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Noninvasive Assessment of Subclinical Atherosclerosis in Children and Adolescents: Recommendations for Standard Assessment for Clinical Research: A Scientific Statement From the American Heart Association

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Noninvasive Assessment of Subclinical Atherosclerosis in Children and Adolescents

Recommendations for Standard Assessment for Clinical Research

A Scientific Statement From the American Heart Association

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Abstract—Deterioration in endothelial function and arterial stiffness are early events in the development of cardiovascular diseases. In adults, noninvasive measures of atherosclerosis have become established as valid and reliable tools for refining cardiovascular risk to target individuals who need early intervention. With limited pediatric data, the use of these techniques in children and adolescents largely has been reserved for research purposes. Therefore, this scientific statement was written to (1) review the current literature on the noninvasive assessment of atherosclerosis in children and adolescents, (2) make recommendations for the standardization of these tools for research, and (3) stimulate further research with a goal of developing valid and reliable techniques with normative data for noninvasive clinical evaluation of atherosclerosis in pediatric patients. Precise and reliable noninvasive tests for atherosclerosis in youth will improve our ability to estimate future risk for heart attack and stroke. Currently, large longitudinal studies of cardiovascular risk factors in youth, such as the Bogalusa and Muscatine studies, lack sufficient adult subjects experiencing hard outcomes, such as heart attack and stroke, to produce meaningful risk scores like those developed from Framingham data. (*Hypertension*. 2009;54:919-950.)

Key Words: AHA Scientific Statements ■ pediatrics ■ elasticity imaging technique ■ brachial artery ■ risk factors ■ vasculature ■ carotid arteries

Cardiovascular disease is the leading cause of death in Western societies.^{1,2} Evidence indicates that atherosclerosis begins in childhood with the accumulation of lipid in the intima of arteries to form fatty streaks.³ Nearly all children have at least some degree of aortic fatty streaks by 3 years of age,⁴ and these fatty streaks increase after 8 years of age,⁵ with atherosclerotic plaques present in the coronary arteries during adolescence.⁶ This atherosclerotic process results in changes in the structure and function of the arterial tree.⁷

Cardiovascular diseases are associated with a number of risk factors, such as diabetes mellitus, hypertension, dyslipidemia, and obesity, and the prevalence of these risk factors has been increasing among children and adolescents⁸⁻¹⁰ in relation to the worldwide epidemic of obesity.^{11,12} In post-mortem studies, intimal surface involvement with atherosclerotic lesions in the descending thoracic aorta, the abdominal aorta, and the right coronary artery was positively associated with risk factors that included abnormal lipid and lipoprotein

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profiles, smoking, glucose intolerance, and obesity.^{13–16} Given that clusters of risk factors in childhood predict the presence of risk factors in adults,¹⁷ an increase in the incidence of cardiovascular disease is likely to occur as current adolescents enter adulthood. Current primary prevention guidelines identify high-risk youth solely by cardiovascular risk factor cut points derived from epidemiological data, such as the Bogalusa¹³ and Muscatine¹⁸ studies, that lack sufficient adult subjects experiencing hard outcomes, such as heart attack and stroke, to produce meaningful risk scores like those developed from Framingham data.¹⁹ Development of validated tools for noninvasive measurement of early atherosclerotic disease has the potential to change the paradigm for evaluation and treatment of elevated cardiovascular risk in youth by focusing on target-organ damage.

Arterial Structure: Carotid Intimal-Medial Thickness

Since the early 1990s, assessment of carotid intimal and medial thickness (cIMT) with high-resolution B-mode ultrasonography has emerged as one of the more powerful tools for the evaluation of subclinical atherosclerosis. Newer ultrasound systems with high-frequency transducers allow easy identification of the lumen-intima interface and intima-adventitia interface and thus easy and reliable measurement of cIMT. Far-wall cIMT accurately represents the intima-media thickness compared with direct histological examination.²⁰

Relationship to Cardiovascular Risk in Adults

In adults, increased cIMT is associated with coronary artery disease and is predictive of future cardiovascular events, including stroke and myocardial infarction.^{21–26} Several cardiovascular risk factors have been associated with cIMT, including age, male sex, diabetes mellitus, total cholesterol, and smoking.^{27–29} cIMT has proved sufficiently robust and reproducible in the evaluation of changes over time to serve as an end point in clinical trials assessing the impact of antihypertensive and lipid-lowering medications on cardiovascular risk.^{30–35} In the Prospective Randomized Evaluation of the Vascular Effects of Norvasc Trial (PREVENT) in patients with coronary artery disease, a decrease in cIMT was demonstrated in the patients who received amlodipine, whereas patients who received placebo had an increase in cIMT over the study period.³¹ In the European Lacidipine Study on Atherosclerosis (ELSA), less of an increase in cIMT, the primary end point of the trial, was seen in hypertensive patients who were given lacidipine than in those given atenolol.³⁵ In the Effect of Aggressive versus Conventional Lipid Lowering on Atherosclerosis Progression (ASAP) trial, patients randomized to aggressive therapy demonstrated a significant decrease in cIMT over the study period, whereas those who received conventional therapy showed an increase.³³ In the Kuopio Atherosclerosis Prevention Study, the rate of progression of atherosclerosis, as assessed with cIMT, was decreased in the group that received pravastatin compared with those receiving placebo.³² Finally, in the Asymptomatic Carotid Artery Progression Study

Table 1. Conditions Associated With Increased cIMT

Condition	Reference
Familial hypercholesterolemia	Aggoun et al, ³⁹ Järvisalo et al, ⁴⁰ Koeijvoets et al, ⁴¹ Tonstad et al, ⁴² Wiegman et al ⁴³
Hypertension	Lande et al, ⁴⁴ Sorof et al ⁴⁵
Obesity	Meyer et al, ⁴⁶ Woo et al ⁴⁷
Diabetes mellitus	Järvisalo et al, ⁵⁰ Singh et al ⁴⁸
Metabolic syndrome	Iannuzzi et al ⁴⁹
Human immunodeficiency virus	Charakida et al ⁵¹
Kawasaki disease	Noto et al ⁵²
Interventions	
Statin therapy	Koeijvoets et al, ⁴¹ Wiegman et al ⁵³
Diet and exercise	Woo et al ⁵⁴

(ACAPS), treatment with lovastatin was shown to decrease cIMT, as well as cardiovascular events, compared with placebo.³⁰ A more extensive review of randomized controlled trials evaluating the effect of an intervention on change in cIMT can be found in the review by Bots et al.³⁶ On the basis of these and other studies, assessment of cIMT in adults is widely accepted as a valid and reliable measure of atherosclerotic burden and may be considered for risk assessment in experienced laboratories.^{37,38}

Data in Children and Adolescents

To evaluate early, subclinical disease, assessment of cIMT also has been used extensively in children and young adults with known risk factors for cardiovascular disease. Increased cIMT relative to normal children has been demonstrated in pediatric patients with familial hypercholesterolemia (FH),^{39–43} hypertension,^{44,45} obesity,^{46,47} type 1 diabetes mellitus,^{40,48} and the metabolic syndrome⁴⁹ (Table 1). Longitudinal studies have demonstrated that increased cIMT in young adults is associated with the presence of cardiovascular risk factors in childhood,^{55–58} including a positive parental history of premature coronary artery disease.^{59,60} Assessment of cIMT has also been used to evaluate cardiovascular risk in populations of children with other chronic medical conditions, including end-stage renal disease,⁶¹ systemic lupus erythematosus,⁶² HIV infection,⁵¹ and Kawasaki disease,⁵² and in patients who have undergone repair of aortic coarctation.⁶³

Although cIMT varies with age, sex, and race in adults,^{64,65} the studies investigating these relationships in children and adolescents have not demonstrated significant sex differences in cIMT.^{66–68} In a series of 160 normal subjects between 10 and 18 years old, Sass and colleagues⁶⁶ did not find a significant correlation between age and cIMT. In a younger population of normal children 5 to 14 years of age, Ishizu et al⁶⁷ demonstrated a weak but statistically significant correlation between age and cIMT. In perhaps the largest cohort of normal children and adolescents reported, Jourdan et al⁶⁸ found a weak positive correlation between age and cIMT ($r=0.15$), with a somewhat stronger correlation between cIMT and both height and body mass index (BMI). Published

Table 2. cIMT in Normal Children and Adolescents

Mean Age, y	No. of Subjects	Sex	Side	Segment	Wall	Mean cIMT, mm	Reference
11±2	30	M, F	R, L	CC	FW	0.42±0.04	Järvisalo et al ⁵⁰
11.1±3.0	27	M, F	R	CC	FW	0.5±0.03	Aggoun et al ³⁹
11±1	28	M, F	R, L	CC	FW	0.42±0.04	Järvisalo et al ⁴⁰
14.2±2.3	20	M	R	CC	FW	0.48±0.06	Tonstad et al ⁴²
13.9±2.4	10	F	R	CC	FW	0.42±0.05	Tonstad et al ⁴²
14.7±2.1	20	M, F	R, L	CC	NW, FW	0.39±0.05	Meyer et al ⁴⁶
15.7±2.7	35	M, F	R, L	CC	FW	0.32±0.08	Woo et al ⁴⁷
13.5±4.0	33	M, F	R	CC	FW	0.38±0.06	Mitsnefes et al ⁶¹
16.3±4.7	20	M, F	R	CC	FW	0.48±0.08	Noto et al ⁵²
11±2	60	M, F	R, L	CC	FW	0.44±0.05	Ishizu et al ⁶⁷

M indicates male; F, female; R, right; L, left; CC, common carotid artery; FW, far wall; and NW, near wall.

normal values for cIMT are shown in Table 2, and normal values for age are summarized in Table 3. It is not known, however, whether some of the changes in cIMT that occur with age represent normal vascular adaptation or a pathological change. Bots et al⁶⁹ compared end-diastolic lumen to cIMT to study the relationship between shear stress and transmural pressure. They found that at lower degrees of cIMT, thickening reflected adaptation that led to a balance between pressure and flow, but beyond a certain level, cIMT did indicate true atherosclerotic change.

Although analysis of cIMT has been used extensively in cross-sectional studies in children, few clinical trials have used cIMT as an end point. Assessment of cIMT was used as a surrogate end point for atherosclerotic burden in randomized clinical trials assessing statin therapy in children with FH.^{41,53} In a trial reported by Wiegman et al,⁵³ children were randomly assigned to receive either pravastatin or placebo,

and the change in mean cIMT after 2 years of therapy was the primary end point. These investigators found a decrease in mean cIMT after 2 years of pravastatin therapy, whereas treatment with placebo was associated with an increase. In addition, a significant difference in the change from baseline between the 2 groups was seen. Koeijvoets et al⁴¹ assessed the relationship between low-density lipoprotein (LDL) receptor genotype and cIMT response to pravastatin therapy. Although children with null alleles had a greater baseline cIMT than those with receptor-defective mutations, both groups demonstrated a similar change in cIMT in response to statin therapy, consistent with the non-receptor-dependent, antiinflammatory mechanisms of this class of drugs.⁷⁰

A linear positive relationship has been demonstrated between obesity in childhood and cIMT in young adults.^{71,72} Individuals who experienced the largest increase in BMI during childhood and adolescence and who remained overweight the longest had the greatest cIMT. In a study investigating the impact of diet and exercise on noninvasive markers of atherosclerosis in obese children, Woo and colleagues⁵⁴ were able to demonstrate a significant decrease in cIMT within 1 year of intervention. The findings of these studies demonstrate the utility of cIMT assessment in interventional clinical trials involving children and adolescents and underscore the importance of efforts to change modifiable cardiovascular risk factors early in life.

In addition to cIMT, a few pediatric studies have measured intima-media thickness in the aorta (aIMT). This technique has proved useful even in neonates and young children. With abdominal ultrasound, increased aIMT has been associated with low birth weight,⁷³ intrauterine growth restriction,⁷⁴ maternal smoking,⁷⁵ and FH.⁴⁰ One case-control study found seropositivity to *Chlamydia pneumoniae* was significantly associated with aIMT but not cIMT in children 7 to 11 years of age.⁷⁶ In these children, aIMT but not cIMT was associated with the number of exposures to antimicrobial therapy that was effective against *C pneumoniae*, as well as elevated C-reactive protein, after adjustment for cardiovascular risk factors.⁷⁷ This would suggest that repeated infections may have the potential to accelerate atherosclerosis and that

Table 3. Normal cIMT by Age Group

Age Range, y	No. of Subjects	Sex	Side	Segment	Wall	Mean cIMT, mm
Sass et al ⁶⁶						
					Unstated*	
10–12	23	M	R, L	CC		0.49±0.04
10–12	23	F	R, L	CC		0.48±0.03
13–14	13	M	R, L	CC		0.50±0.03
13–14	24	F	R, L	CC		0.49±0.03
15–16	20	M	R, L	CC		0.49±0.03
15–16	23	F	R, L	CC		0.49±0.03
17–18	17	M	R, L	CC		0.50±0.04
17–18	17	F	R, L	CC		0.48±0.03
Jourdan et al ⁶⁸						
					Far wall	
10.0–13.9	41	M	R, L	CC		0.38±0.04
10.0–13.9	61	F	R, L	CC		0.38±0.03
14.0–16.9	51	M	R, L	CC		0.40±0.04
14.0–16.9	34	F	R, L	CC		0.39±0.05
17.0–20.0	28	M	R, L	CC		0.39±0.03
17.0–20.0	32	F	R, L	CC		0.40±0.03

*Not specified.

