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Non-Invasive Assessment of Endothelial Function

Which Technique?

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OBJECTIVES
The purpose of this study was to compare 3 non-invasive techniques for assessment of endothelial function in adults and children and evaluate their utility in acute inflammation.

BACKGROUND
Endothelial dysfunction is a key early event in pre-clinical atherosclerosis. Flow-mediated dilation (FMD), although the established technique, is expensive and technically demanding. Measurements of vascular responses to inhaled salbutamol by pulse wave analysis (PWA) or pulse contour analysis (PCA) are potential alternatives.

METHODS
Sixteen adults (mean age 28 years, range 18 to 39) and 16 children (mean age 13 years, range 7 to 17) underwent concurrent vascular function testing on 2 occasions with ultrasound, PWA, and PCA. Eighteen men were also studied before and after typhoid vaccination.

RESULTS
Reproducibility of FMD was high in adults and children (coefficient of variation [CV] = 7.1 and 6.3, respectively). Salbutamol responses were more variable with PWA (adults CV = 11.5, children CV = 17.1) and PCA particularly in children (adults CV = 18.2, children CV = 36.3). Flow-mediated dilation (p < 0.001) and PWA with salbutamol (p = 0.03) responses fell after typhoid vaccination, and PCA (p = 0.7) was unchanged.

CONCLUSIONS
Vascular dysfunction during acute inflammation can be measured by FMD and by PWA with salbutamol. Flow-mediated dilation is less variable than PWA. Variability of PCA makes this technique currently unsuited to serial measures of endothelial function in children. Flow-mediated dilation remains the most reproducible method.

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The vascular endothelium is a key signal transducer in atherogenesis (1). Study of preclinical vascular disease has been facilitated by use of non-invasive ultrasound techniques (2). The vasodilator response to increased conduit arterial flow (flow-mediated dilation [FMD]) is dependent on local nitric oxide (NO) bioavailability, and measurement of this response has been widely used in clinical studies (3,4).

Recently, alternative non-invasive techniques have been developed with beta2 adrenoceptor agonist-mediated endothelial NO release, using measurement of the response with radial artery plethysmography (pulse wave analysis [PWA]) or digital photoplethysmography (pulse contour analysis [PCA]) (5–7). Although the equipment is small, portable, and easy to use, the comparative reproducibility of PWA and PCA and their ability to detect acute vascular changes have not been determined. We designed this study to assess the reproducibility of PWA, PCA, and FMD in children and adults and compared their ability to detect inflammation-induced changes in vascular function.

METHODS

Studypopulation. We studied 16 children (11 boys, mean age 13 years, range 7 to 17) and 16 adults (9 men, mean age 28 years, range 18 to 39) to determine reproducibility and 18 men (mean age 26 years, range 18 to 39) to study vascular responses during acute inflammation. All were healthy and free from cardiovascular risk factors. All adults and parents gave written consent; children gave verbal assent. The study was approved by the local ethics committee.

Studyprotocol. All investigations were performed by 2 experienced investigators. Subjects refrained from caffeine-containing drinks and food for 4 h before study and from vigorous exercise on the day of study. All studies were undertaken in a warm, temperature-controlled room by the same operator at the same time of day on both occasions.

STUDY 1—REPRODUCIBILITY: ADULTS. Adults attended on consecutive days (Fig. 1A). Brachial FMD was assessed. The effect of a 25–μg sublingual dose of glyceryl trinitrate (GTN) was then assessed simultaneously by ultrasound, PWA, and PCA for ≥15 min after administration, until the brachial artery diameter (D) had returned to baseline. The PWA and PCA were assessed before and at 2.5-min intervals.
for 20 min after salbutamol inhalation via a spacer device (adults 400 μg, children 200 μg, Allen & Hanbury’s, GlaxoSmithKline, Uxbridge, United Kingdom). Blood pressure, D, peripheral augmentation index (PAI), and reflection index (RI) were measured at baseline and before each intervention.

STUDY 1—REPRODUCIBILITY: CHILDREN. Children attended on 2 occasions at the same time of day, 1 week apart (to avoid disrupting school attendance). The GTN was omitted to shorten the study and improve recruitment and compliance (Fig. 1B).

STUDY 2—RESPONSE TO AN ACUTE INFLAMMATION. Eighteen men were studied before and 8 h after a typhoid vaccination (TYPHIM Vi 0.5-ml injection, Aventis Pasteur, Merck Sharp & Dohme; Hoddesdon, Hertfordshire, United Kingdom), which transiently attenuates FMD (8) (Fig. 1A).

