Comparison of baseline brachial artery measurements and effect on peak flow-mediated dilation

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Summary

Background Flow-mediated dilation (FMD) following reactive hyperaemia can use different time-point measures for baseline diameter. The aim of this study is to compare preocclusion, occlusion and postocclusion baseline brachial artery measurements on the calculation of peak FMD.

Study Design Ultrasound imaging of the brachial artery following reactive hyperaemia was conducted in 418 children and 533 adults. Baseline brachial artery measurements were a 10-s average before (preocclusion), during (occlusion) and after (post occlusion) hyperaemia. Peak FMD was defined as the greatest percent change from baseline to the peak brachial artery diameter following reactive hyperaemia.

Results Preocclusion, occlusion and postocclusion baseline measures of brachial artery diameter were not significantly different in children (3.15 ± 0.51, 3.14 ± 0.50 versus 3.11 ± 0.50 mm, P = 0.179) or adults (3.81 ± 0.72, 3.81 ± 0.73 versus 3.79 ± 0.73 mm, P = 0.201). Peak FMD values were not significantly different when calculated from preocclusion, occlusion or postocclusion baselines in children (6.77 ± 5.78, 6.93 ± 4.03 versus 7.85 ± 3.62%, P = 0.208) or adults (6.07 ± 5.53, 6.14 ± 3.94 versus 6.62 ± 3.70%, P = 0.266).

Conclusion We found no difference in FMD regardless of the baseline brachial artery diameter used in children and adults. Therefore, compilation of data and comparison of results from studies utilizing different measures of baseline brachial diameter may be able to be conducted.

Introduction

Dysfunction of the endothelium is commonly associated with increased cardiovascular risk and atherosclerosis, (Vita & Keaney, 2002) as well as increasing age (Gerhard et al., 1996), gender (Kapuku et al., 2001; Juonala et al., 2008), cigarette smoking (Lekakis et al., 1998) and obesity (Tounian et al., 2001; Williams et al., 2005). Today, the most widely accepted non-invasive method for measuring endothelial function is ultrasound imaging of the brachial artery following reactive hyperaemia or flow-mediated dilation (FMD; Corretti et al., 2002). Peak dilation of the brachial artery is subsequently calculated as the greatest percent change from baseline brachial artery diameter following hyperaemia (Celermajer et al., 1992).

Although technical aspects of performing FMD studies has been developed and discussed extensively, (Corretti et al., 2002; Barac et al., 2007; Peretz et al., 2007; Williamson et al., 2008; Thijssen et al., 2011; Flammer et al., 2012; Marlatt et al., 2012) there are still diverse techniques implemented among the scientific community (Peretz et al., 2007). Historically, researchers have measured FMD using a blood pressure occlusion cuff at various locations along the arm, from the upper arm above the ultrasound probe (Peretz et al., 2007) to the wrist, (Doshi et al., 2001) yet the most widely accepted technique occludes the forearm (Peretz et al., 2007). Duration of occlusion has also been variable among studies, (Corretti et al., 2002; Harris et al., 2011; Thijssen et al., 2011) although the most widely implemented occlusion time is 5 min as it induces a response predominantly endothelium mediated and nitric oxide dependent (Corretti et al., 2002; Barac et al., 2007; Harris et al., 2010; Thijssen et al., 2011). Baseline brachial artery diameter assessment technique has also been variable among the scientific community (Herrington et al., 2001; Thijssen et al., 2008; Harris et al., 2010). To our knowledge, only one study has investigated the impact of baseline brachial artery diameters determined at differing time points on peak FMD and that study only examined two common brachial...
artery locations (Thijssen et al., 2008). Currently, three differing baseline brachial artery diameter measures are being used in peak FMD determination within the literature (Herrington et al., 2001; Thijssen et al., 2008). In this study, we examined the potential effect of these three diameter measures of the brachial artery on the determination of peak FMD. To our knowledge, the impact of these three time-point measurements of brachial baseline diameter has not been fully investigated. Concurrent with previous research comparing two differing time-point baseline measures, it is hypothesized that the three differing time-point baseline measurements will be significantly different, thus producing a significant difference in subsequent peak FMD calculations.