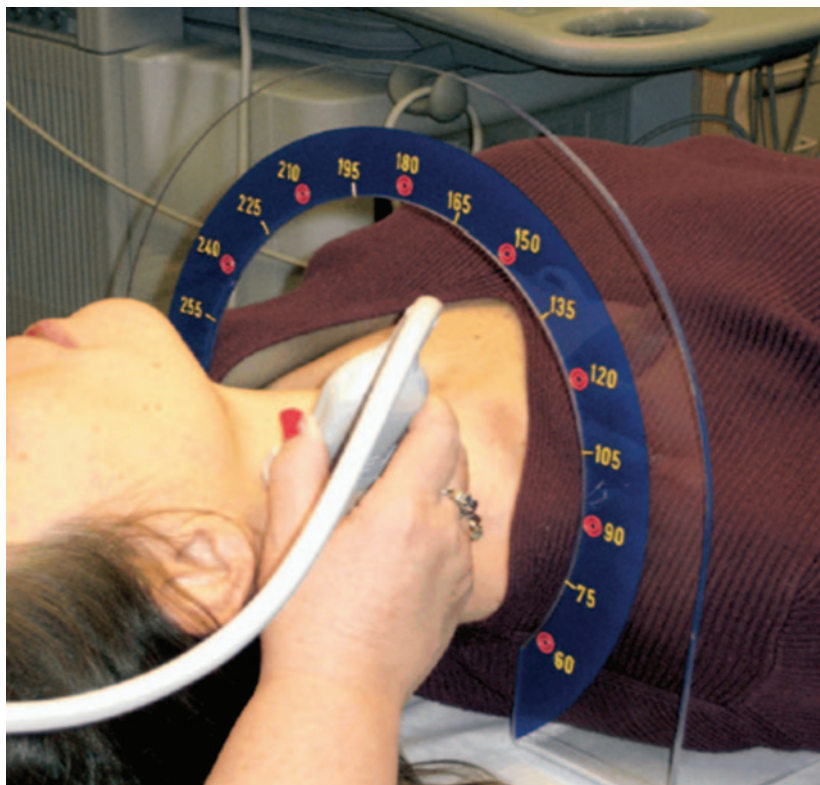


Figure 1. Meyer's arc device used to record angle of insonation used in obtaining carotid intima-media thickness measurements in longitudinal studies.

antibiotic drugs do not prevent this. In the Muscatine offspring study,⁷⁸ the relationships between cardiovascular risk factors and both aIMT and cIMT were compared. Although cardiovascular risk factors were associated with aIMT and cIMT in a similar pattern, it appears that the strength of associations may be greater for aIMT than for cIMT in those <18 years of age, which suggests that measurement of aIMT may allow detection of the atherosclerotic process at an earlier age than cIMT.⁷⁸

Despite the clear value of this tool in the assessment of cardiovascular risk in high-risk children and adolescents,⁵³ its application has been restricted by a number of factors, including limited access to trained vascular technicians and appropriate ultrasound equipment, as well as variable protocols for data acquisition and analysis. No standard recommendations exist, and the different methods used for research purposes limit the ability to make comparisons and generalizations of reported findings.

Methods of Assessment

The individual layers of the carotid artery wall can be distinguished by 2-dimensional (2D) ultrasound in several locations, given the relatively superficial location and limited movement of the vessel. Published studies evaluating cIMT in both children and adults have variably used a number of differing methods of analysis, including measurement of common carotid artery, carotid bulb, internal carotid artery, or an index using multiple sites; near-wall versus far-wall assessment; and differences in timing of measurements dur-

ing the cardiac cycle. A measure of the combined thickness of the intima and media, or intima-media complex, is the end point applied across all imaging protocols. Carotid artery plaques, an anatomic characteristic frequently encountered in older adults and indicative of advanced atherosclerotic disease, are uncommon in children and adolescents and will not be addressed in this statement.

Imaging of the carotid arteries is performed in the cardiovascular ultrasound laboratory with the patient resting comfortably in the supine position. The patient's neck is slightly extended, with the head turned 45° toward the side opposite that being examined. A high-frequency (7- to 12-MHz) linear-array transducer is typically used to image the carotid artery. Care should be taken to have the vessel as perpendicular as possible to the plane of sound to ensure optimal imaging of the vessel wall. The carotid artery is then imaged in its long axis with multiple scanning angles (anterior, lateral, and posterior), and the carotid bifurcation is identified. Generally, images are recorded in the plane where the maximal cIMT can be visualized; however, longitudinal study designs may require measurement at a specific anatomic landmark or the use of an externally applied measuring angle device³⁶ to ensure that follow-up studies are taken at the same angle (Figure 1). Magnification of the vessel wall allows easy identification of the intimal-medial complex, defined by the border between the echolucent vessel lumen and the echogenic intima and the border between the echolucent media and echogenic adventitia (Figure 2). Magnified digital images of the distal 10 mm of the common carotid artery, carotid bulb, and proximal 10 mm of the internal

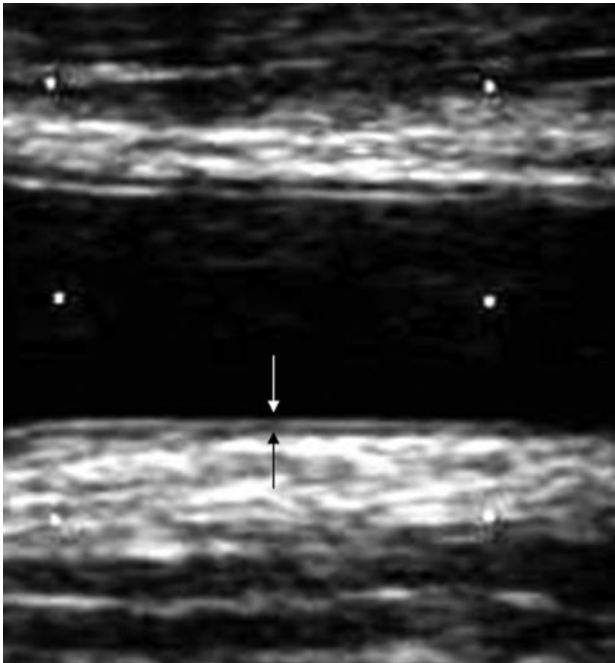


Figure 2. Image taken from the distal common carotid artery demonstrating the intimal-medial complex (between arrows). The intimal-medial thickness is measured from the border between the echolucent vessel lumen and the echogenic intima (white arrow) and the border between the echolucent media and echogenic adventitia (black arrow).

carotid artery are typically acquired for analysis. The carotid segments are generally defined in reference to the flow divider (bifurcation), with 10 mm above the flow divider defined as the internal carotid. The superior extent of the carotid bifurcation begins at the flow divider. The inferior aspect is the beginning of the dilatation associated with the bifurcation. The length of the carotid bifurcation does vary among subjects, so sonographers should be consistent with sites of measurement. The common carotid is defined as 10 mm below the bulb. Doppler ultrasound is a useful tool for distinguishing the internal and external carotid arteries, the former having a more pulsatile flow pattern and the latter exhibiting continuous diastolic flow between systolic pulses.

Finally, consideration should be given to the acquisition of data on carotid stiffness. This is accomplished by obtaining B-mode-guided M-mode images of the common carotid artery.⁷⁹ Maximal and minimal lumen diameters can be measured. These are used with a noninvasively measured blood pressure to calculate various stiffness parameters, such as Pearson’s and Young’s elastic modulus, arterial compliance, circumferential arterial strain, and the beta stiffness index.^{38,80–85} Sonographers should be instructed to use the lightest pressure possible in obtaining the image so as not to obscure the natural pulsations of the vessel.

Several different protocols for measurement of cIMT have been used to assess cardiovascular risk in adult populations. The rigorous protocol used in ACAPS^{30,38} has been used frequently in studies of cardiovascular risk in adults.⁵⁵ The

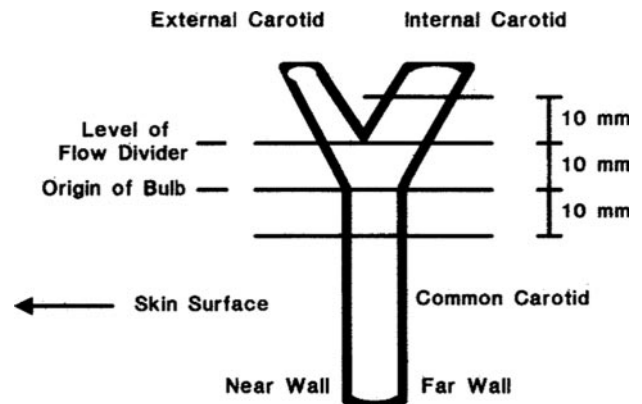


Figure 3. Schematic of the sites used to assess carotid intimal-medial thickness (reprinted with permission from Howard et al⁶⁴).

scanning protocol in ACAPS included imaging of both the right and left carotid arteries, with identification of the near wall (closest to the skin surface) and the far wall (farthest from the skin surface) of 3 arterial segments: the proximal 8 mm of the internal carotid artery, the carotid bifurcation beginning at the tip of the flow divider (site of the division of flow between the external carotid artery and internal carotid artery) and extending 8 mm proximally, and the common carotid artery 8 to 16 mm proximal to the flow divider (Figure 3). The mean value of the 12 maximal cIMT measurements is then used as the primary outcome measure for the ACAPS technique. A similar imaging and analysis protocol was used in the Oral Infections and Vascular Disease Epidemiology Study (INVEST) to assess cardiovascular risk.⁸⁶ ACAPS investigators have also demonstrated highly reliable cIMT assessment and low temporal bias when using this protocol.^{87,88} They stressed the importance of uniform training and performance monitoring throughout the study period to maintain high-quality, reproducible cIMT measurements.

The protocol used in the Atherosclerosis Risk In Communities (ARIC) study has also been cited in other adult studies.⁸⁹ It is similar to the ACAPS protocol; however, only the far-wall measurements from 6 sites (distal common carotid, carotid bifurcation, and proximal internal carotid bilaterally) are used for analysis. The ARIC investigators reported the distribution of cIMT in a large adult population 45 to 64 years of age, stratified by race and sex.⁶⁴ The percentage of patients in whom each carotid artery segment could be assessed was also reported by subgroup (ie, black males, black females, white males, and white females). The common carotid artery segment was adequately imaged in 89% to 92% of patients, the carotid bifurcation in 68% to 82%, and the internal carotid in 37% to 63%. Imaging of the internal carotid artery was particularly limited in women, and it was adequately imaged in only 37% of white women and 41% of black women.⁶⁴ In a report by Crouse and colleagues,⁹⁰ more than 99% of common carotid artery sites and 94% of carotid bulb sites could be evaluated adequately; however, 78% of internal carotid artery sites could be evaluated. These investigators reported that evaluations of the near walls of the right and left internal carotid arteries were the least successful.

The ELSA trial also used an ultrasound protocol that assessed the mean maximal cIMT for 12 arterial wall segments: near and far walls of distal common carotid artery, carotid bifurcation, and proximal internal carotid artery on both the right and left sides.⁹¹ However, the primary efficacy end point for this trial was the mean of the maximum cIMT from the far wall of both the distal common carotid artery and the carotid bifurcation on the right and left sides (mean of 4 sites).³⁵ These investigators demonstrated excellent reproducibility for the analysis of mean maximum cIMT for the distal common carotid artery and carotid bifurcation far walls bilaterally, the mean maximum cIMT for all 12 sites, and the overall maximum cIMT.⁹² The ELSA results underscore the importance of cross-sectional quality control (duplicate scanning by a single sonographer or 2 different sonographers, as well as duplicate interpretation by a single reader or 2 different readers) and longitudinal quality control (rereading selected studies annually) to achieve optimally reliable study measurements.⁹³

Although landmark adult studies have used a combination of near-wall and far-wall measurements to assess cIMT, the majority of pediatric studies have focused on assessment of the more readily visualized far wall of the carotid artery. In an *in vitro* study performed by Montauban van Swijndregt and colleagues,⁹⁴ the correlations between extravascular and intravascular ultrasound analysis and histology of near- and far-vascular wall measurements were assessed. Excellent correlation was demonstrated between extravascular far-wall measurements and both intravascular ultrasound analysis and histological analysis, with correlation coefficients of 0.91 and 0.87, respectively. Correlation was poor, however, between extravascular near-wall versus intravascular and histological measurements, yielding correlation coefficients of 0.49 and 0.37, respectively. These investigators concluded that the inherent limitation and error in the extravascular assessment of trailing edges of echogenic borders of the vessel near wall, as opposed to the leading edges of echogenic structures set off by an echolucent vessel lumen, explained the observed differences. Their findings suggest that assessment of the vessel far wall with the leading edge of the lumen-intimal interface and the medial-adventitial interface more accurately reflects the actual thickness of the intimal-medial complex. In addition, in an analysis by Espeland et al,⁹⁵ the relationships between cIMT and risk factors were stronger for far-wall measurements than for near-wall measurements. These findings suggest that assessment that is focused on the carotid artery far wall may be warranted.

Differences in the relationship between specific carotid artery sites and cardiovascular risk have also been demonstrated. Stronger correlations between risk factors and cIMT measured at the carotid bifurcation and internal carotid artery segments compared with the common carotid artery segments have been demonstrated.⁹⁵ Some studies have demonstrated the value of common carotid artery measurements in predicting coronary events²² and in identifying high-risk patients,²⁵ whereas others have demonstrated that although the aggregate mean of 12 carotid artery sites is probably the most informative in terms of describing overall atherosclerotic

burden, the addition of internal cIMT assessment to analysis of the common carotid artery and carotid bulb yields little additional predictive power.⁹⁰ These findings emphasize the need for the inclusion of analysis of multiple carotid artery segments in a comprehensive evaluation of cIMT.

A number of studies involving the assessment of cIMT do not specifically outline the point in the cardiac cycle at which measurements are performed; rather, the image frames are selected on the basis of areas where the intimal-medial complex is best visualized and appears the thickest. In studies that do specify the timing of measurement, end diastole is typically used.^{25,39,40,42,57,60} Evidence exists that cIMT does vary by $\approx 5\%$ between maximal and minimal lumen diameter, with the cIMT being slightly thicker, as would be expected, when the lumen diameter is at its minimum (end diastole).^{96,97} These findings suggest that standardized cIMT measurements should be made at the same point in the cardiac cycle and, for ease of measurement, should be made at end diastole, when the cIMT is at its thickest.

As outlined above, most pediatric studies have focused on assessment of the carotid artery far wall in assessing cIMT, particularly the far wall of the common carotid artery segment; however, the specific sites and number of sites included for analysis vary significantly from study to study. Averages of measurements of the far wall of the distal 10 mm of the common carotid artery, on both the right and left sides, have been used for cIMT evaluation in a number of cross-sectional studies in pediatric patients with FH,^{39,42,68} hypertension,⁴⁴ diabetes mellitus,^{40,48} and chronic renal disease.⁶¹ Some cross-sectional studies have used averages of both near- and far-wall measurements of the common carotid artery, again on both the right and left sides, for analysis,⁴⁹ and others have incorporated measurements from multiple carotid artery segments.⁴⁶

In the few clinical trials in children that have used cIMT as an end point, the 2 studies that assessed the response to statin therapy in pediatric patients with FH used multiple carotid artery sites to assess cIMT,^{41,53} whereas Woo and colleagues⁵⁴ used assessment of the far wall of both the right and left common carotid arteries for their evaluation of the impact of diet and exercise in obese children. Similar protocols were used in the 2 trials that assessed the impact of statin therapy on cIMT. For these trials, the mean far-wall cIMT from 6 sites (the distal common carotid, carotid bulb, and internal carotid on both the right and left sides) was used as the end point.

