Wu, Brian and Credeur, Daniel and Fryer, Simon M and Stoner, Lee (2015). *The use of shear rate–diameter dose–response curves as an alternative to the flow-mediated dilation test.* Medical Hypotheses, 84 (2), 85-90. ISSN 03069877

THE USE OF SHEAR RATE-DIAMETER DOSE-RESPONSE CURVES AS AN ALTERNATIVE TO THE FLOW-MEDIATED DILATION TEST

Abstract

The brachial artery flow-mediated dilation test (FMD) is the non-invasive gold-standard used to test endothelial function. Reduced FMD precedes the development of atherosclerosis and provides an early marker for predicting future cardiovascular disease events. Although, this test is used extensively, it is somewhat limited by poor reproducibility. By utilizing hand warming and grip exercise combined with hierarchical linear modeling, shear rate-diameter dose-response curves may provide a novel and more accurate way to assess endothelial function in humans. Shear rate-diameter dose-response curves could potentially improve upon the traditional FMD measurement and serve as a clinical and research tool for assessing cardiovascular disease risk in a variety of populations.

KEYWORDS: Flow mediated dilatation; ultrasound; blood flow; shear stress; cardiovascular disease.

Introduction

The vascular endothelium is implicated in many important constructs underlying cardiovascular disease (CVD). Disruption of this monolayer of cells is thought to occur early in the pathogenesis of CVD, and non-invasive clinical bio-markers can play a vital role in early detection of endothelial dysfunction.

Currently, the flow-mediated dilation (FMD) test is the non-invasive gold-standard used to assess endothelial function (1). Duplex Doppler-Ultrasound is used to scan an artery (e.g., brachial or popliteal) to measure the vasodilatory response to blood flow-induced increases in shear stress. Typically, a pneumatic cuff is placed around the forearm distal or proximal to the scanned region and inflated to a supra-systolic blood pressure (i.e., 220 mmHg) for 5 minutes (1). Rapid deflation of this cuff leads to increased blood flow (reactive hyperemia) to the oxygen starved forearm muscles, with a subsequent increase in flow through the up-stream conduit artery. The resultant flow-induced elevation in shear stress stimulates endothelial cell release of vasodilators, most notably nitric oxide (NO) when the occluding cuff is placed distally, with subsequent smooth muscle cell relaxation (2, 3). The FMD is typically expressed as the percentage increase in artery diameter from baseline to peak dilation (Fig. 1). Reduced FMD is an early event in the development of atherosclerosis (1) and provides a bio-marker for predicting future CVD events (4). Despite the FMD test being considered the current gold standard, it is somewhat limited by poor reproducibility and reliability. Specifically, the within-subject variability of FMD has been reported to be as high as 50% (5). Most of the poor reproducibility is accounted for by three major limitations associated with the standard FMD methodology: 1)

inappropriate calculation of FMD, 2) lack of normalization to the stimulus, and 3) short-lived peak diameter response.

Flow-Mediated Dilation Limitations

Firstly, FMD expressed as a percentage limits statistical power (6). FMD can be calculated as: 1) post-only score, 2) delta score, 3) fraction, or 4) co-varied for resting diameter. A simulation study found the analysis of covariance approach (i.e., option 4 above) had the greatest statistical power, with percentage change from baseline producing the lowest statistical power (6). Expressing FMD as a percentage effectively squares the variation due to resting diameter, and may result in a non-normally distributed statistic from normally distributed data. Using resting diameters as a covariate is most likely to adjust for the bias due to baseline values (6-8).

Secondly, most studies still fail to account for the stimulus, i.e., shear stress (9). Shear stress is primarily related to movement of red blood cells close to the endothelial layer (represented by bottom and top-most arrows in Fig. 2.1b). As fluid particles "travel" parallel to the vessel wall, their average velocity increases from a minimum at the wall to a maximum value at some distance from the wall, resulting in a gradient of velocities that form concentric circles in the lumen of the vessel (Fig. 2.1a). This shearing stress therefore acts at a tangent to the wall to create a frictional force at the surface of the endothelium. Mitchell *et al.* (10) demonstrated that reduced FMD may be attributable not only to impaired NO bioavailability, but also to a lesser shear stimulus. Fortunately, the ultrasound technology used to conduct the FMD test can also provide estimates of shear stress.

