Arterial Applanation Tonometry: Feasibility and Reproducibility in Children and Adolescents

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BACKGROUND
Aortic pulse wave velocity (PWV) and augmentation index (AIx) are markers of vascular health and have recently been used in pediatric clinical trials. However, there are limited data on standardization of these measurements in pediatrics. The objective of this study was to prospectively test the feasibility and reproducibility of PWV and AIx in children and adolescents.

METHODS
We performed arterial tonometry on 2 different days within 2 weeks in 40 healthy subjects aged 10–19 years. PWV and AIx were measured in triplicate on each visit.

RESULTS
The visits were separated by a mean of 3.08 ± 3.7 days. We obtained PWV in 77 of 80 (96%) visits and AIx in 76 of 80 (95%) visits in triplicate. Intraclass correlation coefficients (ICCs) for PWV were 0.61 (95% confidence interval [CI] = 0–0.86) when at least 2 measurements and 0.92 (95% CI = 0–1) when 3 measurements were obtained at each visit that met the quality criteria established for adults by the manufacturer (n = 17 and 3 paired visits, respectively). For AIx, ICCs were 0.78 (95% CI = 0.58–0.88) and 0.81 (95% CI = 0.63–0.90) when measurements with an operator index ≥80, a measure of the quality of the waveform, were included (n = 39 and 36 paired visits, respectively).

CONCLUSIONS
Arterial applanation tonometry is feasible and reproducible in healthy children and adolescents. AIx has excellent intervisit reproducibility, whereas the intervisit reproducibility of PWV relies on acquisition of multiple measurements that meet quality criteria established for adults. These results have implications for the methodology of future pediatric clinical trials in a population at increasingly higher risk for premature atherosclerosis.

Keywords: adolescents; augmentation index; blood pressure; children; hypertension; pulse wave analysis; pulse wave velocity; reproducibility.

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Cardiovascular disease is the leading cause of death in Western societies. The Bogalusa and the Pathobiological Determinants of Atherosclerosis in Youth studies demonstrated that pathological changes of atherosclerosis begin in some children in the first decade of life. Obesity, dyslipidemia, hypertension, and hyperglycemia are additive modifiable risk factors as these children reach adulthood.

It has been widely recognized that carotid–femoral or aortic pulse wave velocity (PWV) and augmentation index (AIx) are markers of vascular health and, consequently, of cardiovascular outcome in the adult population. In children, PWV has been assessed in many conditions, such as obesity, chronic renal failure, hyperlipidemia, Kawasaki disease, and diabetes mellitus, as a marker of vascular health.

Traditional endpoints such as stroke, myocardial infarction, and mortality used in adult clinical trials are not suitable to assess the effect of intervention or treatment in pediatric cohorts. More recently, arterial applanation tonometry has been used as a primary outcome in pediatric clinical trials.

There are strict established measurement criteria for quality and reproducibility purposes in the adult population, whereas standardization of arterial tonometry in the pediatric population has not been studied. Many investigators have reported good reproducibility of PWV and AIx measurements within the same visit. There are limited data on intervisit reproducibility of PWV or AIx in the pediatric population.

By assessing the feasibility and intervisit reproducibility of this technology in a group of healthy children and adolescents, we sought to contribute to the design and methodology of clinical studies using PWV or AIx as an outcome variable in children and adolescents.

METHODS
Patient population

Subjects were recruited through an online community listing (www.craigslist.com) and public advertisement (October 2011–June 2012). To be eligible, subjects had to meet each of the following criteria: (i) age 10–19 years; (ii) absence of known

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cardiovascular risk factors such as hyperlipidemia, hypertension, obesity (body mass index (BMI) <85th percentile), diabetes mellitus, renal disease, smoking, and vascular disease such as Raynaud's disease, Kawasaki's disease, or juvenile idiopathic arthritis; and (iii) absence of other significant medical conditions, including pregnancy. Subjects were excluded if they (i) were on medications; (ii) were unable to comply with the requirements of the study, including fasting overnight; or (iii) had a recent febrile illness. Family history was collected. Study approval was obtained from the Stanford University Institutional Review Board, and written informed consent and assent was obtained.