Variability and Limitations

Image Acquisition

The use of cIMT as a noninvasive tool to detect atherosclerosis, predict its sequelae, and detect its progression and regression has been restricted largely to multicenter clinical research trials, but the minimization of variation or error in cIMT measurement is critical for both research and clinical applications. A comprehensive review of the reproducibility of cIMT measurements concluded that consensus on the methodology, analysis, and interpretation of cIMT was urgently needed.⁹⁸ With the caveat that this need has yet to be met,

several well-supported standards, with minimal variability, can be used to guide practitioners and researchers in the use of cIMT.

As outlined above, the measurement variability of near-wall cIMT assessment exceeds that of the far wall, but it has also been reported that a combination of near- and far-wall measures can enhance precision without loss of validity.³⁰ Ultrasound scanning of the same vessel sites at multiple angles can give a more accurate impression of the 3-dimensional anatomy,⁹⁹ a rationale that was incorporated into the ACAPS protocol, which includes 6 sites (common carotid artery, carotid bulb, and internal carotid artery on both the near and far walls) each taken at 4 angles on both the right and left sides.³⁸ The mean maximum cIMT of 12 measurement sites (each yielding a “plaque score” or composite from 4 angles of visualization) is more strongly associated with coronary disease than cIMT from any 1 individual site, including the most frequently imaged common carotid artery.⁹⁰ When cIMT assessment focuses on a smaller number of sites, interobserver variability can be diminished by the inclusion of ultrasound images from both sides in a final mean cIMT calculation.¹⁰⁰

Optimal implementation of a multisite protocol or a more limited assessment of carotid segments is dependent on access to skilled sonographers and implementation of ongoing quality control. Because this is not always practical, the single anatomic site (the common carotid artery, carotid bulb, or internal carotid artery) at a single angle of interrogation that would yield the most reliable and reproducible cIMT measurements on repeat examinations by 2 sonographers on separate visits 1 week apart has been evaluated.¹⁰¹ Only the far wall was evaluated as part of this analysis. The reproducibility of measurements between visits was best for the common carotid artery far wall, with a mean difference of 0.02 versus 0.08 mm for the bulb or internal carotid artery. Investigators attributed this to the relatively linear and superficial location of the common carotid artery, which lends itself to perpendicular interrogation by the transducer in the longitudinal plane, a procedural requirement for optimal cIMT visualization that is challenged by the curvature of the carotid bulb and the variable takeoff angles and depth of the internal carotid artery. Others, using identical ultrasound equipment (Acuson 128 and 7- to 7.5-MHz linear transducers), have also found the least interobserver variability in cIMT measures of the far wall of the common carotid artery (0.08 mm) but considered the greater differences for the common carotid near wall (0.10 mm) and far wall (0.15 mm) of the bulb to be acceptable.¹⁰² Corresponding intraobserver differences were 0.06, 0.10, and 0.15 mm, respectively. Approximately 70% to 80% of the measurement variability was due to differences among sonographers, which underscores the importance of an excellent imaging technique. Although this measurement variability was considered to be generally small, it was noted that error increased proportionally with increasing cIMT, an observation that has been corroborated by others and attributed to the irregular character of advanced atherosclerotic lesions in arterial walls. This suggests that the usefulness of cIMT imaging may be greatest in young and middle-aged individuals.

Image Analysis

Methods of data analysis have varied considerably between cIMT studies. Manual assessment by the sonographer using calipers online increasingly has been replaced by either manual or automated offline analysis of digitally stored images. Because the variability between different sonographers is generally larger than the variability between different readers,³⁸ the separation of data acquisition and analysis improves study reproducibility. Results from offline manual and automated measurements of the same cIMT studies have been compared by several investigators and have consistently shown less interobserver variability with the use of automated analysis software programs.^{99,103–105} Furthermore, when digitally archived images were analyzed retrospectively in a blinded fashion by 4 independent observers, 2 adhering to a manual protocol and 2 using automated computer software, the overall mean cIMT values were significantly higher by manual assessment.¹⁰⁴ Computerized edge-detection sequential multiframe image processing is highly reproducible and affords the investigator an opportunity to track and average serial frames of cIMT measurements. A 5-frame averaging of cIMT reduced the variability of the measurement by 27% relative to single-frame measurement.⁹⁶ The extent to which automated edge detection reduces measurement variability still depends, of course, on the contribution of sonographers and the quality of the images obtained. It has been noted that if ultrasound images have indistinct borders, the automated system may have to be overridden manually, thereby eliminating the principal advantage of automated edge detection.³⁶

In conclusion, ultrasound is a reliable and reproducible method for determining intima-media thickness, especially in young and middle-aged individuals, and can be followed longitudinally for atherosclerotic vessel wall changes. Measurements from the common carotid artery are the most reproducible but not necessarily the most reliable for detection of early disease. Ideally, several segments of the carotid artery should be assessed at multiple angles. Accurate data collection methodology and precise measurement are essential and can be maximized through the use of trained personnel. The need for accepted research and clinical cIMT protocols has become acute, especially because the technique has been recommended by the American Heart Association as a useful tool for risk stratification in adults with unclear or intermediate risk of cardiovascular disease. The increased prevalence of the cardiovascular risk factors of obesity, hypertension, dyslipidemia, and diabetes in children accentuates a parallel need for standardization of accepted pediatric protocols.

Recommendations for Standard Assessment of cIMT

1. Equipment:
 - a. 2D, color, and spectral Doppler imaging.
 - b. High-frequency linear-array transducer (7 to 12 MHz).
 - c. Electrocardiograph (ECG).
 - d. Digital or super VHS recording.
2. Image acquisition:
 - a. Patient should be resting comfortably in the supine position.
 - b. Patient's head should be turned 45° toward the side opposite the side being examined.

- c. Patient's carotid artery should be as perpendicular to the plane of sound as possible.
 - d. Sonographer should record magnified images of the common carotid artery, carotid bulb, and internal carotid artery (as defined above).
 - e. Sonographer should obtain images from lateral and anterior-oblique angles.
 - f. For carotid stiffness, sonographer should use B-mode image to place M-mode cursor across the common carotid artery 1 cm proximal to the bulb. Sonographer also should obtain noninvasive blood pressure.
3. Image analysis:
- a. Measure far-wall cIMT from 3 segments (distal common carotid, carotid bulb, and proximal internal carotid) at end diastole (R wave on ECG).
 - b. Calculate mean of maximal cIMT measurements from the 3 segments outlined above on both the right and left sides from 2 scanning angles (mean of 12 maximal measurements).
 - c. Maximal and minimal lumen diameters from the right and left common carotid from M-mode echocardiography are used in calculation of carotid stiffness.

Gaps in Current Knowledge

cIMT is one of the most widely used noninvasive measures of subclinical atherosclerosis in pediatric research; however, before cIMT can be applied in the clinical setting, many questions must be answered, including the following:

1. What are normal cIMT values by age, race/ethnicity, and sex?
 - How does one account for the effect of differences in body size and growth on cIMT?
 - At what age are changes in cIMT with growth or disease of large enough magnitude to be measurable?
2. At what age or body or arterial size does cIMT become sufficiently reproducible to be considered reliable, and does reproducibility vary by age, race/ethnicity, sex, or underlying disease state?
3. How do cIMT measures relate to anatomic atherosclerosis and other measures of arterial structure and function?
4. Are cIMT measures cost-effective in identifying high-risk youth?
 - Does cost-effectiveness vary by site measured (common carotid artery alone, or must all segments be imaged)?

Arterial Structure: Coronary Calcification

Another method for evaluation of arterial structure is measurement of coronary artery calcification (CAC) with electron-beam computed tomography (CT) or spiral or helical CT imaging (enumerated below). Calcium uptake occurs during the development of atherosclerosis, with calcification ending in the deposition of insoluble calcium apatite crystals within the fibrous plaque by a mechanism similar to that found in active bone formation and remodeling.¹⁰⁶ The presence of calcified lesions, detected *in vivo*, accurately predicts the presence of atherosclerotic plaque, and coronary calcified lesions occur almost exclusively when coronary atherosclerosis is present.¹⁰⁷ Microcrystalline calcium in the lipid core has been detected in the coronary arteries in individuals as young as 23 years of age.¹⁵

Relationship to Cardiovascular Risk in Adults

Electron-beam CT has a high level of sensitivity in detecting and defining the location and extent of CAC, which predicts obstructive coronary artery disease, and has prognostic value for future coronary events.^{108,109} In a study of young adults in the age group 29 to 37 years, 31% of men and 10% of women demonstrated evidence of CAC.¹⁸ Similar prevalence rates (21% in men and 11% in women) were noted in a study of subjects 30 to 39 years of age.¹¹⁰ People with diabetes are at increased risk, with 2- to 3-fold increased odds of having a positive electron-beam CT scan compared with control subjects.¹¹¹ Most studies investigating CAC have documented a greater prevalence with increasing age and a greater prevalence in males versus females in every age group.^{18,108,110,112-118}

Recently, studies have examined the relationship of known risk factors with CAC. In a study of 675 men and 190 women 22 to 85 years of age, Wong et al¹¹⁵ examined the association between the presence and extent of CAC and self-reported coronary risk factor data. They demonstrated a significantly greater prevalence of CAC in both men and women with a self-reported history of hypertension and hypercholesterolemia, as well as in men with a history of diabetes, previous smoking, infrequent exercise, and obesity. A significant relationship of levels of coronary risk factors measured during childhood and young adult life with the presence of CAC was noted.¹⁸ In a recent study,¹¹⁹ the Framingham risk equation underestimated subclinical atherosclerosis risk in asymptomatic women. According to the Framingham risk equation, the majority (84%) of women with significant CAC ≥ 75 th percentile were classified as low risk, and 45% of low-risk women with ≥ 2 coronary heart disease risk factors and a family history of premature coronary heart disease had significant CAC. These authors concluded that determination of CAC may provide incremental value to the Framingham risk equation in identifying women who will benefit from targeted preventive measures.

Data in Children and Adolescents

Experience with these technologies in pediatric patients is limited. Gidding et al¹²⁰ demonstrated the usefulness of electron-beam CT in adolescents and young adults with heterozygous FH. Significant coronary calcium was identified in 7 of 29 subjects with FH. In addition, these investigators demonstrated that overweight increased the likelihood of calcium being present in individuals already at high risk. Similarly, Frey et al¹²¹ demonstrated the utility of ultrafast CT in the noninvasive evaluation of coronary artery aneurysms in patients with Kawasaki disease. In a prospective cohort pilot study, patients with Kawasaki disease with residual coronary abnormalities showed CT evidence of CAC, and the authors suggested that the presence of CAC may be predictive of sudden death.¹²² The degree of coronary artery dilation after Kawasaki disease appears to correlate with the risk of future calcification.¹²³ Young adults receiving maintenance dialysis, especially when dialysis was initiated during the childhood years, develop CAC,¹²⁴ and a recent study documented CAC in 2 pediatric patients receiving

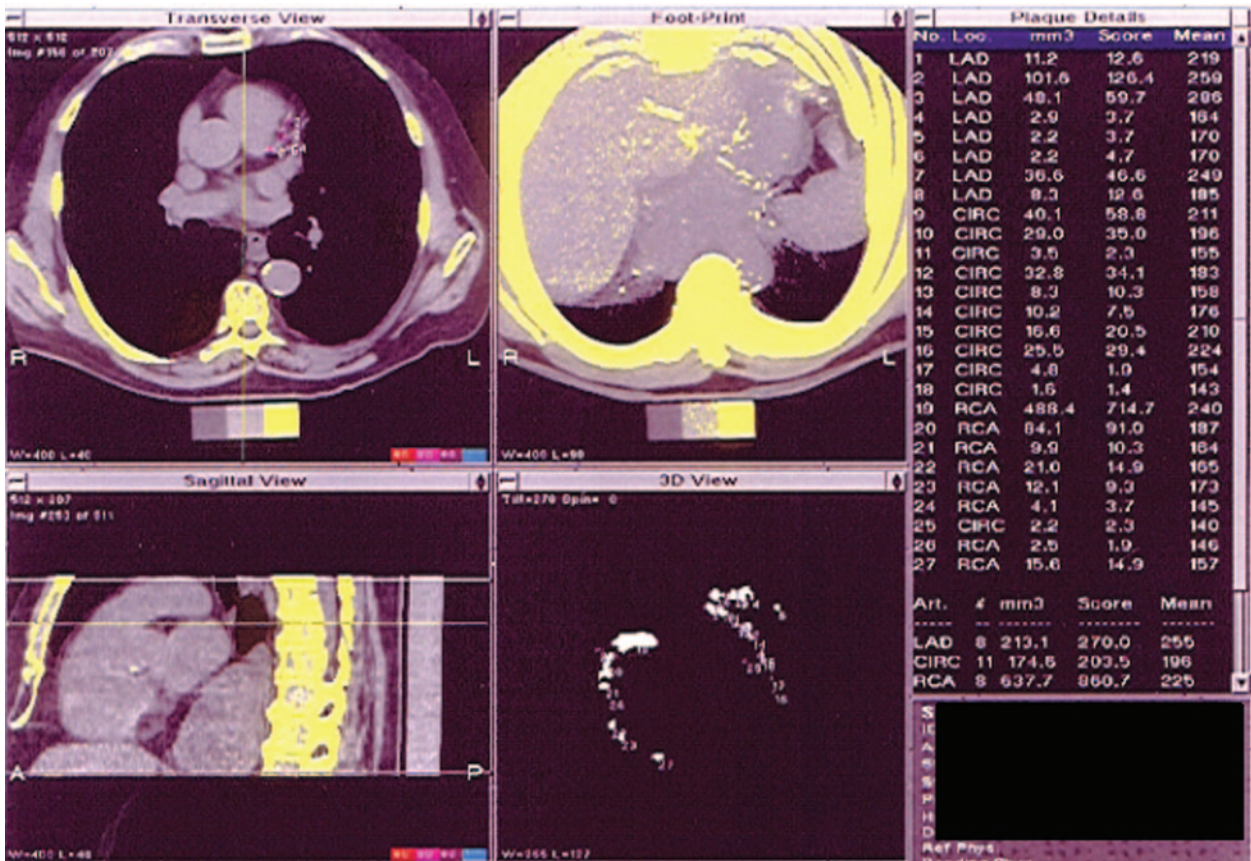


Figure 4. The far right panel lists the individual lesions by vessel location, plaque volume, calcium score, and mean density (in Hounsfield units). The adjacent images illustrate several of the views that can be displayed on the workstation. From standard transverse and sagittal views (left upper and lower panels, respectively), a composite footprint image (center upper panel) is reconstructed and displayed. CT densities exceeding the threshold are color coded in yellow for easier identification. The lower right image shows marked CAC in the distribution of right, anterior descending, and circumflex coronary arteries. LAD indicates left anterior descending coronary artery; CIRC, circumflex artery; and RCA, right coronary artery.

dialysis.¹²⁵ Similarly, pediatric kidney transplantation patients¹²⁶ and subjects with type I diabetes mellitus diagnosed in youth¹²⁷ may also be at increased risk for coronary calcification before they reach middle age. Finally, pediatric heart transplant recipients appear to be at risk for early CAC, likely related to posttransplantation coronary arteriopathy.¹²⁸ The radiation exposure associated with these technologies limits their utility in most pediatric patients.