Thirdly, the peak diameter in response to reactive hyperemia is short lived (see Fig. 1) and, therefore, difficult to capture unless utilizing a high-resolution acquisition and analysis system. Variance in peak diameter measurements may be attributable to differences in the stimulus (i.e., shear stress), or measurement error (Fig. 3). Variance due to change in the stimulus can be accounted for by normalizing FMD to shear stress. To account for measurement error, according to laws governing regression to the mean (11), the FMD test would need to be repeated multiple times in order to obtain a "true" response (Fig. 3). Alternatively, a more accurate assessment of endothelial function can be achieved by estimating shear rate-diameter dose-response curves. The addition of shear rate-diameter dose-response curves may provide a valuable component to assessing endothelial function.

Shear rate-diameter dose-response curves

In the biological sciences, dose-response curves are widely used to understand and model the response of a living organism to a particular stimulus. A dose-response curve is used to relate the magnitude of a stressor (e.g., shear stimulus) to the response of the receptor (e.g., arterial dilation). This approach can be used to assess endothelial function. The following questions would need to be addressed prior to utilizing dose-response curves: 1) how should we estimate the shear stimulus? 2) how should we manipulate the shear stimulus? And, 2) how should we express dose-response outcomes?

Hypothesis

Endothelial function is a necessary and vital component of assessing cardiovascular health. As discussed, the current methods may suffer from potential sources of error variability, limiting test reliability. Here we hypothesize that shear rate-diameter dose-response curves may improve

upon measurement reliability and validity, therefore, serving as a valuable clinical and research tool for the evaluation of endothelial function.

Evaluation of the hypotheses

Shear Stress Calculation

Clinical studies in humans, including FMD studies, typically estimate shear stress by employing a simplified mathematical model based on Poiseuille's law, where shear rate equals:

$$\text{Shear rate } (\gamma) = \frac{2(2+n)\,v}{d}$$

…where d is the internal arterial diameter, v is time averaged mean blood velocity, and n represents the shape of the velocity profile (*for a fully developed parabolic profile, n is 2*).

Poiseuille's law assumes that: 1) the fluid (blood) is Newtonian, 2) blood flows through a rigid tube, 3) whole blood viscosity represents viscosity at the vessel wall and is linearly proportional to shear rate, and 4) the velocity profile is parabolic. First, although blood is non-Newtonian, the effect of the non-Newtonian behavior does not appear to be pronounced in large arteries (12). Second, blood vessels are distensible, meaning that wall shear rate may be ~30% less in a distensible artery as compared with a rigid tube (13). Third, blood viscosity exhibits low intra-subject variability (14), particularly among a healthy, homogeneous group. Thus, shear rate has been used as a surrogate measure of shear stress in a number of previous studies (14-18). Lastly, in arteries, the velocity profile will generally not develop in to a full parabola, as a consequence

of flow unsteadiness and short vessel entrance lengths. However, in the brachial artery, under resting conditions, the underestimation is less pronounced - likely due to a more parabolic velocity profile in this artery, i.e., *n* (velocity profile) is closer to 2 (19). However, this may only be true for resting conditions; occurrence of flow turbulence is possible during reactive hyperemia, which may limit the validity of shear rate estimate under such conditions, i.e., standard FMD test (20).

Manipulating the Shear Stimulus Using Hand Warming and Handgrip Exercise

Since one is assessing a physiological system, an appropriate range of stimuli should create an S-shape dose-response curve (see Fig. 4). Assuming one can use resting diameter to represent the baseline response, at least five progressive intensities of shear stimulus would be needed: one intensity to estimate the onset of the reactive portion of the curve (the slope), two intensities to estimate the slope, and two intensities to estimate the plateau. To overcome the short lived change in diameter induced by reactive hyperemia, shear stress may be progressively increased in a sustained manner, e.g., through local hand warming and low-intensity handgrip exercise (17, 21-23). This approach would also allow for a more accurate assessment of shear rate (see above discussion).

