Author's response to reviews

Title: Flow Mediated Dilation of the Brachial Artery: An Investigation of Methods Requiring Further Standardization

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Author's response to reviews: see over
Deborah Saltman, M.D, PhD
Editorial Director,
BMC Cardiovascular Disorders

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Dear Dr. Saltman:

We are pleased to re-submit this manuscript as directed, and greatly appreciate you ongoing consideration of our manuscript entitled “Flow Mediated Dilation of the Brachial Artery: An Investigation of Methods Requiring Further Standardization.” We have revised the manuscript in response to the excellent comments from reviewers, and believe this is an improved manuscript.

The specific responses to reviewers follow their comments, in a point-by-point fashion below.

Please contact me with any questions.

Respectfully,

Alon Peretz, MD, MHS

Reviewer 1


We thank this reviewer for the extremely relevant articles suggested. They have been discussed in the revised manuscript.

In the 2006 Guidelines of American Society of Echocardiography, it is clearly stated that “when the cuff is placed on the upper part of the arm, reactive hyperemia typically elicits a greater percent change in diameter compared with that produced by the placement of the cuff on the forearm. This may be caused by a greater flow stimulus resulting from recruitment of more resistance vessels, or possibly by direct effects of ischemia on the brachial artery. Compared with forearm cuff occlusion, upper arm occlusion is technically more challenging for accurate data acquisition because of collapse of the brachial artery non visualization of color Doppler flow, and the shift in artery and soft tissue that occur on cuff release.” The confirmatory nature of your findings should be more overtly discussed.

We acknowledge the observation made by this reviewer regarding the confirmatory nature of some of our results. This is explicitly addressed in the discussion of the revised manuscript.
We agree with this reviewer about the fundamental importance of the Guidelines of the American Society of Echocardiography. We are familiar with this review by Roman MJ et al. and feel that although the authors have thoroughly examined many cardinal aspects of testing flow mediated dilation at the brachial artery, they have yet to identify a standardized test for brachial artery reactivity. They state that “standardization and improvement of the measurement technique are needed before this modality can become a routine clinical assessment of CVD risk.” We believe that the study of reproducibility of brachial artery reactivity is essential in determining this study’s clinical role. In the Discussion in our revised manuscript we emphasize that fact, in light of conflicting evidence on variability and repeatability of the test.

Reviewer 2

My main comment involves the discussion part of the part and the inference the authors make. In their study in one center, with one well trained and dedicated sonographer well educated in providing the best images, with one off line reader, again well trained, and trained in all ins and outs of the measurement, with sufficient time dedicated the job, in all healthy subjects, the reproducibility at different time points is very modest with Intraclass correlation coefficients of 0.6. In this study the FMD measurement is probably the best you can get.

We thank this reviewer for acknowledging the methodology we employed to assess brachial artery dilation accurately. We share with the reviewer the perception about the importance of employing an accurate methodology to test brachial artery reactivity, in order to reduce measurement error and increase the reproducibility of the test.

There are many other single center and multicenter studies published that report coefficients of variation of FMD measurements of 25-50%. Also in these studies dedication was present, yet the imaging technique or the off line measurements was less than what has been reported here or there were more technicians involved, clearly leading to increased measurement error. Therefore, my recommendation is to address this further in the discussion on the relevance of the FMD measurement or measurement of change in FMD in multicenter studies, or in studies with more technicians. To me these results, and those of other indicate that either very large sample sizes are needed, or multicenter studies should be abandoned. Also, the authors may want to discuss the value of having duplicate measurements in a study as a potential means to reduce part of the measurement error.

We agree with the reviewer’s comment and have further elaborated on this in the Discussion of the revised manuscript.

Reviewer 3

The magnitude of the shear stress stimulus is a critical determinant of the magnitude of the FMD response. Variability in FMD can have shear stimulus as a contributor. Thus, an assessment of FMD repeatability by necessity must include normalization to shear stress. The shear stress of relevance (peak or total prior to peak dilation) has been a matter for debate, although Pyke and Tschakovsky have just published data indicating that the peak shear does not determine the FMD magnitude, rather it is the area under the curve of the reactive hyperemia that is of critical importance (see J.Appl.Physiol. 2007 e-pub). Thus, simply from the point of view of repeatability in the different occlusion cuff positions: are the differences between proximal and distal occlusion repeatability due to variations in the reactive hyperemia shear stress magnitude?
We thank this reviewer for acknowledging our methodology in this study. The reviewer suggests using the shear stress to normalize the FMD, postulating that variability in FMD may have shear stimulus as a contributor. We fully agree with this assumption. We read the newly published reference mentioned by this reviewer (Pyke and Tschakovsky. J Appl. Physiol. 2007 e-pub) with interest, and have now cited this paper. We are convinced by the evidence that the area under the curve of the reactive hyperemia—and not the peak flow—determine the magnitude of FMD. Unfortunately, we did not measure the pulsed wave Doppler signal continuously due to limitation in the device used (forcing us to choose between Doppler or B-mode), thus we are unable to calculate the area under the curve of the shear rate. It is clearly better to have this available, and we have addressed this limitation in the manuscript.