Materials and methods

Study population

Four hundred eighteen healthy children (228 males, 190 females) and 533 adults (257 males, 276 females) were assessed for peak FMD from three differing time-point baseline brachial artery measurements. All subjects were healthy and were recruited from a community-based sample. The study protocol was reviewed and approved by the University of Minnesota Institutional Review Board, and all participants and parents/guardians gave written informed assent and consent. The procedures followed in this study were in accordance with the institutional review board and HIPAA guidelines. Subjects were fasted for at least 8 h prior to the vascular assessment and were asked to abstain from caffeine ingestion for at least 4 h on the morning of testing and to avoid strenuous exercise or physical activity for 24 h prior to the study visit.

Physical assessments

Measurements for height and weight were obtained with a standard stadiometer (Ayrton, Model S100, Prior Lake, MN, USA) and electronic scale (ST Scale-Tronix, Serial No. 5002-8893, White Plains, NY, USA), respectively. Body mass index (BMI) was calculated as weight in kilograms (kg) divided by height in meters squared (m²).

Vascular assessments

Vascular testing was performed in the Vascular Biology Laboratory in the Clinical and Translation Science Institute on the Minneapolis campus of University of Minnesota. Subjects were tested in a quiet, climate-controlled room (22–23°C). Resting blood pressure was recorded using an automated sphygmomanometer (Colin Medical Instruments Corp., San Antonio, TX, USA) on the right arm prior to FMD assessment. Following 15 min of quiet rest in the supine position, vascular images of the left brachial artery were obtained proximal to the antecubital fossa in the longitudinal plane using a conventional ultrasound scanner (Acuson, Sequoia 512, Siemens Medical Solutions USA, Inc., Mountain View, CA, USA) with a 8–15 MHz linear array probe held at a constant pressure on the skin and at a fixed point over the imaged artery by a stereotactic arm. A blood pressure cuff was then placed on the left forearm. Preocclusion baseline diameter was defined as a 10-s average before blood pressure cuff inflation. Occlusion baseline diameter was established as a 10-s average just prior to blood pressure cuff release. Postocclusion baseline diameter was determined from a 10-s average immediately following the release of the blood pressure cuff.

Following preocclusion baseline diameter measurement, the blood pressure cuff on the left forearm was inflated to a suprasystolic pressure level of 200 mmHg and maintained for 5 min. Vascular images were captured 20 s prior to cuff release until 3 min post-cuff release. All images were digitized and stored on a personal computer for later off-line analysis using an electronic wall-tracking software program (Vascular Research Tools 5, Medical Imaging Application, LLC, Iowa City, IA, USA). Vascular images were assessed, and baseline brachial artery measurements were recorded as averages of specified time points. The electronic wall-tracking software was also used to ascertain maximal brachial artery dilation captured following reactive hyperaemia. To determine peak FMD, each subject’s unique maximum brachial artery diameter was used to calculate the percent change from preocclusion, occlusion and postocclusion baseline diameters.

Statistical analysis

All statistical analysis was performed using IBM SPSS Statistics 21 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.). Repeated measures ANOVA adjusted for age and gender were conducted among preocclusion, occlusion and postocclusion baselines, as well as peak FMD from baselines. Greenhouse–Geisser F-statistic and significance were reported for within-subjects effects to correct for susceptibility of type I error due to statistical significance of assumed sphericity. Bonferroni comparisons among baselines as well as FMD changes were conducted to distinguish statistically significant within-subject effects. An alpha value of 0.05 was denoted as statistically significant. Children and adult baseline and FMD change were statistically analysed separately.

Results

Mean demographic characteristics of healthy children and adults are displayed in Table 1. Children ranged in age from 6 to 17 years, with a mean age of 12 years (±3±2 years). Adults were between the age of 18 and 49 years, with a mean age of 37 years (±6±6 years). Both groups had impartial composition of gender and healthy hemodynamic characteristics. The BMI for the adults was 29±1 and 21±0 kg m⁻² in the children. The mean BMI percentile for children in the study was 64,
which is within a healthy reference range (Kuczmarski et al., 2000).