Local warming of the skin induces localized dilation that is graded with skin temperature with the maximal dilation and blood velocity response occurring at 42°C (24, 25). Local warming of the skin over a small region (i.e., forearm) is thought to increase blood flow locally without significant systemic autonomic influences (24-27). The mechanism responsible for this response is not fully understood, but endothelial NO production is thought to play a central role (26-28).

There is evidence to suggest that this response may be produced through a neurogenic reflex with NO serving a permissive role to some unknown neurotransmitter (29). Under controlled conditions, gradually increasing skin temperature can induce successive, sustained, and reproducible increases in local blood flow (17, 22, 25, 30). To ensure that the brachial artery is not directly heated, the forearm should be encased within an airtight container. Furthermore, the skin temperature of the bicep should be continuously monitored.

Rhythmic handgrip exercise can also be used to increase blood flow. Handgrip exercise increases metabolic demand of the forearm. The role of the endothelium in exercise-induced vasodilatation is not clear. A possible limitation is the potential for recruitment of the bicep muscle, thereby directly activating the region of interest. The exercise intensity has to be low enough to prevent synergistic muscle activity. Electromyography can be used to ascertain that the bicep remains inactivated. Shear rate has been manipulated using this approach (31-33); subjects were able to squeeze a handgrip ergometer to 10% of their maximal voluntary contraction up to twice every 3 seconds without recruiting the bicep.

Recently, we found that the relationship between shear rate and vasodilatation is comparable when shear rate is increased in a sustained manner (local hand warming and handgrip exercise-induced) (31) (see Fig. 5). This is consistent with a recent study by Pyke et al. (34), who similarly found a significant relationship between handgrip exercise-induced FMD when the FMD responses were normalized to shear rate. Consideration has to be given to the mechanism(s) inducing FMD; the mechanisms regulating conduit artery vascular tone may be dependent on the duration of the shear stimulus (21, 35-38), with FMD in response to sustained

shear rate likely being less NO-dependent (32). Nonetheless, the endothelium is still thought to primarily govern conduit artery vasodilation under steady-state shear rate conditions. For instance, hand warming has no effect on brachial artery diameter when flow is not allowed to increase (15, 17, 21). Furthermore, pharmacological blockade of the autonomic nervous system has no effect on radial artery vascular tone in response to hand warming (21), consistent with animal studies suggesting that FMD is preserved after surgical or pharmacological denervation (39, 40).

Expressing Dose-Response Outcomes

A standard dose-response curve for FMD is defined by four parameters: 1) the baseline response (bottom), 2) the maximum response (Top), 3) the slope, and 4) the stimulus which provokes a halfway response between baseline-maximum (EC_{50}). The slope, which would represent the change in diameter per one unit change in shear rate, is likely to be the parameter which most accurately reflects endothelial function. An alternative is to use the EC_{50}; however, this parameter requires that the baseline and maximum are adequately characterized. The maximum response would most likely reflect the degree of arterial stiffness (41-44). To estimate non-biased outcomes each parameter (slope, EC_{50}, and/or maximum) of interest should be co-varied to baseline diameter (6-8).

Statistical Analysis

Shear rate: diameter slopes for each subject can be estimated by regressing shear rate against diameter for each condition (i.e., each intensity of heat/exercise). Between- or within-group

slopes can then be compared using the general linear model approach, e.g., *t*-test or analysis of variance.

An alternative is to normalize the FMD response (i.e., change in diameter) to shear using hierarchical linear modeling (HLM) (45). HLM is a more advanced form of multiple linear regression that accounts for hierarchical (i.e., successive inter-related levels) effects on the outcome variable. This is accomplished in HLM by including a complex random subject effect which can appropriately account for correlations among the data. This approach models different patterns in the data by allowing for the intercepts (initial diameter) and slopes (shear rate-diameter) to randomly vary. A third level may also be specified; this may be the specification of groups (e.g., to delineate differences in endothelial function), an intervention or a modifiable risk factor such as smoking. This approach has been used to compare upper vs. lower extremity arterial health in persons with spinal cord injury (SCI) (43), to assess improvements in arterial health following electrical stimulation-evoked resistance exercise therapy in persons with SCI (46), to look at the effects of occasional cigarette smoking on arterial health (47), to determine whether velocity acceleration is an important contributor to FMD (48), and to assess whether peak- and time-integrated shear rates independently predict FMD (31). The disadvantage of this approach is that multiple stimuli (preferably ranging from minimal to maximal shear stimuli) are required to generate a reliable shear-diameter relationship.