The authors state as part of their rationale for the current study that there is a lack of standardized method to measure brachial artery reactivity based on a 2001 report (their reference #5). That reference does indicate the following:

“If this technique is going to be a valuable clinical and research tool, the following issues regarding method, along with others, need to be addressed to enable accurate data collection and interpretation: (1) location of the occlusion device on the arm (upper versus lower arm), (2) duration of brachial artery occlusion, (3) timing for detection of peak hyperemia.”

However, since that time it has been established that proximal occlusion results in FMD that is largely nitric oxide independent (eg. Doshi et al Clin Sci 101:629-35, 2001), 2) that the timing of the peak FMD is delayed in proximal vs. distal occlusion (Doshi et al Clin Sci 101:629-35, 2001) and that the magnitude of FMD is consistently greater in proximal occlusion (see any reference that compares the two). Thus, the hypothesis in the current study regarding proximal occlusion resulting in the greatest FMD magnitude has already been extensively tested and confirmed. Given that it is also fairly evident that the proximal occlusion site is not appropriate due to the minimal NO contribution to its FMD, it is puzzling to this reviewer why the authors wished to compare proximal and distal occlusion. Let us put it in this context: what would the authors have recommended if the statistical analysis revealed that the proximal occlusion exhibited better repeatability? Would they then have advocated for the use of proximal occlusion to evaluate endothelial function? This reviewer would argue strongly against a statistical underpinning guiding the choice of the FMD technique, when that technique is not measuring the physiological determinant of interest.

We greatly appreciate the reviewer’s important comment. There is a growing consensus—derived from recently published papers—that the distal occlusion provides more information on the nitric-oxide mediated aspects of FMD. Our project was initiated before this consensus had developed, and we believe that our study provides an additional insight into informing methodological considerations. Some investigators have continued to use proximal occlusion because of the larger magnitude of response, on the premise that this will permit a larger “signal-to-noise” ratio and hence have advantages when it comes to statistical analysis of the results. Our data indicate, and have bolstered this concept in the discussion, that there is now not only physiological but also statistical reasons to prefer distal occlusion in the assessment of FMD as an outcome.

One of the hypotheses of the current study stated in the last sentence of the Background section has been tested numerous times and it has been consistently demonstrated that proximal occlusion results in a greater FMD. (See any of the literature in which this comparison has been made). Thus, testing of this hypothesis is redundant.

We agree with this reviewer’s comment that a proximal occlusion result in a greater and delayed response compared to a distal occlusion, and that this was shown extensively
before. We have addressed this in the revised manuscript. Our study aims were reworded accordingly, and the results of these outcomes were underlined as confirmatory, rather than original.

Having voiced these concerns, this reviewer would ask the authors to consider their work in the following perspective.

We find the reviewer’s suggestions thoughtful and essential for understanding certain aspects of endothelial function.

First, one aspect of the data that is novel is the carefully collected information on the time course of FMD. The reviewer appreciates the careful approach taken to document the time course of FMD. It is clear that the old approach of choosing an arbitrary (60 s post) time for assessment of peak FMD is misguided. With regard to this point, the authors have a robust data set in terms of characterizing the variability of time course responses in this particular group. The authors would be encouraged to expand this data set in the distal cuff position and investigate factors that might affect the timing of the peak FMD. This would provide critical direction for investigators who base their FMD assessment on the initial studies that seemed to indicate a peak FMD at ~60 s.

The reviewer has brought up an exciting opportunity for additional research using the data we have collected, and continue to collect. While this is beyond the scope of the work in this paper, we will develop this further in our ongoing project.

Second, there has been up to now a virtually exclusive emphasis on FMD as a bioassay of endothelial nitric oxide function. However, shear stress…and indeed other physiological stimuli…evoke endothelial release of numerous other known (and potentially unknown) vasoactive and vasoprotective factors. Understanding how all aspects of endothelial function might be related to disease seems critical. Therefore, the particular aspect of endothelial function that is interrogated with the proximal cuff position may yet have important implications. Thus, while a comparison of the two cuff positions may not be appropriate because they interrogate nitric oxide to different degrees, there may be important applications for the proximal cuff intervention. In this regard, an approach which frames the assessment of repeatability and characterization of magnitude and time course of the current data set (and adds shear stress measurement) in terms of application to measures of different components of endothelial function may be of considerable value.

We fully agree with the reviewer’s insight into this question. We have added a paragraph to the discussion and a conclusion point to highlight this issue and the importance of this question for future research.