Vascular assessment measures for children and adults are found in Figs 1 and 2. Baseline measurements in children (3.15 ± 0.51, 3.14 ± 0.50 versus 3.11 ± 0.50 mm) were not significantly different (df = 1.273, F = 1.797, P = 0.179) (Fig. 2). Similarly peak FMD (6.77 ± 5.78, 6.93 ± 4.03 versus 7.85 ± 3.62%) was also not significantly different in children (df = 1.304, F = 1.602, P = 0.208) (Fig. 2). Correcting the model for BMI and systolic blood pressure (SBP) did not result in a significant difference for baseline measures of the brachial artery (df = 1.278, F = 2.821, P = 0.083) or peak FMD (df = 1.312, F = 2.346, P = 0.117) in children.

As observed in children, baseline measurements of the brachial artery in adults (3.81 ± 0.72, 3.81 ± 0.73 versus 3.79 ± 0.73 mm) were not significantly different (df = 1.295, F = 1.641, P = 0.201) (Fig. 1). Peak FMD (6.06 ± 5.53, 6.12 ± 3.94 versus 6.62 ± 3.70%) was also not significantly different in adults (df = 1.312, F = 1.292, P = 0.266) (Fig. 1). Correcting the statistical model for BMI and SBP did not result in a significant difference for baseline measures of the brachial artery (df = 1.295, F = 1.477, P = 0.229) or peak FMD (df = 1.311, F = 1.382, P = 0.248) in adults (Figs 2–4). A strong correlation was found among baseline measures for both children and adults (Figs 3–4).

### Discussion

The aim of this study was to examine the effect of the three different baseline measures of the brachial artery in the calculation of peak FMD. The findings of the present study indicate no statistical difference among preocclusion, occlusion and postocclusion baseline measurements and corresponding determination of peak FMD in healthy children or adults.

Unlike Thijssen et al. (2008) who observed preocclusion and occlusion baselines and peak FMD significantly different for both children and adults, no significant difference was found between these baselines or their corresponding peak FMD in children or adults in the present study. The contradiction between the two study results may be explained in the

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**Table 1** Demographic characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Children</th>
<th>Adults</th>
</tr>
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<tbody>
<tr>
<td>n (% male)</td>
<td>418 (55%)</td>
<td>533 (48%)</td>
</tr>
<tr>
<td>Age, years</td>
<td>12.2 ± 3.2</td>
<td>37.0 ± 6.6</td>
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<tr>
<td>Blood pressure, mmHg</td>
<td></td>
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<tr>
<td>Systolic</td>
<td>111.7 ± 11.8</td>
<td>124.5 ± 15.8</td>
</tr>
<tr>
<td>Diastolic</td>
<td>58.2 ± 7.8</td>
<td>71.6 ± 10.9</td>
</tr>
<tr>
<td>Height, cm</td>
<td>153.5 ± 17.7</td>
<td>171.2 ± 10.8</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>51.6 ± 21.1</td>
<td>85.6 ± 23.8</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>21.0 ± 5.2</td>
<td>29.1 ± 7.6</td>
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</table>

Values are means ± SD; n, number of participants/group.

**Figure 1** Mean (±standard error) baseline brachial artery diameter and percent change from baseline for 533 adults (square) for preocclusion, occlusion and postocclusion measures.

