Reviewer's report

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

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Reviewer: Michael Tschakovsky

General

This study by Peretz et al. was conducted with the intent of establishing a consistent method for brachial artery reactivity assessment. The authors compared what they describe as commonly used techniques to establish characteristics of magnitude, time course, and repeatability of FMD. It appears to this reviewer that the study was carefully and thoroughly conducted in terms of measurement and analysis of data. The continuous measure of vessel diameter with automated edge detection is particularly useful. The authors are to be commended on their careful approach. There are however a number of serious concerns that substantially undermine the impact of the current study. These are as follows: 1) The omission of shear stress measurements, 2) The issue of whether the use of proximal vs. distal occlusion remains open for debate, therefore warranting the present comparison. The guiding determinant here would be whether both techniques are assessing the same mechanistic FMD response to elevations in shear stress. 3) That the magnitude of FMD is greater with proximal occlusion, one of the main hypotheses tested by the authors, has already been clearly established. These issues are addressed in detail below.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. Omission of shear stress

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?

2. Is the proximal vs. distal cuff position still a matter of debate?

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) the timing of the peak FMD is delayec 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.

3. Issue of FMD magnitude difference between cuff positions
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.

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

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.

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.