**Pulse wave analysis**

Pulse wave analysis was performed on 2 days separated by no more than 15 days under the same physiologic and environmental conditions to the greatest degree possible. The subject's weight and height were measured on testing day 1 (Scaletronix scale, White Plains, NY; Seca stadiometer, Columbia, MD). On both testing dates, 4 sets of resting blood pressures (BPs) were measured on the testing arm in a sitting position with the oscillometric method (Dinamap-GE, Waukesha, WI) after at least 5 minutes of rest. The average of the last 3 BP measurements was used as per laboratory protocol, and z scores were calculated. The testing room was arranged to provide a quiet, restful environment with a comfortable temperature at 72–75 °F. Before testing, subjects were asked to fast overnight, except for the consumption of water. PWV and AIx were measured in triplicate on each participant on both days. The single operator (A. Lowenthal) was trained by the manufacturer (SphygmoCor, Atcor-Medical, Sydney, Australia) and blinded to measurements on prior visits. Data entry was performed exclusively by the research assistant, who was also responsible for setting up the computer with the study ID before testing to ensure the operator did not have access to prior study visit results. Electrocardiography leads were applied.

**Pulse wave velocity.** The distance from the carotid artery pulse to the sternal notch and the distance from the sternal notch to the femoral artery pulse were measured at each visit. A pressure tonometer the size of a pencil was placed on the proximal artery (carotid) then on the distal artery (femoral) to obtain arterial waveforms gated to the R-wave. PWV (m/sec) was calculated by the software as the relative difference in path length between the 2 arterial sites divided by the difference in transit time. Arterial waveforms were captured over a 10-second interval, and each PWV measurement was reported as mean ± SE by the software.

**Augmentation index.** The pressure sensor was applied to the right radial artery to sample arterial pressure waves, and the AIx measurements were collected by autocapture. A generalized transfer function validated in adults against invasive catheterization data was used by the software to calculate and reconstruct the central aortic pressure curve. AIx was computed as the ratio (%) of the aortic augmentation to the pulse pressure. The software has an incorporated operator index calculation for AIx measurements where ≥80 is considered optimal based on the waveform variation. The software also calculated the AIx adjusted to a heart rate of 75 beats per minute (bpm; AIx75).

**Blood work and dietary recall**

Fasting plasma lipid profile and C-reactive protein were obtained. The 24-hour dietary recall on each testing day was analyzed (Elizabeth Stewart Hands and Associates, V.10.1, Salem, OR) to assess for average daily intake of saturated and unsaturated fats and selected vitamins.

**Statistical analysis**

Summary statistics are presented as mean ± SD or median (range) for parametric and nonparametric data, respectively. Comparisons between the 2 testing days were made using either the paired t test or the Wilcoxon signed-rank test for continuous variables and the McNemar test for dichotomous variables. Intraclass correlation coefficient (ICC) was calculated as a measure of reproducibility. Spearman correlation was used to explore the relationship of heart rate difference between the arterial sites and PWV. P ≤ 0.05 was used to determine statistical significance. Subgroup analyses were made on PWV that fit criteria established by the manufacturer for adults (PWV<sub>carotid-femoral</sub>) as ≤5 bpm difference between the heart rate at the carotid and femoral sites and <10% carotid–femoral pulse transit time SD during the 10 seconds of capture time.

To analyze the effects of clinical variables on reproducibility of PWV, percent difference was used, defined as the absolute value of the difference in the average of the measurements on testing day 1 and 2 divided by the mean of the average of the measurements multiplied by 100. To analyze the effects of clinical variables on reproducibility of AIx, the absolute value of the difference in the average of the measurements on testing days 1 and 2 was used. Spearman correlation and multivariable regression analysis were used to explore the relationship of age, weight, BMI, BP, and cholesterol levels with the percent difference of PWV and with the difference in AIx measurements between visits. Analyses were performed with SPSS software, version 20 (IBM Corporation, Armonk, NY). Study data were collected and managed using REDCap electronic data capture tools hosted at Stanford University.