Methods of Assessment

The criteria used for electron-beam CT determination of coronary calcium (CAC) were established by Agatston et al¹²⁹ (Figure 4). These threshold criteria are firmly established and are in wide use around the world. A focus of CAC is considered to exist if there are ≥3 contiguous pixels (area=1.03 mm²) of ≥130 Hounsfield units in density. The Agatston score is calculated by multiplying the area (in millimeters squared) of each lesion by a weighted CT attenuation score that is dependent on the maximal CT attenuation (in Hounsfield units) within the lesion.¹²⁹ The volume score is calculated for each calcified lesion by multiplying the voxel volume (in cubic millimeters) calculated with isotropic interpolation¹³⁰ by the number of voxels

>130 Hounsfield units in the lesion. With the attenuation conversion, the number of voxels >130 Hounsfield units may be increased or decreased. Moselewski et al¹³¹ determined the relationship between CT attenuation (based on phantom measurements) and calcium concentration based on a single set of phantom measurements made at the level of the left main coronary artery. Because of the high cost and relatively limited availability of electron-beam CT, spiral or helical CT imaging began playing an increasingly important role in screening for CAC. Recent advances in helical CT imaging with the development of multirow detector CT have shown great improvement in temporal resolution.

Variability and Reproducibility

The reproducibility of the interpolated volume score has been shown to be consistently superior to that of the traditional Agatston score, with an overall reduction in error of 39.5%.¹³⁰

Recommendations for Standard Assessment of CAC

CT technology does not provide evaluation of the lumen of the coronary arteries except with contrast, although it does provide excellent morphological evaluation of the coronary arteries in individuals at high risk for atherosclerosis. In adults, high-risk status is defined with validated cardiovascu-

lar risk factor scores such as the Framingham risk score¹³² or Reynolds risk score.¹³³ Unfortunately, data with sufficient longitudinal follow-up relating cardiovascular risk factors measured in youth to hard cardiovascular outcomes in adults are not available. Therefore, no such cardiovascular risk score exists for use in the pediatric population. However, autopsy data from the Pathobiological Determinants of Atherosclerosis in Youth study have been used to develop a score based on traditional cardiovascular risk factors that predicts extent of atherosclerosis in adolescents and young adults.^{134,135} For this reason, the American Heart Association suggests more aggressive risk factor reduction in patients with high-risk conditions that predispose to coronary artery disease at ages <30 years, such as homozygous FH, type 1 diabetes mellitus, chronic kidney disease, and Kawasaki disease with aneurysm, as well as after heart transplantation.¹³⁶ Because of the risk of radiation exposure from CAC measurements, this technique should be limited to very high-risk children and adolescents participating in research studies and clinical trials.

Gaps in Current Knowledge

1. What is the best method for measuring arterial calcification in youth?
 - Can calcification imaging techniques be applied to the aorta, where autopsy studies show early development of atherosclerosis in youth?
2. Do measures of arterial calcification correlate with other measures of arterial structure and function?
3. Are measures of arterial calcification reproducible in youth?
4. What is the threshold for identifying an abnormality in calcification in specific arteries by age, race/ethnicity, and sex?
5. In which pediatric conditions will the prevalence of CAC be high enough to provide cost-effectiveness to its measurement?

Arterial Stiffness

The arterial properties that have been well studied as markers of structural change are arterial compliance, distensibility, and stiffness. Although both compliance and distensibility can be used as measures of stiffness, they individually represent different facets of arterial structure and function. Distensibility is a measure of the elastic properties of an artery, whereas compliance is a measure of the local vessel capacity to respond to changes in blood volume.¹³⁷ Arterial stiffness is the reciprocal of distensibility.

Arterial stiffness is a dynamic property that is dependent on vascular structure and function and arterial pressure. These factors function independently and in concert to effect changes in arterial stiffness. Arterial pressure is a major determinant of arterial stiffness, and elevated arterial pressure increases arterial stiffness.¹³⁸ Increases in arterial pressure arise from elevated heart rate, higher stroke volume, elevated vascular resistance, and early wave reflections.^{139,140} Given this dynamic interaction, the necessity for standardization in measurement procedures becomes obvious.

Although facets of vascular structure and function have been addressed elsewhere in this statement, it is important to

discuss the structure of the arterial wall. Collagen and elastin, as well as smooth muscle, are the major components of the arterial wall. The central arteries have a relatively high elasticity as a result of the high elastin-to-collagen ratio and decreased influence of smooth muscle tone. Elasticity decreases toward the peripheral vessels as the ratio of elastin to collagen in the wall declines. Furthermore, the effect of smooth muscle bulk and tone becomes increasingly important to the elasticity of the vessel.^{141,142}

Three groups of noninvasive methods typically are used in the assessment of arterial stiffness: (1) analysis of the arterial pressure waveforms; (2) calculation of the change in diameter (or area) of an artery with respect to the distending pressure; and (3) measurement of pulse-wave velocity (PWV), which appears to be emerging as the “gold standard” in studies of adults. Here, we discuss the individual methods and, where applicable, summarize the somewhat limited published pediatric experience.

Relationship to Cardiovascular Risk in Adults

Arterial stiffness has become increasingly important in the pathogenesis of cardiovascular disease. Increasing stiffness is an antecedent factor in elevated systolic blood pressure and pulse pressure. Elevated systolic blood pressure and pulse pressure play integral roles in cardiovascular outcomes such as left ventricular hypertrophy, ventricular failure, and the atherosclerotic process. Furthermore, systolic hypertension is a major risk factor for coronary heart disease,^{143,144} stroke,¹⁴⁵ and cardiovascular¹⁴⁶ and total¹⁴⁷ mortality. Therefore, it is not surprising that measures of arterial stiffness also predict future cardiovascular risk in adults.¹⁴⁸

Abnormalities (decreases) in arterial distensibility measured with ultrasound are known to be present in adult populations with hypertension,^{149,150} diabetes mellitus,¹⁵¹ or dyslipidemia^{39,150} and are more prevalent with clustering of cardiovascular risk factors.¹⁵² In adults, reduced distensibility predicts future hypertension¹⁵³ and adverse cardiovascular outcomes.^{148,154,155} Physical training has been shown to improve arterial distensibility.¹⁵⁶ Brachial artery distensibility measured with a non-ultrasound-based technique (described below) is also highly correlated with cardiovascular risk factors¹⁵⁷ and declines with development of metabolic syndrome in adults.¹⁵⁸

PWV increases with increasing arterial stiffness and vascular damage.^{159,160} In adults, aortic PWV is strongly associated with the presence and extent of atherosclerosis¹⁴⁸ and is increased in the presence of various cardiovascular risk factors, including diabetes,¹⁶¹ hypertension,¹⁶² end-stage renal disease,¹⁶³ hyperlipidemia,¹⁶⁴ increasing age,¹⁶⁵ and sedentary lifestyle.¹⁶⁶ PWV is an important, perhaps even the strongest, independent predictor of cardiovascular events.¹⁴⁸

Data in Children and Adolescents

Regardless of the technique used to measure arterial stiffness, few data are available on pediatric use. Augmentation index, a parameter derived from systolic pulse contour analysis, was found to be elevated (increased stiffness) in children with conditions that predispose to cardiovascular disease, such as type 1 diabetes mellitus¹⁶⁷ and type 2 diabetes mellitus,¹⁶⁸ compared with normal control subjects. Arterial distensibility

Table 4. Effect of Disease States on Arterial Distensibility

Disease	No. of Subjects	Method	Finding	Reference
Dyslipidemia	361	Ultrasound	Decreased with increasing cholesterol*	Leeson et al ¹⁶⁹
Obesity	294	Ultrasound	Decreased with increased fat mass†	Singhal et al ¹⁷²
Hypertension	471	Ultrasound	Decreased with increased adiposity and DBP‡	Whincup et al ¹⁷³
Type 1 diabetes mellitus	33	Ultrasound	Decreased in diabetes† and correlates well with HbA1C*	Parikh et al ¹⁷⁶
Obesity and insulin resistance	969	Oscillometric	Decreased with obesity and insulin resistance*	Urbina et al ¹⁷⁵

DBP indicates diastolic blood pressure; HbA1C, hemoglobin A1C.
 * $P=0.05$.
 † $P=0.01$.
 ‡ $P<0.001$.

measured with ultrasound is impaired in the settings of positive family history of myocardial infarction,³⁸ elevated total and LDL cholesterol,¹⁶⁹ obesity,^{170,171} elevated leptin levels,¹⁷² increased blood pressure,^{173,174} obesity-related hyperinsulinemia,¹⁷⁵ and type 1 diabetes mellitus¹⁷⁶ (Table 4). Pediatric data related to the nonultrasound method for assessment of arterial distensibility (described below) are now available. A decline in brachial distensibility is seen with the development of obesity, with further deterioration in adolescents with both obesity and hyperinsulinemia.¹⁷⁵ This suggests that the technique may be helpful in identifying early cardiovascular change in youth before development of the full metabolic syndrome or progression to type 2 diabetes mellitus.

A limited number of studies have evaluated PWV in children, but they each have reported relatively few subjects. The bulk of the available PWV research in pediatric subjects includes those

with diseases such as type 1 diabetes mellitus,^{177,178} neurofibromatosis,^{179,180} primary snoring,¹⁸¹ Kawasaki disease,¹⁸² polyarteritis nodosa,¹⁸³ and coarctation of the aorta after surgical repair.^{184–186} Most of these studies have control groups of varying mean ages (5 months to 22 years), with patient numbers ranging from 28 to 155 subjects. Furthermore, a few published population studies^{165,187,188} have included a small number of pediatric subjects (Table 5). In these studies, PWV was increased in the studied disease states and, as has been shown in other studies,¹⁸⁷ increased with age.

A recent study¹⁹² using brachial-ankle PWV (baPWV, described below) evaluated 205 normotensive, healthy American adolescents with the intent of establishing mean PWV values for further comparison studies. Sex and racial differences in baPWV were present, with males having values greater than females ($P<0.003$) and black subjects having values greater than white

Table 5. Published Normal Pediatric PWV Values

Age, y	No. of Subjects	Method	PWV, m/s	Reference
9.5±2.87	30	Photoplethysmography*	9±2.0	Kwok et al ¹⁸¹
6 to 18	155	Photoplethysmography†	Unstated	Cheung et al ¹⁸³
11	30	Pressure transducers	6.5±1.2	Tedesco et al ¹⁸⁰
9.1±2.6	36	Photoplethysmography‡	5.9±1.4	Cheung et al ¹⁸²
11 to 20	30	Photoplethysmography,§ photoplethysmography¶	6.5±1.2 5.5±0.6	Eliakim et al ¹⁸⁹
8.4±3.4	28	Doppler ultrasound	Unstated	Stella et al ¹⁷⁷
5 to 20	21	Microphonic transducer	7.2	Woolam et al ¹⁷⁸
12.9±0.2	110	Applanation tonometry	4.1 to 8.2	Ahimastos et al ¹⁹⁰
0.2 to 20	125	Doppler ultrasound	4.4 to 7.9	Avolio et al ¹⁸⁷
3 to 20	108	Doppler ultrasound	6.2 to 10.2	Avolio et al ¹⁶⁵
0 to 19	38	Uncertain	4.9±1.2	Okada et al ¹⁸⁸
0.3 to 12	11	Pressure transducers	9.1±2.5	Hsieh et al ¹⁹¹
15.8±2.4; 15.9±2.5	99 M; 106 F	Oscillometric cuffs	10.9±1.4; 10.4±1.3	Collins et al ¹⁹²
14.8±2.5; 14.5±2.6	500 M; 470 F	Oscillometric cuffs	9.97±1.3; 9.47±1.2	Niboshi et al ¹⁹³
14.5±1.2; 14.8±1.5	178 M; 84 F	Oscillometric cuffs	10.3±0.9; 9.6±1.7	Im et al ¹⁹⁴
6 to 23	133	Applanation tonometry	5.02±0.89	Kis et al ¹⁹⁵

Plus/minus values are mean±SD.
 *Brachial artery.
 †Carotid-femoral artery.
 ‡Brachial-radial artery.
 §Femoral-dorsalis pedis artery.
 ¶R wave gated to dorsalis pedis.