Validity and Reliability

Prior to the application of a physiological test, the validity and reliability should be ascertained. To test the validity, the proposed method should be directly compared against the standard FMD

methodology. Discriminant function analysis may be used to determine which dependent variable (current FMD test or proposed test) is the most important for classifying groups who are known to be either disease free of afflicted with CVD. To test reliability, intra-class coefficients may be calculated by conducting each test on three separate mornings under standardized conditions (20). Optimally, to ensure subsequent studies can be appropriately powered, using between-day reliability will be calculated for at least two groups: with and without known CVD.

Consequences of the hypothesis

Advantages

The use of parameters from dose-response curves would offer a number of advantages over standard FMD methodology: 1) the stimulus (shear) is directly accounted for in a manner that does not violate statistical assumptions, 2) improved sensitivity, i.e., the slope (endothelial function) can be clearly identified (with the standard FMD test it cannot be ascertained at which point on the slope endothelial function is being estimated), 3) improved reliability, i.e., the dose-response slope is more resistant to measurement error when compared to a single measurement (11), and 4) more information is provided, i.e., the slope isolates endothelial function whereas the maximum response more likely reflects the degree of arterial stiffness (41-44).

Clinical Implications

A recent meta-analysis by Green et al. (49), assessed the CVD prognostic strength of FMD by conducting a meta-analysis of observational studies which examined the associations between brachial artery FMD and future cardiovascular events. Green et al. found that FMD resulting from a more intense and prolonged shear stimuli, using proximal cuff placement (50) provided a

better prognosis for CVD risk. Recently, Green et al. (49) re-analyzed the meta-analysis conducted by Inaba and colleagues (51) by assessing the prognostic strength of those studies that used distal versus proximal cuff placement. For studies in which a distal cuff placement was selected (the standard and widely advocated approach), a 1% increase in FMD was associated with a relative risk of 0.91, that is, a 9% (95% CI: 4% to 13%) decrease in the future risk of cardiovascular events. For studies involving proximal cuff placement, the relative risk improved to 0.83, that is, a 17% (95% CI: 12% to 22%) decrease in cardiovascular risk for every 1% increase in FMD. The difference between these two relative risks was found to be statistically significant ($P = 0.01$), indicating that FMD conducted using proximal cuff placement, which has been demonstrated to be less NO-dependent (50), provides a more accurate prognosis for CVD risk. Therefore, it appears probable that endothelial function has prognostic value beyond the narrow limits attributable to NO bioavailability.

Conclusions

Shear rate-diameter dose-response curves may prove to be a more reliable and sensitive marker of endothelial function compared to the standard FMD test. The use of progressive shear stimuli - isolated heating and exercise - would make it more challenging to ascertain the mechanism[s] responsible for dilation. Nonetheless, the health of the endothelium remains an important construct. Further study is required to ascertain whether shear-rate diameter dose-response curves offer greater statistical power and prognostic capacity for predicting cardiovascular events.