**Figure 2** Mean (±standard error) baseline brachial artery diameter and percent change from baseline for 418 children (circle) for preocclusion, occlusion and postocclusion measures.
slight difference in methods and/or differing sample sizes. The current study utilized a stereotactic arm to stabilize the arm and secure the placement of the ultrasound transducer. Thijssen et al. (2008) did not specify the use of a stereostatic arm or a device to hold the subject’s arm. It is imperative to image the same section of the brachial artery throughout the

Figure 3 Correlation and Bland–Altman comparison plots between preocclusion, occlusion and postocclusion baseline measures for 533 adults.
vascular assessment and to ensure image quality and a stereotactic arm aids in image acquisition (Flammer et al., 2012). The difference in sample size between the current study (418 children and 533 adults) and Thijssen et al. (2008) (45 children and 31 adults) could also explain the conflicting results. When using a smaller sample size, statistical significance

Figure 4 Correlation and Bland–Altman comparison plots between preocclusion, occlusion and postocclusion baseline measures for 418 children.
could occur by chance and thus provide little consequential support to the research (Carver, 1978). This could explain the significance found between preocclusion and occlusion baselines and subsequent FMD reported by Thijssen et al. (2008). Statistical significance in research is usually dependent on sample size, and when using a very large sample size, cautious interpretation of results can avoid reporting statistically significant mean difference when, from a research standpoint, the detected mean difference is small and insignificant (Carver, 1978). Both studies could thus be prone to type I errors, where a significant mean difference was reported when in reality there is no difference. The logic of ‘statistically significant but clinically insignificant’ would only solidify the reported results of the current study due to no significant differences among baselines or FMD changes, following adjustment for age and gender, even with a large sample.

Sample demographics, specifically age, may also explain the inconsistency in results between studies. Thijssen et al. (2008) examined children that on average were 2 years younger (10.0 ± 0.8 years) than the children examined in our study (11.2 ± 3.2 years). When comparing mean baseline brachial artery measures between studies, the current study had about 0.5 mm larger diameter than Thijssen et al. (2008) for both preocclusion and occlusion baselines in children. The stage of adolescent development of the children sampled could attribute to the inconsistency in results between studies, although preocclusion and occlusion baselines were not significant for either children or adults in the current study. An alternative consideration could be the larger artery size of children in the current study allowed for less user error.

The lack of statistical significance among differing time-point baseline measurements is an interesting finding in the current study. This would lead researchers to conclude that preocclusion, occlusion and postocclusion baselines could theoretically be interchangeable in methodology when using healthy children and adult subjects. Consequently, as significant difference was not found between the investigated baseline measures or effect on peak FMD, then comparison of results utilizing these time-point baseline measures could be conducted. Of course, these comparisons should be made with careful consideration, specifically the prognostic value of the FMD from the three differing baselines.

The findings of this present study aid in the development of standardized methodology for clinical assessment of vascular health using ultrasound imaging following FMD. Current FMD research techniques should consider capturing data concerning all three baseline brachial artery measures for analysis, as the incorporation of this into FMD procedures would take minimal effort. Once available, prognostic value of FMD from the three differing baselines could be assessed and the proper baseline method could be chosen for future studies.

**Study strengths** include the considerably large sample size for detection of differences in baseline measures in healthy children and adults. One limitation of the present study is that all subjects were healthy children and adults. Generalization to less healthy populations is limited. Secondly, FMD techniques were performed by four trained professionals. Ideally, all subjects in the study would have had flow-mediated dilation techniques performed by one trained professional. Furthermore, techniques that vary from 5 - min occlusion distal to the ultrasound probe cannot be compared with the findings of this study. The variance of time-point baseline measures could not be determined due to the cross-sectional nature of this study.

**Conclusion**

Preocclusion, occlusion and postocclusion baseline brachial artery diameters were not statistically different in our populations of healthy children and adults. Furthermore, no statistically significant difference among peak FMD change was observed. Therefore, comparison and compilation of data from studies utilizing different measures of baseline brachial diameter in healthy children and adults could possibly be conducted. However, careful consideration should be used when choosing a baseline measure for non-healthy children and adult populations. Future studies could investigate non-healthy children and adults peak FMD from the three time-point baseline measures. Finally, examination of the variance of the three time-point baseline measurements by analysing multiple brachial artery diameter assessments from the same subject to determine consistency of the three baseline measurements would clarify the ideal baseline measurement technique to implement.

**Conflict of interest**

The authors have no conflict of interests.

**References**


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