**RESULTS**

**Patients**

We enrolled 40 healthy children and adolescents in a period of 9 months (Table 1). Patient characteristics were compared on the 2 testing days. The visits were separated by a mean of 3.08 ± 3.7 days. Systolic BP z scores were higher in the participants during visit 1 compared with visit 2 (−0.33 ± 0.5 vs. −0.59 ± 0.08; P = 0.02). There were no significant differences in diastolic BP z scores, hours since last meal, medications in the last 24 hours, intake of caffeinated beverages or alcohol,
Tables 2 and 3 show summary statistics of the average of PWV and AIx measurements collected on both testing days.

**Pulse wave velocity**

**Feasibility.** We obtained PWV in triplicate in 77 of 80 visits (96%). The missing tonometry measurements (n = 4 of 240; <2%) were during testing day 1 and in the earlier period of the study (within the first 6 study subjects); there were 2 participants with only 2 PWV measurements, and 1 with only 1 PWV measurement. Sixty-one percent (n = 144 of 236) of these measurements fit the adult criteria.

PWV<sub>o</sub> measurements were captured in 73 of 116 (63%) measurements on testing day 1 vs. 71 of 120 (59%) measurements on testing day 2 (P = 0.69). PWV<sub>o</sub> measurements were captured in significantly fewer of the first 20 participants vs. the last 20 participants (n = 63 of 116 (53%) vs. n = 81 of 120 (68%); P = 0.03). Overall, 21 (26%) of the 80 subject visits had 3 PWV<sub>o</sub> measurements; 30 (38%) had 2 PWV<sub>o</sub> measurements; 21 (26%) had 1 PWV<sub>o</sub> measurement; and 8 (10%) had no PWV<sub>o</sub> measurement.

Reproducibility. The ICC coefficients for within-visit reproducibility were 0.84 (95% confidence interval (CI) = 0.72–0.91) and 0.81 (95% CI = 0.67–0.89) for testing day 1 and 2, respectively. Table 2 presents the intervisit reproducibility of the average of all PWV measurements and the average of PWV<sub>o</sub> measurements including visits with at least 1, 2, or 3 measurements that fit the criteria. When all measurements were included, there was a significant difference in the median PWV on testing day 1 vs. 2. As stricter criteria were applied, the level of agreement was superior and the difference in the median PWV disappeared.

Heart rate difference between arterial sites. The analysis of the carotid–femoral heart rate difference of each measurement and the PWV (n = 236) demonstrated that an increased absolute carotid–femoral heart rate difference is associated with a decreased PWV (r = −0.16; P = 0.01).

Reproducibility and other factors. Sex, weight, BMI, BMI percentile, total cholesterol, low-density cholesterol levels, and presence of family history of hypertension did not have an effect on intervisit PWV reproducibility. Older age and lower average systolic and diastolic BP z scores on the first visit were associated with better reproducibility (r = −0.33, P = 0.04; r = 0.48, P = 0.02; r = 0.42, P = 0.007, respectively). By multivariable regression analysis, systolic BP z score on the first visit was the only independent predictor of reproducibility (P = 0.01).
**Augmentation index**

**Feasibility.** We successfully obtained Alx in 76 of 80 visits in triplicate (95%). All visits where we could not capture 3 measurements occurred during testing day 1 and in the earlier period of the study (n = 4 of 4 (100%) within the first 20 subjects); there was 1 participant visit with only 2 Alx measurements and 3 with only 1 measurement. Measurements with an operator index ≥80 (AIx_{o80}) were made in 218 of 232 (94%) of the measurements. AIx_{o80} measurements were captured in 102 of 113 (90%) measurements on testing day 1 vs. 116 of 120 (97%) on testing day 2 ($P = 0.003$). AIx_{o80} measurements were captured in significantly fewer of the first 20 vs. the last 20 participants (n = 100 of 113 (88%) vs. n = 118 of 120 (98%); $P < 0.001$). Overall, 68 subject visits (85%) had 3 AIx_{o80} measurements; 6 (8%) subject visits had 2; 2 (2%) subject visits had 1; and 4 (5%) subject visits had no AIx_{o80} measurement.