References

1. Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet. 1992;340(8828):1111-5.
2. Furchgott RF, Zawadzki JV**.** The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature. 1980;299:373-6.
3. Moncada S, Higgs A**.** The L-arginine-nitric oxide pathway. N Engl J Med. 1993(329):2002-12.
4. Schroeder S, Enderle MD, Ossen R, Meisner C, Baumbach A, Pfohl M, et al. Noninvasive determination of endothelium-mediated vasodilation as a screening test for coronary artery disease: pilot study to assess the predictive value in comparison with angina pectoris, exercise electrocardiography, and myocardial perfusion imaging. Am Heart J. 1999;138(4):731-9.
5. De Roos NM, Bots ML, Schouten EG, Katan MB**.** Within-subject variability of flow-mediated vasodilation of the brachial artery in healthy men and women: implications for experimental studies. Ultrasound Med Biol. 2003;29(3):401-6.
6. Vickers AJ**.** The use of percentage change from baseline as an outcome in a controlled trial is statistically inefficient: a simulation study. BMC Med Res Methodol. 2001;1:6.
7. Tu YK, Blance A, Clerehugh V, Gilthorpe MS**.** Statistical power for analyses of changes in randomized controlled trials. J Dent Res. 2005;84(3):283-7.
8. Twisk J, Proper K**.** Evaluation of the results of a randomized controlled trial: how to define changes between baseline and follow-up. J Clin Epidemiol. 2004;57(3):223-8.
9. Koller A, Sun D, Kaley G**.** Role of shear stress and endothelial prostaglandins in flow- and viscosity-induced dilation of arterioles in vitro. Circ Res. 1993;72(6):1276-84.
10. Mitchell GF, Parise H, Vita JA, Larson MG, Warner E, Keaney JF, Jr., et al. Local shear stress and brachial artery flow-mediated dilation: the Framingham Heart Study. Hypertension. 2004;44(2):134-9.
11. Shephard RJ**.** Regression to the mean. A threat to exercise science? Sports Med. 2003;33(8):575-84.
12. Chien S, Usami S, Taylor HM, Lundberg JL, Gregersen MI**.** Effects of hematocrit and plasma proteins on human blood rheology at low shear rates. J Appl Physiol. 1966;21(1):81-7.
13. Duncan DD, Bargeron CB, Borchardt SE, Deters OJ, Gearhart SA, Mark FF, et al. The effect of compliance on wall shear in casts of a human aortic bifurcation. J Biomech Eng. 1990;112(2):183-8.
14. Gnasso A, Carallo C, Irace C, Spagnuolo V, De Novara G, Mattioli PL, et al. Association between intima-media thickness and wall shear stress in common carotid arteries in healthy male subjects. Circulation. 1996;94(12):3257-62.
15. Joannides R, Bakkali el H, Richard V, Benoist A, Moore N, Thuillez C**.** Evaluation of the determinants of flow-mediated radial artery vasodilatation in humans. Clin Exp Hypertens. 1997;19(5-6):813-26.
16. Betik AC, Luckham VB, Hughson RL**.** Flow-mediated dilation in human brachial artery after different circulatory occlusion conditions. Am J Physiol Heart Circ Physiol. 2004;286(1):H442-8.