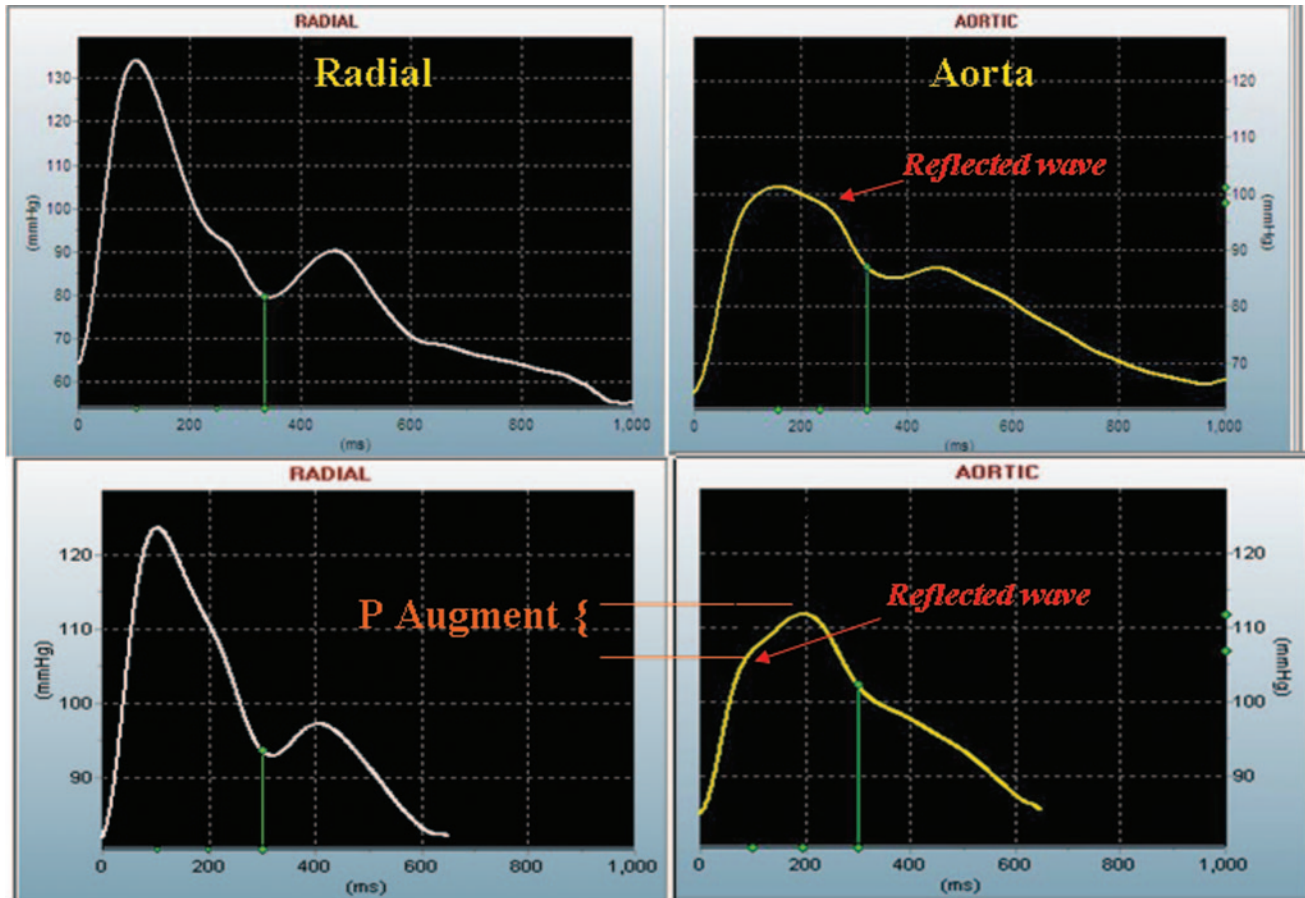


Figure 5. Pulse waveform analysis obtained from radial artery tonometer demonstrating measured radial artery tracings (left) and calculated central aortic tracings (right). The adolescent with type 2 diabetes mellitus has an increased augmentation index, adjusted to a heart rate of 75 beats per minute (Alx-75) with wave reflection occurring early in systole (bottom right) compared with the healthy subject whose reflected wave arrived in diastole (top right).

subjects ($P < 0.05$). These findings are consistent with those reported by Li et al in a similar population of young adults.¹⁹⁶ The mean age and baPWV for the adolescent study population were 15.9 years and 10.7 m/s, respectively.¹⁹²

In a large cohort of 970 healthy Japanese children, Niboshi et al¹⁹³ recently demonstrated similar findings in baPWV. Sex and age differences in baPWV were significant. The study further established normal percentile values for baPWV in Japanese males and females between the ages of 9 and 17 years. These mean values were statistically significantly lower than in our US cohort. More recently, Im et al¹⁹⁴ corroborated the finding of sex differences in a group of 262 healthy Korean adolescents. In that study, correlations with blood pressure and BMI also were demonstrated. Although a correlation with blood pressure was demonstrated in the American cohort¹⁹² and that of Niboshi et al,¹⁹³ neither study demonstrated significant correlations with BMI.

Methods of Assessment

Arterial Pulse Waveform Analysis

Analysis of the pulse waveform can be accomplished via evaluation of either systolic or diastolic pulse contour or of the digital volume pulse. Both systolic and diastolic pulse contour analysis use applanation tonometry of a given peripheral arterial segment.

Systolic pulse contour analysis uses a transfer function to derive central aortic waveforms from measurements obtained at a peripheral artery, such as the radial artery.¹⁹⁷ This transfer function is generalized instead of individualizing the transfer function for particular subject characteristics, such as sex (Figure 5). Although some data demonstrate that systolic pulse contour analysis measurements are nearly identical to those acquired with a high-fidelity intra-arterial transducer,¹⁹⁸ other data have shown poorer correlations.^{199–201} To the best of our knowledge, this technique has not been validated in pediatric subjects, who generally have compliant vessels.

Diastolic pulse contour analysis uses a modified Windkessel model to derive information on proximal and distal arteries by analyzing the diastolic portion of the pressure pulse contour.²⁰² The diastolic waveform in diastolic pulse contour analysis consists of 2 components, typically referred to as C1 and C2. C1 represents large-artery compliance, whereas C2 provides a measure of the compliance of the small arteries.²⁰² This technique has been subject to multiple criticisms because of a lack of consistent correlation with invasively measured values.^{203–205} Furthermore, uncertainty exists as to what the C2 component actually represents.²⁰⁰ These questions raise doubt as to the reliability of this technique for evaluation of arterial stiffness.

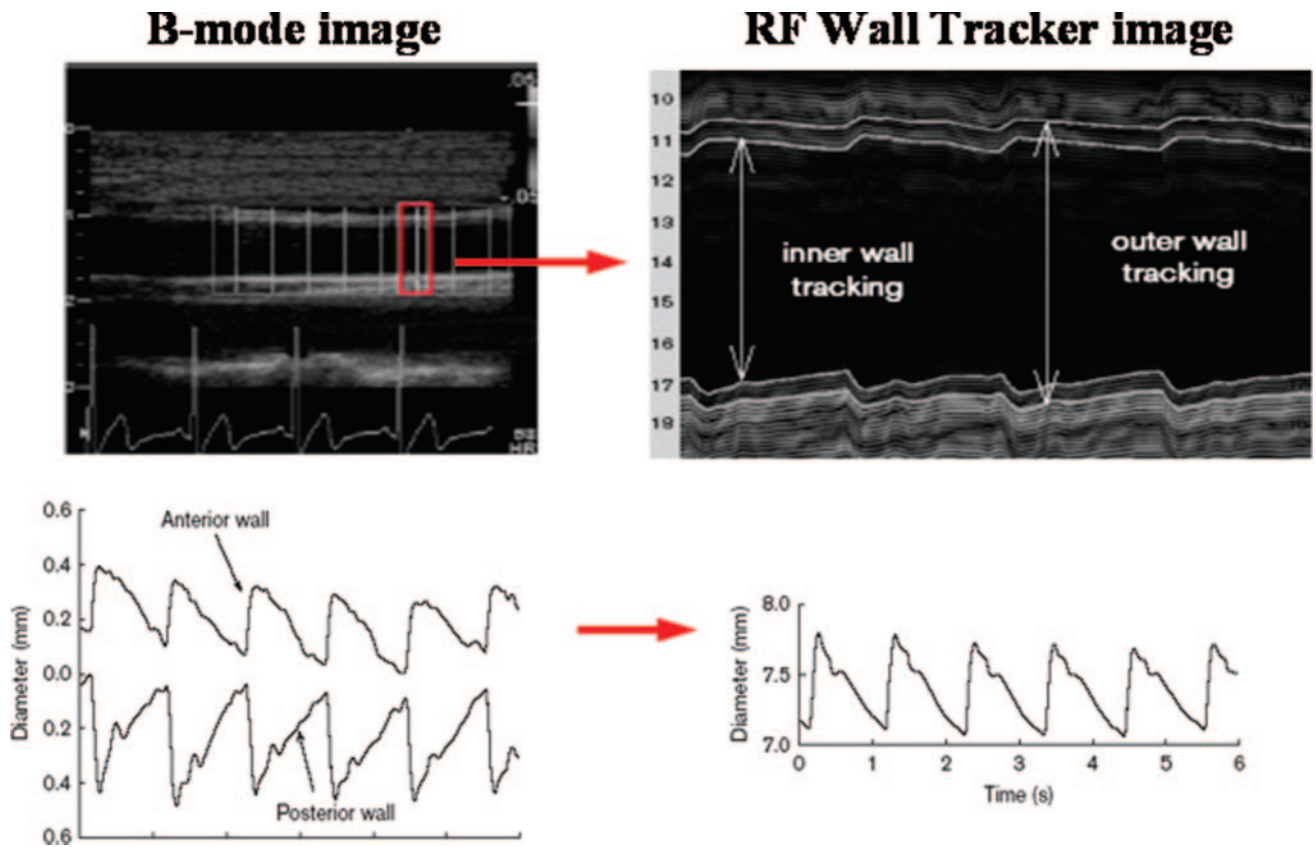


Figure 6. Ultrasound evaluation of resting arterial distensibility with radiofrequency (RF) echo tracking. A segment of the artery wall is selected on the B-mode image. The RF signal tracks the anterior and posterior wall to measure the distance from the transducer to calculate lumen diameter throughout the cardiac cycle. Reprinted from Segers et al,²⁰⁷ with permission from the *Journal of Hypertension*.

The pulse contour measured photoplethysmographically at the pulp of a digit closely resembles that measured at the radial artery²⁰⁶ and can be used to derive an estimate of arterial stiffness. Nevertheless, this methodology has not been validated in either adult or pediatric populations as a means of arterial stiffness measurement, nor has its exact relationship to the central arterial waveform been established.

Arterial Diameter Change and Distensibility

Measurement of the change in arterial diameter as it relates to distending pressure provides a reciprocal of arterial stiffness, defined as arterial distensibility. As stated, arterial distensibility is a measure of the elastic properties of an artery. Distensibility is defined as the fractional change in arterial cross-sectional area per unit change in (local) pulse pressure—that is, it describes the amount of diameter expansion expressed as a percentage of the initial diameter of the artery in relation to the force that causes the expansion (transmural pressure).¹³⁷ This change in diameter can be measured by B-mode-guided M-mode⁷⁹ or A-mode radiofrequency tracking²⁰⁷ of a regional arterial segment such as the carotid, brachial, radial, or femoral artery (Figure 6); however, a nonultrasound method for measuring brachial artery distensibility is also available.²⁰⁸

The nonultrasound method for evaluating brachial artery distensibility derives brachial artery distensibility by use of

waveform analysis of the arterial pressure signals obtained from a standard cuff sphygmomanometer. The pressure waveform is calibrated and incorporated into a physical model of the cardiovascular system, which assumes a straight-tube brachial artery and T-tube aortic system. Brachial artery compliance is derived from waveform parameters, as published previously.²⁰⁸ Baseline brachial artery diameter (D_0) is estimated with an empirically derived model based on sex, height, weight, and mean arterial blood pressure and validated with B-mode ultrasound ($n=1250$, $R=0.63$, $P\leq 0.05$). Brachial artery distensibility is then calculated as compliance normalized to baseline diameter. In adults, validation of this method to derive arterial compliance demonstrated excellent correlation between compliance measurements obtained during cardiac catheterization and those derived with the noninvasive method ($r=0.83$).²⁰⁸

The advantages of this noninvasive device include ease of use (a nonsonographer can perform the measurement), lack of observer bias (no manual readings are performed), and ability to perform measurements in the field (the device is portable). Because the blood pressure cuff exerts an even pressure along a large portion of the upper arm, concerns that direct pressure over the brachial artery might induce reflex changes in vessel tone are minimized. Furthermore, blood pressure is measured simultaneously at the same site where distensibility is calculated.

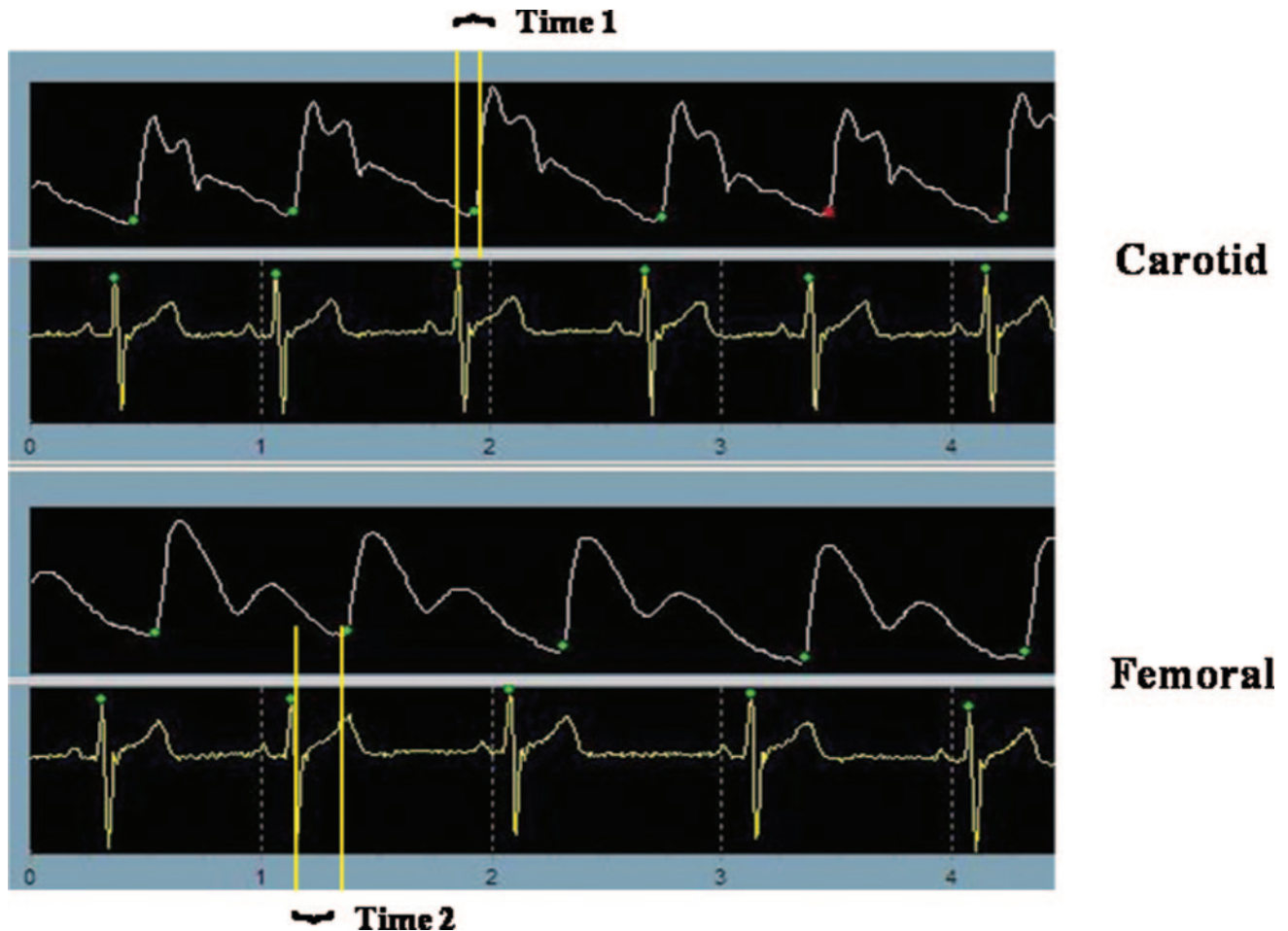


Figure 7. PWV obtained from tonometry at the carotid and femoral arteries. The time from the peak of the R wave on the ECG to the foot of the pressure curves is used to calculate the difference in time of arrival of the pressure wave. The arterial distance is measured manually and entered into the device, and PWV is calculated.