Vascular function assessment. BRACHIAL ARTERY FMD. The right brachial artery was imaged, 5 to 10 cm above the antecubital fossa, with a high-resolution ultrasound probe (Acuson Aspen, Siemens, Malvern, Pennsylvania) held in a stereotactic clamp. Brachial artery FMD was induced by a 5-min inflation of a pneumatic cuff placed around the forearm immediately below the medial epicondyle (adults 300 mm Hg, children 200 mm Hg) followed by rapid deflation with an automatic air regulator (Logan Research; Rochester, Kent, United Kingdom). Brachial artery diameter (D) was measured with edge detection software (Brachial Tools, Iowa City, Iowa) from electrocardiogram-triggered images captured every 3 s throughout the 11-min recording protocol (9). The FMD was expressed as maximal percentage change in vessel D from baseline (with the average of 20 baseline [D_b] and 3 peak [D_p] D readings: FMD% = (D_p − D_b)/D_b × 100). Blood flow was recorded continuously by pulsed-wave Doppler. Reactive hyperemia (RH%) was calculated from the maximal flow within the first 15 s after deflation of the pneumatic cuff, relative to the baseline flow. All ultrasound measures were performed offline by one experienced investigator (A.E.D.) masked to the order of the study visit.

PWA. The radial pressure-pulse waveform was acquired with a micromanometer (SPC-301, Millar Instruments, Houston, Texas) (10). Data were recorded directly onto a computer running proprietary software (SphygmoCor version 7.0, Scanmed; Moreton-in-Marsh, Gloucestershire, United Kingdom). Peripheral and central augmentation index can be derived from the peripheral pressure pulse (11). We used PAI, because changes in the central pulse waveform with salbutamol are more subtle and the transfer function used to derive the central pressure-pulse waveform has not been validated in children.

PCA. The RI was obtained with a photoplethysmograph (Micro Medical; Gillingham, Kent, United Kingdom), on the index finger of the left hand and measured as previously described (5).

Pulse wave analysis and PCA were performed simultaneously; 3 baseline readings, 1 min apart, were averaged, and the maximal changes in PAI and RI after GTN and salbutamol were calculated.

Statistical analysis. For PWA and PCA, results are expressed as the maximal change in PAI (ΔPAI_b; ΔPAI_c) and RI (ΔRI_b; ΔRI_c) after salbutamol and GTN administration.
tion, respectively. Values are expressed as mean ± SD unless otherwise stated. Within-method reproducibility is expressed as the technical error of the measurement (TEM) (12) and as the percentage of the coefficient of variation (SD of the paired differences/the overall mean)/√2 × 100.

Data are also presented with Bland-Altman plots. Relationships between vascular responses were examined with the Pearson correlation. Student paired t tests were used to assess the effect of typhoid vaccination on vascular function. Statistical analyses were performed with SPSS v12 (SPSS, Chicago, Illinois).

### Table 1. Summary of the Baseline Data and Endothelial-Dependent and Endothelial-Independent Function for Three Methods in Adults and Children

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Endothelial Function</th>
<th>Endothelial-Independent Function</th>
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<tbody>
<tr>
<td></td>
<td>D (mm)</td>
<td>PAI (%)</td>
<td>RI (%)</td>
</tr>
<tr>
<td>Adults</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Visit 1</td>
<td>3.5 ± 0.5</td>
<td>48.8 ± 9.1</td>
<td>68.4 ± 9.2</td>
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<tr>
<td>Visit 2</td>
<td>3.6 ± 0.5</td>
<td>47.1 ± 9.1</td>
<td>66.9 ± 9.5</td>
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<tr>
<td>TEM</td>
<td>0.06</td>
<td>2.1</td>
<td>2.7</td>
</tr>
<tr>
<td>CV (%)</td>
<td>1.7</td>
<td>4.3</td>
<td>3.9</td>
</tr>
<tr>
<td>Children</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Visit 1</td>
<td>3.1 ± 0.6</td>
<td>42.7 ± 9.7</td>
<td>64.9 ± 11.2</td>
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<tr>
<td>Visit 2</td>
<td>3.0 ± 0.6</td>
<td>44.7 ± 9.7</td>
<td>61.2 ± 16.4</td>
</tr>
<tr>
<td>TEM</td>
<td>0.05</td>
<td>1.8</td>
<td>3.6</td>
</tr>
<tr>
<td>CV (%)</td>
<td>1.6</td>
<td>4.1</td>
<td>5.8</td>
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CV = coefficient of variation; D = diameter; FMD = flow-mediated dilation; GTN = Glyceryl trinitrate mediated dilation; PAI = peripheral augmentation index; ΔPAI<sub>C</sub> = change in PAI with GTN; ΔPAI<sub>S</sub> = change in PAI with salbutamol; RI = reflection index; ΔRI<sub>C</sub> = change in RI with GTN; ΔRI<sub>S</sub> = change in RI with salbutamol; TEM = technical error of the measurement.
RESULTS

Study 1—reproducibility. All adults and all but 2 children completed both assessments (Table 1, Fig. 2). One child did not return and one engaged in strenuous exercise immediately before visit 2. Reproducibility of the baseline measures before endothelial stimulation was highest for D and similar for PAI and RI both in adults and children. Baseline flow and reactive hyperemia were the same on both visits in adults and children.