17. Pyke KE, Dwyer EM, Tschakovsky ME**.** Impact of controlling shear rate on flow-mediated dilation responses in the brachial artery of humans. J Appl Physiol. 2004;97(2):499-508.
18. Padilla J, Johnson BD, Newcomer SC, Wilhite DP, Mickleborough TD, Fly AD, et al. Normalization of flow-mediated dilation to shear stress area under the curve eliminates the impact of variable hyperemic stimulus. Cardiovasc Ultrasound. 2008;6:44.
19. Dammers R, Stifft F, Tordoir JH, Hameleers JM, Hoeks AP, Kitslaar PJ**.** Shear stress depends on vascular territory: comparison between common carotid and brachial artery. J Appl Physiol. 2003;94(2):485-9.
20. Stoner L, Sabatier MJ**.** Use of ultrasound for non-invasive assessment of flow-mediated dilation. J Atheroscler Thromb. 2012;19(5):407-21.
21. Mullen MJ, Kharbanda RK, Cross J, Donald AE, Taylor M, Vallance P, et al. Heterogenous nature of flow-mediated dilatation in human conduit arteries in vivo: relevance to endothelial dysfunction in hypercholesterolemia. Circ Res. 2001;88(2):145-51.
22. Joannides R, Costentin A, Iacob M, Compagnon P, Lahary A, Thuillez C**.** Influence of vascular dimension on gender difference in flow-dependent dilatation of peripheral conduit arteries. Am J Physiol Heart Circ Physiol. 2002;282(4):H1262-9.
23. Stoner L, Sabatier M, Edge K, McCully K**.** Relationship between blood velocity and conduit artery diameter and the effects of smoking on vascular responsiveness. J Appl Physiol. 2004;96(6):2139-45.
24. Johnson J, M., O'Leary DS, Taylor WF, Kosiba W**.** Effect of local warming on forearm reactive hyperemia. Clin Physiol. 1986;6:337-46.
25. Taylor WF, Johnson JM, O'Leary D, Park MK**.** Effect of high local temperature on reflex cutaneous vasodilation. 1984:191-6.
26. Kellogg DL, Jr., Liu Y, Kosiba IF, O'Donnell D**.** Role of nitric oxide in the vascular effects of local warming of the skin in humans. J Appl Physiol. 1999;86(4):1185-90.
27. Shastry S, Reed AS, Halliwill JR, Dietz NM, Joyner MJ**.** Effects of nitiric oxide synthase inhibition on cutaneous vasodilation during body heating in humans. J Appl Physiol. 1998(85):830-4.
28. Kellogg DL, Jr , Crandall CG, Liu Y, Charkoudian N, Johnson JM**.** Nitric oxide and cutaneous active vasodilation during heat stress in humans. J Appl Physiol. 1998;85:824-9.
29. Crandall CG, MacLean DA**.** Cutaneous interstitial nitric oxide concentration does not increase during heat stress in humans. J Appl. Physiol. 2001;90:1020-4.
30. Savage MV, Brengelmann GL**.** Reproducibility of the vascular response to heating in human skin. J Appl Physiol. 1994;76(4):1759-63.
31. Stoner L, McCully K**.** Peak- and time integrated-shear rates independently predict flow-mediated dilation. Journal of Clinical Ultrasound. In Press.
32. Stoner L, McCully KK**.** Peak and time-integrated shear rates independently predict flow-mediated dilation. J Clin Ultrasound. 2012;40(6):341-51.
33. Wray DW, Witman MA, Ives SJ, McDaniel J, Fjeldstad AS, Trinity JD, et al. Progressive handgrip exercise: evidence of nitric oxide-dependent vasodilation and blood flow regulation in humans. Am J Physiol Heart Circ Physiol. 2011;300(3):H1101-7.

34. Pyke KE, Jazuli F**.** Impact of repeated increases in shear stress via reactive hyperemia and handgrip exercise: no evidence of systematic changes in brachial artery FMD. Am J Physiol Heart Circ Physiol. 2011;300(3):H1078-89.
35. Frangos JA, Eskin SG, McIntire LV, Ives CL**.** Flow effects on prostacyclin production by cultured human endothelial cells. Science. 1985;227(4693):1477-9.
36. Kuchan MJ, Jo H, Frangos JA**.** Role of G proteins in shear stress-mediated nitric oxide production by endothelial cells. Am J Physiol. 1994;267(3):C753-8.
37. Frangos JA, Huang TY, Clark CB**.** Steady shear and step changes in shear stimulate endothelium via independent mechanisms--superposition of transient and sustained nitric oxide production. Biochem Biophys Res Commun. 1996;224(3):660-5.
38. Macarthur H, Hecker M, Busse R, Vane JR**.** Selective inhibition of agonist-induced but not shear stress-dependent release of endothelial autacoids by thapsigargin. Br J Pharmacol. 1993;108(1):100-5.
39. Lie M, Sejersted OM, Kiil F**.** Local regulation of vascular cross section during changes in femoral arterial blood flow in dogs. Circ Res. 1970;27(5):727-37.
40. Hilton SM**.** A peripheral arterial conducting mechanism underlying dilatation of the femoral artery and concerned in functional vasodilatation in skeletal muscle. J Physiol. 1959;149:93-111.
41. Harris LM, Faggioli GL, Shah R, Koerner N, Lillis L, Dandona P, et al. Vascular reactivity in patients with peripheral vascular disease. Am J Cardiol. 1995;76(3):207-12.
42. Black CD, Vickerson B, McCully KK**.** Noninvasive assessment of vascular function in the posterior tibial artery of healthy humans. Dyn Med. 2003;2(1):1.
43. Stoner L, Sabatier M, VanhHiel L, Groves D, Ripley D, Palardy G, et al. Upper vs lower extremity arterial function after spinal cord injury. J Spinal Cord Med. 2006;29(2):138-46.
44. Sabatier MJ, Stoner L, Reifenberger M, McCully K**.** Doppler ultrasound assessment of posterior tibial artery size in humans. J Clin Ultrasound. 2006;34(5):223-30.
45. Raudenbush SW, Bryk AS**.** *Hierarchical Linear Models: Applications and Data Analysis Methods (Advanced Quantitative Techniques in the Social Sciences)*. Thousand Oaks, CA: SAGE Publications; 2001.
46. Stoner L, Sabatier MJ, Mahoney ET, Dudley GA, McCully KK**.** Electrical stimulation-evoked resistance exercise therapy improves arterial health after chronic spinal cord injury. Spinal Cord. 2007;45(1):49-56.
47. Stoner L, Sabatier MJ, Black CD, McCully KK**.** Occasional cigarette smoking chronically affects arterial function. Ultrasound Med Biol. 2008;34(12):1885-92.
48. Stoner L, McCully K**.** Velocity acceleration as a determinant of flow-mediated dilation. Ultrasound in Medicine and Biology. In Press.
49. Green DJ, Jones H, Thijssen D, Cable NT, Atkinson G**.** Flow-mediated dilation and cardiovascular event prediction: does nitric oxide matter? Hypertension. 2011;57(3):363-9.
50. Doshi SN, Naka KK, Payne N, Jones CJ, Ashton M, Lewis MJ, et al. Flow-mediated dilatation following wrist and upper arm occlusion in humans: the contribution of nitric oxide. Clin Sci (Lond). 2001;101(6):629-35.
51. Inaba Y, Chen JA, Bergmann SR**.** Prediction of future cardiovascular outcomes by flow-mediated vasodilatation of brachial artery: a meta-analysis. Int J Cardiovasc Imaging. 2010;26(6):631-40.