Pulse Wave Velocity

First described by Bramwell and Hill,²⁰⁹ the use of PWV as an index of cardiovascular health has increased tremendously, and it is recognized as likely the best clinical measure of stiffness over an arterial segment.²¹⁰ PWV, as a measure of arterial stiffness, is based on the principle that the pressure pulse, generated by ventricular ejection, is propagated along the arterial tree at a speed determined by the geometric and elastic properties of the arterial wall.²¹¹ PWV is defined by the Moens-Korteweg equation as $PWV = \sqrt{Eh/2\rho R}$, where E is Young's modulus of the arterial wall, h is wall thickness, ρ is blood density, and R is arterial radius at the end of diastole.

Several methods of measuring PWV have been described, including the use of pressure transducers,²¹² Doppler ultrasound,²¹³ applanation tonometry²¹⁴ (Figure 7), magnetic resonance imaging,²¹⁵ and oscillometric pressure cuffs.²¹⁶ The arterial pulse wave is measured at both a proximal and a distal artery, such as the common carotid and femoral arteries, respectively. These relatively superficial arteries readily allow for noninvasive measurements of arterial waveforms. The pulse waveform is recorded at each site, and the time delay between the arrival of a predefined point on the

waveform (typically the “foot”) at the 2 sites is obtained by gating to the peak of the R wave on the ECG. Measurement of distances on the body surface, a practice somewhat dependent on body habitus, allows an estimate of the distance traveled. PWV is then calculated as distance/time (m/s).

Recently, a technique has been described, validated, and reproduced that uses oscillometric pressure cuffs to evaluate PWV via measurements at the brachia and ankles (baPWV).^{216,217} To determine the PWV with baPWV, oscillometric cuffs are placed at the brachia and ankles to measure changes in arterial pressure. An ECG is used to gate ventricular ejection at the aortic root. The lengths of the arterial segments from the aortic root to the brachium and from the aortic root to the posterior tibial artery at the ankle are derived on the basis of the patient's height. The time from the gated ventricular ejection until pressure pulse arrival at the oscillometric cuff is measured. The distance traveled is then divided by the time required to give the PWV over the segment, be it across the aortic root-to-brachium or the aortic root-to-posterior tibial artery segment. Simultaneous measurement at both the brachium and the ankle results in the determination of PWV across 2 arterial segments. The differences between the aortic root-to-posterior tibial artery and aortic root-to-

brachium distances and times can be used to derive a third arterial segment over which the baPWV can be calculated. Therefore, a single measurement obtained by using cuffs at the brachium and ankle can result in 3 PWV measures. This method is known to produce higher values for PWV because it measures both central and peripheral arterial segments; however, baPWV has been shown to correlate well with and can be used to estimate carotid-femoral PWV (cfPWV) by the equation:

$$\text{cfPWV} = 0.833 \times \text{baPWV} - 2.333,$$

where baPWV is measured in meters per second.²¹⁸ Yu and colleagues²¹⁹ have recently shown that baPWV correlates better with left ventricular mass, diastolic function, and other indices of arterial function than carotid-femoral PWV. These results were suggested to be due to baPWV reflecting arterial stiffness from a greater percentage of the arterial tree.

Variability and Limitations

Validation studies of arterial stiffness measures have not been reproduced in children and adolescents. Reproducibility studies in children are also lacking. In adults, pulse-wave analysis was found to be highly reproducible.²²⁰ The nonultrasound method for measurement of brachial artery distensibility also demonstrated excellent correlation between repeat blind duplicate recordings (intraclass correlation coefficient 0.72).¹⁵⁷

Each technique for measurement of arterial stiffness suffers from unique limitations to its use. Simonetti et al,²²¹ in a group of 79 healthy children, has shown that accuracy of arterial stiffness measured by photoplethysmographic analysis of the finger pulse may be limited in subjects with increased vascular tone, because they observed poor correlation to the standard carotid to femoral artery PWV.

The use of ultrasound techniques for assessment of changes in arterial diameter has limitations. Given that changes in arterial diameter in some studies are measured in hundredths of a millimeter,^{169,172,173} correct placement of the ultrasound transducer over the peripheral artery is mandatory to ensure accurate measurements. Highly trained personnel are necessary to perform the studies in a rigorously consistent manner. The greatest limitation in the use of this methodology is likely the direct, local effect of the distending pressure on the change in arterial diameter. Pulse pressure changes cannot be measured at a site that is the same as or near the site of measurement of the arterial diameter changes. In addition, measurement of local pulse pressure by tonometry is limited, because even modest pressure applied to the artery by the tonometer alters the pressure waveform.¹⁵⁶ The effect of modest variations in pulse pressure on the change in distensibility of a discrete arterial segment cannot be predicted. The utilization of a pulse pressure measured at an alternative site, therefore, makes the interpretation of this technique questionable.

Another inherent limitation of measurement of arterial stiffness by tracking changes in arterial diameters is the paucity of data examining the effects of changes in arterial size with growth and changes in arterial properties with pubertal maturation on distensibility. In adults, sex differ-

ences in arterial compliance with aging have been observed.²²² Similar sex differences in arterial stiffness were found in prepubertal children. These differences were not present after maturation had occurred.¹⁹⁰ Further study is needed to determine the significance of these findings.

Although the measurement of PWV has become the leading modality, if not the gold standard, for the measurement of arterial stiffness in adult groups, some issues limit the use of PWV as a tool for cardiovascular assessment in pediatric populations. The most significant issue is that no study has demonstrated the validity of any of the available assessment modalities specifically in pediatric groups. The available validation studies have been performed in adults. Although this casts some doubt on measures performed in pediatric groups, it should be recognized that true validation studies in pediatric populations would be impossible. Although there is no true gold standard for local or regional in vivo measurement of arterial stiffness, to validate the noninvasive measurements of PWV, simultaneous intra-arterial measurements with catheters and pressure manometers are required. These procedures have been performed in healthy, consenting adults to allow validation of some methodologies; however, it would be unethical to subject healthy children to such invasive testing for the sole purpose of a validation study. Thus, it appears quite unlikely that such validation studies will ever be performed.

A significant issue that limits PWV interpretation in children is the lack of data in normal subjects. A few studies have attempted to provide normal ranges for populations that include children,^{165,187,188} but they have been hampered by the small numbers of pediatric subjects. A recent publication included PWV data on a control group of 133 children, adolescents, and young adults,¹⁹⁵ but further confirmatory data comparing various populations are needed. Although the recent work by investigators using baPWV to evaluate arterial stiffness in pediatric subjects has greatly increased the available data in normal adolescents, a need remains for further research in such groups before true normative values can be produced. Further investigation of racial differences is needed, because it has been shown that significant differences in arterial stiffness exist between presumably healthy American and Japanese adolescents.^{223–225} Furthermore, the available pediatric data have been collected by use of various methodologies that have not been shown to be equivalent. Therefore, the establishment of cut points for normal PWV in youth is fraught with difficulty.

As discussed, there are advantages and limitations to the use of noninvasive methods for the evaluation of arterial stiffness in pediatric patients. The noninvasive nature of the various arterial stiffness measurements makes their use in pediatric patients ideal. Although no study has specifically validated the various modalities in pediatric populations, there is no reason that these modalities should be any less valid or reproducible in children than they are in adults. Given the low likelihood of true validation studies being performed in pediatric patients, it is prudent to understand that precision is more important than accuracy (validity) when attempting to reveal differences between groups of patients or between arterial territories.²²⁶

In summary, increasing experience in pediatric patients suggests that the evaluation of arterial stiffness parameters in youth is not only reproducible and valid but also important. Although no true gold standard for local or regional *in vivo* measurement of arterial stiffness has been established, PWV has become the most widely studied, reproducible, accepted, and utilized method. With the limited available data in pediatric populations, more studies, including those of a longitudinal design, are required.

Recommendations for Standard Assessment of Arterial Stiffness

1. Patient preparation:
 - a. Patients should refrain from the ingestion of vasoactive medications for a period of 12 hours before the study.
 - b. Patients should refrain from using caffeine-containing products or smoking²²⁷ for a period of 4 hours before the study.
2. Measurement:
 - a. To allow patients to reach a basal resting state, patients should rest in the supine position in a quiet room for ≥ 10 minutes before measurement.
 - b. Depending on the arterial stiffness method used, it may be appropriate to use blood pressure to control for baseline distending pressure when performing mathematical modeling.
 - c. Patients should not speak during the measurements to prevent the possible influence of speaking on arterial tone.
 - d. When patients are to have multiple, longitudinal measurements, each individual patient should be measured at the same time of day to control for the diurnal variation of arterial stiffness.²²⁸
 - e. When there is an opportunity for interobserver variation, such as may occur with the placement of ultrasound probes, measurements should be conducted by the same observer.
 - f. Irrespective of the modality used, ≥ 2 consecutive measurements should be performed.
3. Available modalities:
 - a. 2D and Doppler ultrasound.
 - b. Magnetic resonance imaging.
 - c. ECG-gated oscillometric cuff technology.
 - d. Pressure tonometers.

Gaps in Current Knowledge

1. Which is the best method for assessment?
 - What do the tests measure (large- or medium-artery stiffness, wave reflections)?
 - What is the pathophysiology underlying the methodology?
 - How do the measures relate to each other?
 - Which measures correlate most strongly with cardiovascular risk factors and anatomic atherosclerosis?
 - Which is the most reproducible measure, and does reproducibility differ on the basis of age, sex, underlying disease state, or other factors?
2. What is the range of normal values for each measurement?
 - Do they differ by age, race/ethnicity, and sex?
 - Do they differ by body/arterial size and with normal growth?
3. Which method is the most cost-effective in predicting future cardiovascular disease?

Endothelial Function

Arterial function implies an optimal balance between vasodilating and vasoconstricting substances produced by or acting on endothelial cells.²²⁹ A variety of methods have been developed to evaluate arterial function, with the ultrasound-based measurement of brachial reactivity emerging as the most well-established technique used in adults. Flow-mediated dilation (FMD) assessed with this technique measures the nitric oxide-mediated vasodilation produced by increased flow after a period of ischemia. Non-endothelium-dependent (NED) dilation measures the arterial changes induced by administration of a sublingual dose of nitroglycerin, which reflects predominantly the smooth muscle response.

Relationship to Cardiovascular Risk in Adults

Patients with coronary artery disease and abnormalities in both FMD and NED dilation have the highest rate of adverse cardiovascular events,²³⁰ and the abnormalities predict cardiovascular morbidity independently of traditional cardiovascular risk factors²³⁰ and the Framingham risk score.¹⁹ Not only does FMD predict adverse cardiovascular outcomes, but impaired FMD in asymptomatic individuals also correlates with cardiovascular risk factor levels such as low high-density lipoprotein cholesterol levels,²³¹ hypertension,²³¹ elevated atherogenic LDL subfractions,²³² insulin resistance,^{23,24} type 2 diabetes mellitus,²³³ tobacco exposure,^{24,25} sedentary behavior,²⁴ and inflammation.²³⁴

Interventions to reduce cardiovascular risk in adults demonstrate a parallel improvement in FMD. Treatment of obstructive sleep apnea with nasal continuous positive airway pressure is highly effective in reversing impaired endothelial function,²³⁵ although the improvement is dependent on continued use.²³⁶ Aerobic exercise,²³⁷ but not resistance training,²³⁸ increases FMD in adults, but again, continued compliance with the intervention is needed to sustain the benefit.²³⁷ Dietary change combined with exercise appears to result in a multiplicative response,²³⁹ and the improvement in FMD appears to be independent of improvement in glucose tolerance.²³⁹ Investigators believe that attenuation of insulin resistance rather than change in carbohydrate tolerance is more important in effecting improvement in endothelial function. Diet interventions without exercise have provided less consistent improvements in FMD. Omega-3 fatty acids have alternately shown acute improvement in brachial FMD²⁴⁰ or no change with chronic administration.²⁴¹ However, a "Mediterranean-type" diet rich in oleic acids improved FMD in both patients with both type 2 diabetes mellitus²⁴² and those with ischemic heart disease.²⁴³ Antioxidants such as vitamin C²⁴⁴ and the natural antioxidant flavanoids found in wine,²⁴⁵ grape juice,²⁴⁶ and dark chocolate²⁴⁷ restore endothelial function via a reduction in oxidation and inflammation. This improvement in FMD can also be accomplished by administration of a selective cyclooxygenase-2 inhibitor in adults with severe coronary artery disease.²⁴⁸ Homocysteine metabolism is also closely linked to systemic oxidation and inflammation.²⁴⁹ Adults with hyperhomocystinemia showed increased FMD after 8 weeks of folate administration, and

this improvement was accompanied by a concomitant rise in serum folate levels and a reduction in total plasma homocysteine levels.²⁵⁰

Drug therapies such as the administration of statins to lower cholesterol are efficacious in improving FMD in a variety of underlying states, including heterozygous FH^{251,252} and type 1 diabetes mellitus,²⁵³ as well as in smokers.²⁵⁴ Some data suggest a direct correlation between cholesterol levels and vasoreactivity.²⁵⁵ Others have not found an association in multivariate analyses, which suggests that the non-lipid-lowering, "pleiotropic" effects of statins account for the improvement in brachial FMD.²⁵⁴ In a placebo-controlled trial with metformin in subjects with the metabolic syndrome, improvement in brachial FMD occurred with reduction in insulin resistance.²⁵⁶ In postmenopausal women, supplementation with estrogen improved brachial FMD in both diabetic and nondiabetic females.²³³ Data relating to antihypertensive therapy and brachial artery function are less clear. In 1 study of hypertensive adults, baseline brachial FMD was reduced compared with control subjects; however, neither nifedipine nor captopril treatment improved endothelial function.²⁵⁷ In subjects with coronary artery disease, only mild impairment in brachial FMD was seen at baseline, and only administration of quinapril (and no other agent, including other angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and calcium channel blockers), resulted in improvement in FMD in a crossover study design.²⁵⁸ No vascular benefit was found with drug-induced weight loss combined with exercise in one study,²⁵⁹ whereas another did see increased FMD with the use of the drug orlistat alone.²⁶⁰

