the FMD debate. Their conclusion, that “FMD in response to any shear stress profiles is not sensitive to NO blockade”... is qualified (“...many...”), but true. However, the point remains that when the appropriate FMD approach is adopted, it provides a valid index of NO bioactivity in vivo.

REFERENCES

REBUTTAL FROM DRS. TSCHAKOVSKY AND PYKE

We have taken the sage advice of our erudite colleague Dr. Green to heart... namely indulging in a bottle of robust Australian Shiraz. We, first off, thank him for his suggestion as it has helped us to identify within this debate a classic blunder. Equating FMD with NO-mediated endothelial function repeats the folly of the simple tourist who sees one black sheep and concludes that black is the color of all the sheep in England. The first well-characterized FMD response in humans (2) was found to be NO dependent (4, 11) and this has led to the assumption that all FMD must share this mechanism. Recent publications do not support this position (12) and it is time to... “shear” erroneous assumptions from our concept of FMD.

The argument by Dr. Green could be partially correct if the statement to be defended qualified the type of FMD as 1) brachial or radial artery specific (4, 11), 2) specific to 5 min of distal occlusion reactive hyperemia without ischemic handgrip exercise (1, 12), and 3) in healthy subjects (11). This is critical because primarily NO-mediated FMD has been confirmed only under this specific combination of conditions (4, 11). Furthermore, it has been demonstrated that slight deviations from these conditions resulting in small stimulus profile alterations can dramatically alter the NO dependence of the FMD response (12). Readers! Pay attention to the following statement in the very guidelines that Dr. Green cites to support a consensus on FMD assessment of endothelial NO function: “Studies have variably used either upper arm or forearm cuff occlusions and there is no consensus as to which technique...
provides more accurate or precise information... The change in diameter is similar after 5 and 10 min of occlusion; therefore the more easily tolerated 5-min occlusion is typically used” (3). These statements by experts in the field do not acknowledge the critical importance of the stimulus creation technique in determining the NO dependence of the FMD response.

Finally, we reiterate that, even when the methodological constraints that allow for an NO-dependent FMD response are followed, other factors may influence the magnitude of vasodilation. Specifically, the elevated sympathetic activation, common in pathologies also associated with endothelial dysfunction, may blunt the FMD response (10). In these groups, FMD in response to even 5 min of distal occlusion reactive hyperemia is a reflection of combined NO bioavailability and sympathetic activation.

Finally, it has been clearly demonstrated that some FMD responses in humans are not NO mediated (12). Under these circumstances, to state that FMD as a whole reflects NO-mediated endothelial function is to ignore this or to imply that all other mechanisms of FMD are irrelevant. This is inappropriate on two counts. First, FMD is an important vasoregulatory mechanism in both the coronary and peripheral vasculature systems (6, 9) and thus all mechanisms of FMD should be studied. Second, it has not been clearly established that all coronary FMD is NO dependent (13). In atherosclerotic coronary arteries dilation in response to increases in blood flow (regardless of the mechanism responsible) can help to attenuate myocardial ischemia (5, 7, 8). Therefore from a clinical perspective, all mechanisms of FMD in this vascular bed require focused research.

In conclusion, at present FMD can only be said to reflect NO-mediated endothelial function if it is in response to a very narrowly defined stimulus in only the radial or brachial arteries. To imply that FMD in response to other stimulus profiles in other areas of the vasculature is NO dependent might be akin to, dare we say, embracing the only black sheep in the family.

REFERENCES

POINT-COUNTERPOINT CALL FOR COMMENTS

Readers are invited to give their views on this issue by submitting a brief (250 word maximum, 5 references) Letter to the Editor (please upload to APSCentral: http://www.apscentral.org), which, if accepted, will appear in the earliest possible issue. If you have any questions about this call for comments, please contact Dr. Jerome Dempsey, Editor-in-Chief (608-263-1732 or jdempsey@wisc.edu).