Figure Legends

Figure 1. *Shear rate and diameter responses to 5 minutes ischemia.* The horizontal line represents resting diameter. Flow-mediated dilation (FMD) is typically represented as the peak percentage increase in diameter above rest. Note that the peak diameter occurs at ~40 sec whereas the bulk of the hyperemic (shear) response occurs within the initial 20 sec.

Figure 2. *Endothelium-dependent dilation.* (1) Blood flowing through an artery creates a shearing stress at the endothelial surface. A composite of superimposed concentric circles is shown in 1a (i.e., transverse plane) to correspond with the gradient of increasing RBC velocity from the periphery to the center of the lumen. RBC velocity is represented as a parabola (i.e., longitudinal plane) in 1b using the same color coding as in 1a. The magnitude of the parabola (left to right) corresponds with the gradient of increasing RBC velocity from the periphery to the center of the lumen. (2) Shear stress-induced deformation of the endothelial cells is detected by mechanoreceptors on the cell membrane. (3) In response to mechanotransduced shear stress, a signaling cascade results in the production of NO, PGI_2 and EDHF. (4) The vasodilators diffuse cross the interstitial space and enter the vascular smooth muscle cells. (5) A signaling cascade is initiated which lowers Ca^{2+} concentration and results in smooth muscle cell relaxation (i.e., vasodilation). Ca^{2+} = calcium; eNOS = endothelial NO synthase; COX-2 = cyclooxygenase; EDHF = endothelial-derived hyperpolarizing factor; NO = nitric oxide; PGI_2 = prostaglandins; RBC = red blood cell.

Figure 3. *Flow-mediated dilation (FMD) measurement variance.* Open circles represent multiple FMD measurements (hypothetical data). The closed circle represents mean FMD. Variance due to change in stimuli (shear rate) can be accounted for by normalizing to shear rate. Variance due to measurement error can be minimized by multiple FMD measurements (or by calculating a shear rate : diameter dose response curve).

Figure 4. *Theoretical shear rate-diameter dose-response curve.* Six data points are shown: baseline, and the responses to 5 intensities of heating/exercise.

Figure 5. Hierarchical linear model (HLM) estimates for shear rates regressed against peak diameters. HLM models without (A) and with (B) peak shear being co-varied are shown.