**Reproducibility.** The ICC coefficients for within-visit reproducibility of Alx were 0.95 (95% CI = 0.92–0.97) and 0.97 (95% CI = 0.94–0.98) for testing day 1 and 2, respectively. The ICC coefficients for within-visit reproducibility when Alx75 was used were 0.94 (95% CI = 0.89–0.96) and 0.96 (95% CI = 0.92–0.98) for testing day 1 and 2, respectively. Table 3 presents the intervisit reproducibility analysis. There was no significant difference in the operator index between visits.

**Reproducibility and other factors.** The age, weight, systolic BP $z$ scores, total cholesterol, low-density cholesterol, and triglyceride levels, sex, and presence of family history of hypertension did not have an effect on the difference in Alx or Alx75 between visits. The intervisit difference in Alx correlated significantly with BMI percentile ($r = 0.41; P = 0.01$) and diastolic BP $z$ score.

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**Table 2.** Pulse wave velocity measurements and intervisit reproducibility analysis

<table>
<thead>
<tr>
<th>Pulse wave velocity (n = 40)$^a$</th>
<th>Pulse wave velocity with increasing quality criteria</th>
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</thead>
<tbody>
<tr>
<td>PWV, m/s</td>
<td></td>
</tr>
<tr>
<td>PHRA, bpm, mean ± SD</td>
<td>4.60 (3.30–7.60)</td>
</tr>
<tr>
<td>Carotid femoral pulse transit time SD, %, mean ± SD</td>
<td>4.24 ± 3.75</td>
</tr>
<tr>
<td>Testing day 1, median (range)</td>
<td>6.95 ± 2.42</td>
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<tr>
<td>Testing day 2</td>
<td>0.03</td>
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<tr>
<td>$P$ value$^b$</td>
<td>0.54 (0.13–0.76)</td>
</tr>
<tr>
<td>Intraclass correlation coefficient (95% CI)</td>
<td></td>
</tr>
<tr>
<td>PWV (n = 40)</td>
<td>4.67 (3.65–7.00)</td>
</tr>
<tr>
<td>PWV_{o80} (n = 33)</td>
<td>4.99 (3.65–7.00)</td>
</tr>
<tr>
<td>PWV_{75,o} (n = 17)</td>
<td>4.94 (3.65–7.00)</td>
</tr>
<tr>
<td>PWV_{75,o} (n = 3)</td>
<td>4.97 (3.67–7.00)</td>
</tr>
</tbody>
</table>

| Abbreviations: CI, confidence interval; HR_{PHRA}, heart rate difference between the carotid and femoral arterial sites; PWV, pulse wave velocity; PWV_{o80}, pulse wave velocity measurements that fit adult criteria established by the manufacturer (i.e., ≤5 heart beats difference between the carotid and femoral sites and <10% carotid–femoral pulse transit time standard deviation during the 10 seconds of capture time). |

$^a$Average of all measurements on both days.  
$^b$Wilcoxon rank-sum test.  
$^c$At least 1 PWV measurement that fits adult criteria.  
$^d$At least 2 PWV measurements that fit adult criteria.  
$^e$At least 3 PWV measurements that fit adult criteria.