Flow-mediated dilation was the most reproducible measure between visits, followed by $\Delta$PAI$_S$, then $\Delta$RI$_S$. $\Delta$PAI$_S$ and particularly $\Delta$RI$_S$ were considerably less reproducible in children. Between-visit differences versus mean values for the 3 measures in adults and children show no obvious trends in mean or variability. Individual estimates of endothelial function by the 3 methods were uncorrelated with each other ($\text{FMD/}\Delta$PAI$_S$ r = $-0.05$, $\text{FMD/}\Delta$RI$_S$ r = $-0.08$, $\Delta$PAI$_S$/$\Delta$RI$_S$ r = 0.03). Reproducibility of GTN-mediated changes was less good for all 3 methods.

Study 2—response to acute inflammation. Baseline diameter, PAI, and RI were unchanged after typhoid vaccination (Fig. 3). Baseline flow ($p = 0.53$) and reactive hyperemia ($p = 0.45$) were unchanged before and after typhoid. Flow-mediated dilation and $\Delta$PAI$_S$ fell by 24% ($p = 0.0005$) and 22% ($p = 0.03$), respectively 8 h after vaccination. $\Delta$RI$_S$ was unchanged ($p = 0.7$). Responses to GTN with all modalities remained the same.

DISCUSSION

This study shows that FMD is the most robust of these non-invasive measures of endothelial function in both children and adults. The PWA was less reproducible in children, and PCA performed acceptably only in adults. Flow-mediated dilation and PWA can detect changes in vascular function during acute inflammation.

Endothelial dysfunction is central to early atherogenesis (1,4). The development of non-invasive FMD as a surrogate measure of arterial health has enabled the study of children and healthy populations, providing an opportunity for early detection and prevention. This technique is conceptually simple, NO dependent, and can measure the effect of interventions (2,4,13). Although methodological advances have diminished operator dependence, it remains expensive and technically demanding (14), prompting the search for simpler and cheaper validated techniques.

Salbutamol, a beta$_2$ agonist, causes vascular endothelial NO release, enabling measurement of global rather than local NO-dependent vascular changes (5,6). The drug was administered via a spacer, providing reproducible pulmonary delivery in healthy subjects (7). Pulse wave and contour responses with salbutamol are reproducible in adults and impaired in diabetes, hypercholesterolemia, and coronary artery disease (5–7). However, there are few data on their ability to detect changes with interventions and no data on their applicability in children. Similarly, these methods have not been assessed in smokers, asthmatic patients, and those with other pulmonary conditions, limiting their potential applicability in such subjects.

Typhoid vaccination has been shown to induce transient conduit and microvascular endothelial dysfunction in ≤12 subjects (8,15). In contrast to the findings of Vlachopoulos et al. (16), baseline augmentation and reflection indexes were unaffected by vaccination, but $\Delta$PAI$_S$ fell by 22%, consistent with the extent of endothelial dysfunction seen with FMD. The lack of change in $\Delta$RI$_S$ after typhoid might be due to the greater variability of this technique, resulting in a type 2 error. Alternatively, the vascular effects of vaccination might be less readily detected by $\Delta$RI$_S$ as a result of differences between the volume and pressure-pulse waveforms.

We have been able to demonstrate lower reproducibility of PWA and particularly PCA compared with FMD, clearly distinguishing between the methods even within this relatively small study. This might have important consequences for clinical use of these techniques. Thus studies based on PWA and PCA must be larger than for FMD to achieve the...
same power to detect a given effect size. Taking account of the intrinsic variability demonstrated for each method, power calculations indicate that studies using PWA and PCA would need to be 2 and 6 times larger, respectively, than those using FMD.

The strengths and weaknesses of available methods for endothelial function measurement should be considered in relation to any proposed clinical application. Flow-mediated dilation is accurate and reproducible after appropriate training and applicable to large population studies (3,17). Pulse wave analysis is cheap and portable but requires considerable expertise, remaining in part operator dependent, and current analysis software algorithms are limited in the presence of sinus arrhythmia, common in children and young adults. Pulse contour analysis requires little training and is operator independent; however, it is more variable, possibly owing to the greater influence of sympathetic tone on hand circulation physiology.

We were interested in noting the lack of correlation between the 3 techniques. Although the greater variability of the newer methods is a possible explanation, it is more likely that our observations reflect distinct differences in the pathways responsible for endothelial regulation of arterial tone in different vascular beds. These mechanisms and their pathophysiologic implications require further investigation.

**Conclusions.** Flow-mediated dilation remains the non-invasive technique of choice for study of endothelial function in adults and children. In addition, acute endothelial dysfunction is detectable with PWA, supporting its potential use in mechanistic vascular studies. Pulse wave analysis and PCA are promising methods for assessment of vascular function, but their reproducibility needs to be improved, especially if contemplating use in children. Furthermore, these methods might provide different information on vascular pathophysiology. The larger subject numbers required to demonstrate clinically significant differences reduce the financial and practical advantages of these newer techniques.

**REFERENCES**

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