NED dilation is less strongly related to cardiovascular risk factors, with independent associations found only with age, baseline diameter,²³¹ and BMI.²⁶¹ NED dilation is not impaired in patients with FH, essential hypertension,²⁶² or inflammatory periodontal disease,²³⁴ which suggests that endothelium-dependent mechanisms may be more important in assessing cardiovascular risk than the biological processes that affect smooth muscle in the arterial wall. NED vasodilation does not appear to be affected by hydroxymethylglutaryl-coenzyme A reductase inhibitor therapy²⁵⁴ or treatment with antihypertensive agents.^{257,258} Measurement of NED dilation, however, may add additional information in certain situations. In subjects with type 1 diabetes mellitus and microalbuminuria, a manifestation of more severe microvascular dysfunction,²⁶³ both FMD and NED dilation are compromised. In middle-aged adults, Jensen-Urstad et al²⁶¹ found that NED dilation, but not FMD, correlated with multirisk score in women. Furthermore, although FMD did relate to increased left ventricular mass, the relationship was not independent of NED dilation. This suggests that the overall reduction in vasodilatory capacity may be more important than endothelial function in the origin of cardiac hypertrophy.²⁶⁴

Data in Children and Adolescents

FMD and NED dilation measurements in children have been studied less extensively, although the literature is growing as more researchers adapt these techniques for use in the

pediatric age group (Table 6).^{47,50,248,263,265–287} Abnormalities in endothelial function have been noted in children affected with a variety of conditions. Even in the absence of elevated cardiovascular risk factors, children infected with the human immunodeficiency virus demonstrated decreased FMD.²⁷⁶ Whether an association exists between abnormalities in FMD and treatment with protease inhibitors^{51,276} is controversial. Children with homozygous homocystinuria, even those as young as 4 years of age, demonstrate abnormal FMD.²⁸⁸ Kawasaki disease is another condition in which decreased brachial FMD has been observed.²⁷⁷ In fact, some investigators hypothesize that any condition that produces systemic inflammation, as manifested by elevated high-sensitivity C-reactive protein, may produce endothelial dysfunction.²⁷⁰ Another disorder that appears to promote vascular dysfunction in children is chronic renal failure, both before²⁷⁸ and after²⁷⁹ renal transplantation. Importantly, changes in FMD in chronic kidney disease could not be explained by classic cardiovascular risk factors such as hyperlipidemia.²⁷⁸ Diabetes is another chronic medical condition known to impair vascular function in children. Decreases in both FMD⁵⁰ and NED dilation²⁶⁶ have been observed in children with type 1 diabetes mellitus. However, 1 study found these changes only in subjects with a concomitant elevation in lipid levels,⁵⁰ whereas another demonstrated them only with concurrent infection-related inflammation,²⁶⁵ which suggests that traditional cardiovascular risk factors played a role. On the other hand, glycemic control, as measured by hemoglobin A1c, has not been proved to relate to these abnormalities in children.²⁸⁹ It is clear that endothelial dysfunction is an early index of target-organ damage incurred with many different chronic diseases of childhood.

As more normative data in asymptomatic children become available, noninvasive measurement of vascular function has the potential to become an important addition to traditional cardiovascular risk factor assessment in them. Adolescents with a positive family history of cardiovascular events have lower FMD than do control subjects, with further reduction seen in children with FH.²⁹⁰ These abnormalities are not restricted to the more severe FH cases but are also found in children with familial combined hyperlipidemia.²⁶⁷ It is not clear, however, whether these changes can be related directly to LDL cholesterol levels. Although Aggoun et al¹⁷¹ found a negative correlation between FMD and LDL cholesterol, other investigators have found only lipoprotein(a) to be a significant correlate.²⁶⁸ Reduced FMD has also been correlated with blood pressure levels in prepubertal children and with insulin resistance in obese children.²⁹¹

As in adults, unhealthy lifestyle habits also have been associated with endothelial dysfunction. Asymptomatic overweight children demonstrated reduced FMD compared with control subjects matched for blood pressure, cholesterol, and glucose levels.⁴⁷ Furthermore, the magnitude of endothelial dysfunction correlated with BMI.⁴⁷ In a study of severely obese children (average BMI *z* score >2 standard deviations above normal), both FMD and NED dilation were impaired relative to control values.¹⁷¹ In these obese children, FMD showed a negative correlation with fasting insulin and apolipoprotein A-I, but in multivariate models, only apolipoprotein

Table 6. Conditions Associated With Reduced Brachial FMD

Condition	Childhood Reference	NED Reference
Disease or condition		
Diabetes mellitus	Järvisalo et al, ⁵⁰ Aburawi et al ²⁶⁵	Lee et al, ^{*233} Donaghue et al ²⁶⁶
Dyslipidemia	De Jongh et al, ²⁵² Mietus-Snyder and Malloy, ²⁶⁷ Aggoun et al, ³⁹ Sorensen et al, ²⁶⁸ Tounian et al ¹⁷¹	Atkov et al, ^{*262} Aggoun et al, ^{*39} Järvisalo et al ²⁶⁹
Family history of CVD or risk factors	Gaeta et al ⁵⁹	
Inflammation	Järvisalo et al, ²⁷⁰ Aburawi et al ²⁶⁵	Amar et al ^{*234}
Age		Jensen-Urstad and Johansson ²⁶¹
Adiposity or adipocytokines	Woo et al, ⁴⁷ Tounian et al, ¹⁷¹ Singhal et al ²⁷¹	Jensen-Urstad and Johansson, ²⁶¹ Fernández-Real et al ²⁷²
Target-organ damage (LVH, increased cIMT, microalbuminuria)		Olsen et al, ²⁶⁴ Papaioannou et al ²⁶³
Folate or homocysteine	Wiltshire et al ²⁷³	Chambers et al ^{*274}
Sedentary behavior or increased physical activity	Abbott et al ²⁷⁵	
HIV infection	Bonnet et al, ²⁷⁶ Charakida et al ⁵¹	Bonnet et al ²⁷⁶
Kawasaki syndrome	Deng et al ²⁷⁷	
Chronic renal disease	Kari et al, ²⁷⁸ Lilien et al ²⁷⁹	Kari et al ^{*278}
Low birth weight	Leeson et al, ²⁸⁰ Leeson et al ²⁸¹	Leeson et al ²⁸⁰
Interventions		
Statins	De Jongh et al ²⁵²	Beckman et al ²⁵⁴
Antihypertensive agents		Ghiadoni et al, ^{*257} Anderson et al ^{*258}
Healthy diet (omega-3 fatty acid, Mediterranean diet, plant sterol, L-arginine, folate)	Engler et al, ²⁸² de Jongh et al, ^{*283} Bennett-Richards et al, ^{*284} Bennett-Richards et al ²⁸⁵	Bennett-Richards et al ^{*284,285}
Antioxidants (vitamin C, Cox-2 inhibitor)	Deng et al, ²⁷⁷ Mietus-Snyder and Malloy, ²⁶⁷ Engler et al ²⁸⁶	Deng et al, ^{*277} Williams et al, ^{*244} Chenevard et al ^{*248}
Exercise	Kelly et al ²⁸⁷	

CVD indicates cardiovascular disease; LVH, left ventricular hypertrophy; and Cox-2, cyclooxygenase-2.

*Nonsignificant associations are indicated with an asterisk.

tein A-I had an independent influence.¹⁷¹ Exercise and diet may also affect endothelial function in youth. Habitual physical activity was correlated with higher FMD in healthy children.²⁷⁵ Better baseline folate status, as measured by red blood cell folate levels, was associated with higher FMD in children with type 1 diabetes mellitus.²⁷³ Environmental influences have also been evaluated. A recent large study of 402 healthy children 11 years of age demonstrated reduced brachial FMD in subjects with a higher serum cotinine concentration.²⁹² This study provides further evidence of the dangers of second-hand smoke.

Low birth weight, a manifestation of an adverse prenatal environment, has also been associated with reduced FMD. There appeared to be a graded positive effect of increasing birth weight on endothelial function in a cohort of adults 20 to 28 years of age²⁸⁰ and in young children 9 to 11 years of age.²⁸¹ Early postnatal factors may also affect vascular function, because greater weight gain in the first 2 weeks of life was associated with lower FMD during adolescence, independent of birth weight.²⁷¹ These data suggest that fetal “programming” may have an adverse effect on vascular function later in life.²⁹³

As in adults, intervention studies in children provide evidence for the importance of arterial function measurements in assessing the success of risk factor reduction. This

becomes especially important in pediatric subjects, for whom the hard end points of cardiovascular morbidity and mortality are lacking. Docosahexaenoic acid, one of the main active ingredients in fish oil, resulted in an increase in FMD after 6 weeks of therapy. Unfortunately, the improvement was lost after a 6-week washout period.²⁸² However, administration of the plant sterols sitosterol and campesterol did not improve FMD in children with FH despite improvement in LDL cholesterol levels,²⁸³ and supplementation with the nitric oxide substrate L-arginine did not improve FMD in children with chronic renal disease.²⁸⁵ It is possible that differences in technique (use of A-mode²⁹⁴ versus B-mode imaging), problems with reproducibility, or differences in the intervention used and disease state being studied may account for these disparate results.

Antioxidant administration has also been investigated as a therapy for vascular dysfunction. In children with Kawasaki disease, acute intravenous vitamin C injection significantly improved brachial FMD nearly to the level seen in control subjects.²⁷⁷ Vitamins C and E also restored endothelial function in children with dyslipidemia.²⁸⁶ However, the mechanism responsible for the improvement is unclear, because there was no change in biomarkers for oxidative stress or inflammation.²⁸⁶ Oral folate supplementation in children with chronic renal disease, however, did



Figure 8. Photograph demonstrating the positioning of the patient and blood pressure cuff for evaluation of brachial reactivity, with the blood pressure cuff positioned below the antecubital fossa.

result in an increase in red cell folate levels, a reduction in total homocysteine level, and significant improvement in brachial FMD.²⁸⁴

Lifestyle modifications have also proved efficacious in modifying vascular function. In obese children, a bicycle training program that led to an increase in peak oxygen uptake ($\dot{V}O_2$) was accompanied by a significantly increased area under the curve for the FMD of the pediatric patients.²⁸⁷ The combination of dietary change and exercise produced the greatest vascular improvement in a study of 9- to 12-year-old obese children.⁵⁴ It is not clear, however, which component is more important. Some pediatric studies have suggested that the benefit of exercise for improvement of endothelial function is independent of weight change,²⁹⁵ whereas others have shown the exercise-related increase in FMD was associated with decreased central adiposity.^{54,296}

Changes in FMD with drug administration have not been routinely studied in children. One trial did find improved endothelial function with the administration of statins in children with FH.²⁵²

As in adults, the utility of NED dilation measures is controversial. Although some studies have shown no difference in NED dilation between control subjects and children with FH,¹⁷¹ human immunodeficiency virus,²⁷⁶ or renal disease²⁷⁸ or after vitamin C administration in Kawasaki disease,²⁷⁷ others have found oxidized LDL to be independently associated with a decreased NED response in healthy children and those with diabetes and FH.²⁶⁹

Methods of Assessment

The ability to demonstrate significant differences in vascular function among groups of individuals is dependent on accurate measurements. Both precision (reliable measurements do not vary from each other greatly) and validity (the average of multiple measurements approximates the true and accurate value) are important. Measurement of brachial FMD and

NED dilation is technically challenging and involves a significant learning curve for both image acquisition and analysis.²⁹⁷ Therefore, meticulous attention to technique, with frequent reassessment of quality control, is essential in vascular assessment studies. Much controversy exists as to the best method for imaging the brachial artery for reactivity studies. Multiple sets of guidelines have been published in respected peer-reviewed journals, and each proposes a slight variation in methodology.^{229,297,298} Regardless of the choice of protocol, the proper equipment must be available. The minimal imaging modalities needed include 2D, color, and spectral Doppler packages. Specialized vascular software can make reading studies more efficient, although it is not mandatory. High image resolution is crucial and is best achieved with multifrequency linear-array transducers (usually 7 to 12 MHz).²⁹⁷ All images must be gated to a simultaneously obtained ECG recording, because brachial diameter varies significantly during the cardiac cycle.²⁹⁹ Digital acquisition is preferred, with super VHS video recommended as the next best alternative.²²⁹

Image acquisition is performed with the subject in the supine position. The baseline brachial artery 2D image is obtained in the longitudinal axis. The International Brachial Reactivity Task Force has recommended that imaging may be performed above or below the antecubital fossa²⁹⁷ (Figure 8), whereas the European Society of Hypertension suggests that imaging be done only below the antecubital fossa.²²⁹ The site of imaging is influenced by placement of the blood pressure cuff. Although upper-arm cuff placement generally results in greater flow-mediated change,³⁰⁰ the imaging is more challenging, because the artery tends to collapse and shift in the soft tissues.^{301,302} Therefore, lower-arm placement is generally recommended. The interface between the lumen and vessel wall must be clearly defined for both the anterior and posterior walls. Anatomic landmarks are used to help maintain the position of the transducer, or a stereotactic probe-holding device may be used. Although most published normative data were obtained from B-mode imaging, both M-mode and A-mode (wall tracking) have been used to measure the wall continuously throughout the cardiac cycle.^{303–305}

First, a resting image is obtained and a pulse Doppler velocity signal recorded. The artery is then occluded by inflating the blood pressure cuff to ≈ 50 mm Hg above the subject's resting systolic blood pressure. The cuff is left inflated for a standard length of time (usually 4.5 minutes),²²⁹ then rapidly deflated, either manually or with a specialized automatic pneumatic tourniquet device. Images are then recorded immediately after deflation and, at minimum, at intervals of 60, 90, and 120 seconds, or continuously, for up to 5 minutes after deflation.²²⁹ The site of cuff placement may also affect time to peak dilation, with an average 22-second delay for peak dilation with cuff placement on the upper arm.³⁰⁶ A midartery Doppler signal is also obtained no more than 15 seconds after deflation to measure maximal hyperemic velocity.²⁹⁷ Reactive hyperemia is calculated as the percentage flow change compared with baseline. Flow-mediated dilation measured at end diastole²⁹⁹ is the percentage increase in lumen diameter with hyperemia compared

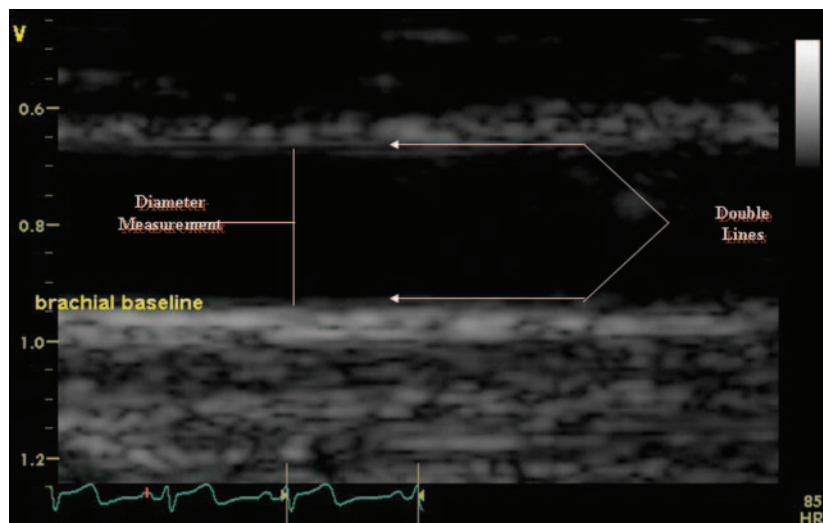


Figure 9. This image demonstrates the typical image of the brachial artery obtained at baseline. The double-layered appearance of the vessel wall can be appreciated and the vessel lumen easily measured.