**Table 3.** Augmentation index measurements and intervisit reproducibility analysis

<table>
<thead>
<tr>
<th>Augmentation index (n = 40)$^a$</th>
<th>Alx and Alx75 with increasing quality criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alx, %</td>
<td>3.06 ± 9.51</td>
</tr>
<tr>
<td>Alx75, %</td>
<td>0.33 ± 8.72</td>
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<tr>
<td>Operator index</td>
<td></td>
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<tr>
<td></td>
<td>91.42 ± 11.45</td>
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<tr>
<td>Testing day 1</td>
<td></td>
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<tr>
<td>Testing day 2</td>
<td></td>
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<tr>
<td>$P$ value$^b$</td>
<td></td>
</tr>
<tr>
<td>Intraclass correlation coefficient (95% CI)</td>
<td></td>
</tr>
<tr>
<td>Alx, % (n = 39)</td>
<td>2.76 ± 8.70</td>
</tr>
<tr>
<td>Alx_{o80}, % (n = 36)</td>
<td>2.72 ± 8.85</td>
</tr>
<tr>
<td>Alx75, % (n = 39)</td>
<td>0.47 ± 7.64</td>
</tr>
<tr>
<td>Alx75_{o80}, % (n = 36)</td>
<td>0.25 ± 7.81</td>
</tr>
</tbody>
</table>
| Values are mean ± SD unless otherwise noted.  
Abbreviations: Alx, augmentation index; Alx_{o80}, augmentation index measurement with an operator index ≥80; Alx75, augmentation index adjusted for a standard heart rate of 75 beats per minute, Alx75_{o80}, augmentation index adjusted for the heart rate of 75 beats per minute with an operator index ≥ 80; CI, confidence interval.  
$^a$Average of all measurements on both days.  
$^b$Paired t test.
(r = 0.32; P = 0.05); however, intervisit difference in AIx75 did not correlate with any factors. By multivariable regression analysis, BMI percentile remained as a significant independent predictor of intervisit difference in AIx (P = 0.05).

**DISCUSSION**

This study demonstrated that PWV acquisition is feasible in children and adolescents, with excellent within-visit reproducibility as shown in prior studies, although with less optimal intervisit reproducibility unless strict adult criteria (see Methods) were met and 3 measurements were obtained. PWV measurements that fit the adult criteria were obtained more frequently during the second half of the study, demonstrating a learning curve. The results showed that AIx analysis is feasible with excellent reproducibility, both within the same visit and between visits. When only measurements with an operator index ≥80 were included, reproducibility was further enhanced. This is most likely a result of the autocapture function, which accepts the waveform only if it meets all of the established AIx adult quality criteria.

**Pulse wave velocity**

In recent years there has been a significant increase in the number of clinical studies using PWV as an outcome variable, which has prompted experts to establish methodological standards for PWV measurement techniques. Wilkinson *et al.* studied reproducibility of PWV in 24 adults within the same visit and reported an intraobserver variability of 0.07 ± 0.24 m/s for PWV. More recently, Frimodt-Moller *et al.* studied 19 adults with chronic kidney disease on 2 different days within a week. The investigators did not report the heart rate difference between the carotid and femoral sites; however, they specified that they only accepted the PWV measurements if the carotid–femoral pulse transit time SD was <10%. The authors reported a mean interobserver difference of −0.7 ± 1.9 m/s (P = 0.008) for PWV with an ICC of 0.94. The investigators speculated that the discrepancy between the high ICC and the found bias could be due to high population variability, which might contribute to an artificially high ICC. There was a difference in systolic BP in the same direction as PWV (less hypertensive and less stiff) between day 1 and 2. Our results showed a similar difference in systolic BP between the 2 days, but we did not observe a relationship between the difference in systolic BP and PWV. However, systolic BP did influence reproducibility since a higher systolic BP z score on the first visit was the only independent predictor of worse reproducibility. Papaiannou *et al.* studied 60 adults and demonstrated that there is a substantial, clinically relevant difference between single PWV measurements compared with the average of 2 measurements during the same visit. The investigators did not find a difference between 2 vs. 3 measurements. Our data suggest that 2 measurements result in moderate intervisit reproducibility in children and adolescents.

Millasseau *et al.* demonstrated that the heart rate increase induced by isoproterenol (18 ± 3 bpm) and pacing (40 bpm) resulted in increased PWV in 43 adults (0.7 ± 0.2 and 2.1 ± 0.5 m/s, respectively). These findings are similar to prior studies on adults with pacemaker leads. The potential explanation for this observation is that tachycardia shortens the time available for recoil, which results in vessel stiffening. Reusz *et al.* reported in a large cohort (n = 1,008; mean age = 15.2; range = 6.5–19.9 years) that PWV decreases as the heart rate increases. However, multiple regression analysis in their study showed that heart rate was not as significant of a predictor of PWV as age, height, or BP. In this study, we demonstrated that the higher the absolute difference between the heart rates at the carotid and femoral sites is the lower the PWV is. This observation highlights the importance of minimizing environmental stimuli that can result in heart rate changes.

There are less published data on reproducibility of PWV in the pediatric population. Currie *et al.* obtained PWV on 2 separate days (average of 8 days apart) in 20 children aged 2–6 years by using a whole-body PWV using the R wave peak and the foot of the dorsalis pedis artery waveform using a plethysmograph (ADInstruments, Dunedin, New Zealand) and reported an ICC of 0.76. Reusz *et al.* reported interobserver and intraobserver coefficients of variation of 5.7% and 6.1%, respectively, for PWV measurements within the same visit. They specified in their study methods that they discarded any measurements in which the heart rate varied >10% between the carotid and femoral sites.

The application of adult quality criteria reduces the volume of PWV measurements in children and adolescents. However, in our study, the number of PWV measurements gathered was significantly greater in the second half of the study, indicating that a more experienced operator can compensate for the strict quality criteria required.

**Augmentation index**

Filipovsky *et al.* studied reproducibility of AIx on 88 adults aged 19–53 years and reported excellent reproducibility with a mean intervisit difference of 1 ± 0.9%. Crilly *et al.* studied the AIx of 20 adults in which they had 2 operators measure the AIx a total of 4 times during 1 clinic visit in alternate order. The inter- and intraobserver variability for AIx75 was excellent for both with ICCs >0.90. Wilkinson *et al.* obtained AIx on 33 adults by 2 observers and reported an intraobserver difference of 0.49 ± 5.37% and an interobserver difference of 0.23 ± 3.8%. Frimodt-Moller *et al.* reported a day-to-day difference of 0.9 ± 24.7% of the AIx and 1.4 ± 15.9% for AIx75 in 19 adults with chronic kidney disease. The investigators only included measurements with an operator index >80. In our study, AIx and AIx75 had excellent within-visit and intervisit reproducibility. Frimodt-Moller *et al.* demonstrated a significant correlation between older age and lower heart rate and AIx but did not analyze the effects of these on reproducibility. In our study, higher BMI percentile resulted in worse reproducibility.

In the current study, reproducibility of systolic and diastolic BP (see Supplementary Appendix) was comparable with reproducibility of PWV measurements and similar to reproducibility of BP measurements reported in prior studies. Overall, reproducibility of the AIx measurements...
seems to be higher than reproducibility of both PWV and BP measurements in our study.

This study should be viewed in light of its limitations. We did not measure Tanner stage or serum cotinine levels for secondhand smoke exposure but expect that they would not have varied significantly within the short period of testing dates. The study cohort did not include obese or hypertensive subjects, so extending these results to the study of patients with these conditions would require some caution.

In conclusion, arterial applanation tonometry is feasible and reproducible in healthy children and adolescents. AXt has excellent intervisit reproducibility, whereas the intervisit reproducibility of PWV relies on acquisition of multiple measurements that meet quality criteria established for adults. This technology provides an easy tool to assess arterial stiffness in the pediatric population, which is at an increasingly higher risk for premature atherosclerosis. These results highlight the importance of standardization of the technical protocol of arterial applanation tonometry and offer guidance in the methodology of future pediatric clinical trials using PWV and AI as outcome measures.

SUPPLEMENTARY MATERIAL

Supplementary materials are available at American Journal of Hypertension (http://ajh.oxfordjournals.org).

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DISCLOSURE

The authors declared no conflict of interest.

REFERENCES