with baseline. NED vasodilation may be measured after a 10-minute rest period after reactive hyperemia studies. A second baseline scan is obtained, and then an exogenous nitric oxide donor, such as a nitroglycerine spray or tablet, is administered. Controversy exists about the optimal dose to be used, with recommendations ranging from as high as 400 μg ²⁹⁷ to as low as 25 μg .²²⁹

Variability and Limitations

Many external and patient-related factors can influence FMD results. First, all studies should be performed in the fasting state, because high-fat foods are known to induce acute endothelial dysfunction.³⁰⁷ Second, subjects should have refrained from ingesting vasoactive substances such as tobacco, vitamin C, and caffeine for 8 to 12 hours before the examination.²⁹⁷ Medications known to affect the vasculature, including decongestants, aspirin, antihypertensive agents, and oral contraceptives, should be withheld for ≥ 4 half-lives, if possible.²⁹⁷ Because hormones may affect vascular function, females should be questioned as to the phase of their menstrual cycle.^{308,309} Finally, environmental stimuli should be standardized, with subjects refraining from exercise for 8 to 12 hours, and all procedures should be performed in a quiet, temperature-controlled room. Time of day for testing should be standardized, because circadian variation in FMD may exist.^{310,311} Arterial size also influences FMD results. Brachial arteries smaller than 2.5 mm in diameter are difficult to measure.²⁹⁷ Smaller arteries are associated with a greater percent FMD,³¹² and therefore, baseline size may be considered as a covariate in data analyses. Some researchers hypothesize that the increase in shear stress caused by flow through a smaller vessel results in greater nitric oxide release, thereby accounting for this observation.³¹³

Careful attention to detail is essential in reading brachial FMD studies. A clear “double-line” sign (Figure 9) should be visible at the site measured. The absence of this feature may indicate that the sonographer is not imaging precisely perpendicular to the vessel wall.²⁹⁸ Similarly, cross-sectional images are believed to be less reliable and are not recommended. Measurements should be performed at the same time

during the cardiac cycle. The R wave on the ECG is a useful tool to identify end diastole.²⁹⁷ Variability is greatest when measurements are taken point-to-point in a single frame and least when multiple measurements along the vessel are averaged.²⁹⁷ As mentioned previously, maximal flow and diameter will be missed if not measured at the appropriate interval after cuff deflation. Adult guidelines suggest that this occurs ≈ 60 seconds after the end of occlusion³¹⁴; however, studies in children suggest that the time to peak dilatatory response varies considerably, thus complicating data collection even further.³¹⁵

Studies of replicate readings of brachial FMD measurements in 253 normal adults found the correlation coefficient between 2 observers was 0.88 (95% confidence interval 0.82 to 0.94). Bland-Altman analyses demonstrated no significant systematic differences between the measurements (mean difference of -0.30%); however, the limits of agreement were wide (-4.48% to 3.87%) compared with the average FMD value (11.98%).³¹⁶ Furthermore, subjects were not reimaged; therefore, no assessment of biological or rescanning variability could be made. In one published guideline for FMD, repeated measures were deemed acceptable when mean differences were no more than 2% to 3% (for an average FMD of 10%).^{297,317}

The difficulty in obtaining precise measurements makes the definition of “normal” values problematic. Published estimates of “normal” brachial FMD from large studies of adults range from $8.23 \pm 4.51\%$ ²²³ to $11.98 \pm 0.6\%$ ³¹⁶ (Table 7).^{281,315,316,318,319} As expected, even fewer data are available in healthy children. Jarvisalo et al³¹⁵ studied 105 healthy children and found that peak FMD was $7.7 \pm 4.0\%$. Leeson et al¹⁶⁹ published a slightly larger population-based study of 361 normal children; however, the wall-tracking (A-mode) method used may not be comparable to the more commonly used B-mode technique. Until better normative data are available, it may be prudent to design case-control studies when brachial FMD is used to measure endothelial function. Reproducibility studies should also be conducted periodically during pediatric research trials that use this technique. Power calculations that use the variance calculated from data ob-

Table 7. Normal Values Reported for Brachial FMD

Age Range, y	No. of Subjects	Technique	Cuff Position	Brachial FMD, Mean % Change \pm SD	Reference
14–98	4040	B-mode, automatic	Forearm	3.58 \pm 0.10	Herrington et al ³¹⁸
9–11	333	A-mode, automatic	Forearm	4.73 \pm 4.38	Leeson et al ²⁸¹
38–48	19	B-mode, manual	Forearm	6.9 \pm 1.5	Coretti et al ³¹⁹
9–16	105	B-mode, manual	Forearm	7.7 \pm 4.0	Järvisalo et al ³¹⁵
38–48	19	B-mode, manual	Upper arm	11.3 \pm 5.4	Accini et al ³¹⁶
29–50	253	B-mode, manual	Forearm	11.98 \pm 0.6	Coretti et al ³¹⁹

tained from an investigator's own laboratory would also prove helpful in providing accurate estimates of the sample size needed to demonstrate significant differences between groups of subjects.²⁹⁷

Nonultrasound methods for assessment of endothelial function have also been developed in an attempt to overcome the difficulties of obtaining reliable results with traditional imaging. Strain-gauge plethysmography is an older method that is rarely used in pediatric studies because of the need for invasive catheters and drug infusion.²²⁴ However, in adults, it has been useful in demonstrating endothelial dysfunction in patients who smoke or have high cholesterol¹⁴⁹ or obstructive sleep apnea.²²⁵

One commercially available device uses peripheral arterial tonometry to measure blood flow in the fingertips at baseline and after an ischemic stimulus. Thimble-like cups are placed on the finger of each hand to collect beat-to-beat plethysmographic recording of the finger arterial pulse-wave amplitude. Pulsatile volume signals are recorded as optical density changes from the finger's palmar surface within the applied pressure field. The volume changes that accompany the pulse waves are analyzed before and after induced ischemia to determine endothelial function. The reactive hyperemia index generated by this device is highly correlated to ultrasound-based FMD,³²⁰ and it has been shown to be reproducible when analyzed by the technique of Bland and Altman.³²¹ The reactive hyperemia index is reduced in patients with coronary artery disease, with a linear decline seen with clustering of cardiovascular risk factors.³²⁰ Recent data from the Framingham Heart Study demonstrate an independent relationship between the peripheral arterial tonometry ratio (the natural logarithm of the ratio of postdeflation to baseline pulse amplitude in the hyperemic finger divided by the same ratio in the contralateral finger) and male sex, BMI, total-to-high-density lipoprotein cholesterol ratio, type 2 diabetes mellitus, smoking, and lipid-lowering therapy.³²² Few data are available in children, although with the use of this technique, abnormalities have been found in children with congenital central hypoventilation syndrome,³²³ obstructive sleep apnea,³²⁴ and type 1 diabetes mellitus.^{325,326} Although this device shows promise and appears to be relatively operator independent, with good reproducibility in children,³²⁵ a single finger cuff size may limit its usefulness in smaller children.

Doppler laser flowmetry is another technique that may be applicable to smaller children. Doppler laser probes can measure changes in blood flow in the microcirculation in response to a variety of stimuli.³²⁷ Although microdialysis catheters have been used in adults, noninvasive iontophoresis

of drugs or heat or ischemic stimuli can also be applied. Data are lacking on the use of this technique in children, but adult studies show a linear decline in stimulus-induced flow with increasing Framingham risk score.³²⁸ In children, the technique has been used to demonstrate improvement in obstructive sleep apnea, a risk factor for hypertension, with removal of tonsils and adenoids.³²⁹ Abnormalities have also been found with children and adolescents with type 1 diabetes mellitus.^{330,331} However, more pediatric data are needed before the use of any of these nonultrasound techniques can be recommended for evaluation of endothelial function in children.

Recommendations for Standard Assessment of Brachial Artery Reactivity

1. Patient preparation. For 8 to 12 hours before the study, the subject should refrain from:
 - a. Eating.
 - b. Ingesting vasoactive substances (drugs and foods).
 - c. Exercising.
2. Environment:
 - a. Effort should be made to standardize the temperature in the examination room.
 - b. Effort should be made to standardize the time of day the examinations are performed.
 - c. Menstrual cycle of females should be noted.
3. Equipment. Ultrasound equipment should include:
 - a. 2D, color, and spectral Doppler imaging.
 - b. High-frequency linear-array transducer (7 to 12 MHz).
 - c. ECG.
 - d. Digital or super VHS recording.
 - e. Automatically deflating pneumatic blood pressure cuff (preferred).
 - f. Stereotactic probe-holding device (may be helpful).
4. Image acquisition for FMD:
 - a. Patient should rest supine with arm extended in a comfortable position.
 - b. Sonographer should place blood pressure cuff below the antecubital fossa; an arm board may be used.
 - c. Sonographer should obtain optimal resting 2D longitudinal image of the brachial artery \approx 2 to 15 cm above the antecubital fossa. Doppler signal should be recorded. The transducer should be perpendicular to the vessel wall.
 - d. Sonographer should inflate cuff to 50 mm Hg above patient's resting systolic blood pressure (maximum 300 mm Hg) for 4.5 minutes.
 - e. Sonographer should deflate the cuff rapidly.
 - f. Sonographer should record 2D and Doppler images immediately after deflation and at 60, 90, and 120

seconds after deflation. Recording may continue for as long as 5 minutes after deflation.

5. NED vasodilation:
 - a. Data in children do not demonstrate sufficient association between NED vasodilation and cardiovascular risk factors. Furthermore, there are small risks associated with nitroglycerin administration. Therefore, this type of brachial artery reactivity testing is not recommended at this time unless a compelling indication is present.
 - b. If NED vasodilation studies are performed, a resting period of 15 minutes is recommended after FMD measurements.
 - c. A second baseline 2D image and Doppler signal should be recorded.
 - d. Nitroglycerin should be administered (dose given to adults is usually 400 μg , although doses as low as 25 μg have been used).
 - e. Images should be recorded 3 minutes after the dose.
6. Analysis of images:
 - a. Careful attention to image quality is essential.
 - b. Select images with a clear "double-line" sign.
 - c. Read images from the same time during the cardiac cycle (R wave on ECG at end diastole is preferred).
 - d. Identify the intima-lumen interfaces (preferred), although media-adventitial interfaces can be used provided the method used is applied consistently.
 - e. Measure the diameter by tracing the wall (preferred), or use an average of several point-to-point measurements.
 - f. Measure the Doppler flow velocity at baseline and at each point after deflation.
 - g. Calculations:
 - i. $\%FMD = (LDp - LDb) / LDb \times 100$, where LDp=lumen diameter after inflation and LDb=lumen diameter at baseline.
 - ii. $\text{Reactive hyperemia} = (Vp - Vb) / Vb \times 100$, where Vp=Doppler velocity after inflation and Vb=Doppler velocity baseline.
7. Training for sonographers and readers:
 - a. Intensive training according to rigid written protocols is required.
 - b. Formal calculation of reproducibility is needed for certification of all sonographers and readers.
 - c. Retraining with written protocols should occur periodically, with recertification required for any observers who have had a significant time lapse since performing duties (scanning or reading).
8. Quality controls:
 - a. Periodic assessment of variability for both rescanning and rereading should be performed, with calculation of:
 - i. Intraobserver and interobserver variability.
 - ii. Temporal comparisons, for laboratory drift.
 - iii. Comparisons between laboratories, for multicenter trials.
 - b. Coefficients of variability, intraclass correlation coefficients, and Bland-Altman plots may be useful in assessing variability.
 - c. Acceptable variability is not well established, although published reports would suggest that correlations greater than 0.8³¹⁶ or a mean difference between measurements of no more than 2% to 3% would be acceptable.^{297,317}

Gaps in Current Knowledge

Many data need to be collected before assessment of arterial function can be integrated into a comprehensive primary prevention program aimed at reducing future cardiovascular events in children and adolescents. The major gaps in our current knowledge base include the following:

1. Which is the best method for assessment?
 - What do the tests measure (function in a medium muscular artery, peripheral bed, skin microvasculature)?
 - What is the pathophysiology underlying the methodology?
 - How do the measures relate to each other?
 - Which measures correlate most strongly with cardiovascular risk factors and anatomic atherosclerosis?
 - Which is the most reproducible measure, and does reproducibility differ on the basis of age, sex, underlying disease state, or other factors?
2. What is the range of normal values for each measurement?
 - Do they differ by age, race/ethnicity, and sex?
 - Do they differ by body/arterial size and with normal growth?
3. Which method is the most cost-effective in predicting future cardiovascular disease?

Summary

Risk factors associated with the development of cardiovascular disease, including diabetes mellitus, hypertension, dyslipidemias, and obesity, are becoming increasingly prevalent in children and adolescents. Therefore, noninvasive methods of assessing atherosclerotic risk in youth need to be developed and standardized. In this statement, we have summarized current knowledge of noninvasive vascular assessment in children and adolescents and outlined recommendations for standard assessment in the pediatric population for clinical research purposes.

Additional data are needed before these methods can be adopted in clinical evaluation. The pressing needs for research in this area include the following: (1) collection of more normative data across age, race, and sex for all devices; (2) longitudinal studies to determine age- and puberty-related changes in the measures in a low-risk population for comparison; (3) correlations to better-established pediatric intermediate target-organ end points (left ventricular hypertrophy, microalbuminuria); and (4) additional interventional studies to demonstrate improvement in vascular function with treatment of cardiovascular risk factors.

The ability to use noninvasive methods to accurately measure vascular damage related to atherosclerotic processes in youth will substantially improve our ability to risk-stratify individuals by traditional assessment, especially in this youthful population in which hard cardiovascular end points are unlikely to occur. Improved risk profiling will allow targeting of the highest-risk youth for the most aggressive interventions to prevent myocardial infarction and stroke. A paradigm shift in our thinking about cardiovascular diseases needs to occur. The emphasis should be on prevention both from a population basis and by better identification of high-risk individuals decades before clinical cardiovascular disease occurs.

Disclosures

Writing Group Disclosures

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*Modest.

†Significant.

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John R. Crouse	Wake Forest University	None	None	None	None	None	None	None
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†Significant.

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