Ultrasound assessment of endothelial function in children

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Abstract: Although the clinical complications of atherosclerosis arise from developed lesions in old age, the atherosclerotic disease is a lifelong process with roots in childhood. Endothelial dysfunction is currently considered an early stage in the pathogenesis of atherosclerosis, which precedes the formation of structural atherosclerotic changes. Improvements in noninvasive imaging modalities, mainly in ultrasound imaging, have made it possible to assess the endothelial health of asymptomatic children with or without cardiovascular risk factors. By using noninvasive ultrasound for endothelial function, important insights have been gained into the early stages of atherosclerosis and the effects of cardiovascular risk factors on vasculature in childhood. The ultrasound test of endothelial function is affordable, available, and safe and may be considered a potent aid in clinical risk stratification of children at high risk for subsequent clinical atherosclerosis in adulthood. At present, this methodology serves only research purposes, as many issues including reproducibility and normal values for healthy children need to be solved before clinical use can be considered. In adults, however, recent studies have shown that attenuated endothelial function predicts the occurrence of future cardiovascular events.

Keywords: atherosclerosis, endothelial dysfunction, ultrasound imaging, childhood vasculature

Endothelial function

The vascular endothelial layer was first considered an inert border that discriminates the vascular wall from the circulating blood stream (Florey 1966). Today the endothelium is recognized as an active organ, responsible for the maintenance of vascular homeostasis in the physiological state. The intact endothelial layer has many antiatherogenic properties: it takes part in the control of arterial tone (Furchgott and Vanhoutte 1989), helps to control proliferation of the underlying arterial smooth muscle cells (Scott-Burden et al 1992), and regulates cell-to-cell interactions by inhibiting thrombocyte aggregation and monocyte adhesion, which constitute important steps in the pathogenesis of atherosclerosis (Vanhoutte 1988, 1991). Research by Furchgott and Zawadzki (1980) demonstrated a pivotal role for endothelial cells in the maintenance of vasodilatation by releasing vasoactive mediators and led to the appreciation of the endothelial layer as a natural inhibitor of atherogenesis.

Under normal conditions, the endothelial layer favors vasodilation over vasoconstriction by releasing vasodilatory substances, mainly nitric oxide (NO) and prostacycline (Ignarro et al 1987; Cohen and Vanhoutte 1995). The physiological atheroprotective function of the endothelium is, however, rapidly attenuated in the presence of atherosclerotic risk factors and toxic substances and conditions (Vanhoutte 1998). The attenuation of endothelial function may be a key event in atherogenesis, which precedes the development of structural and clinical atherosclerosis (Ross 1986).

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In addition to being an early event in atherogenesis, endothelial dysfunction is important in the later stages of atherosclerotic diseases, predisposing individuals to complications, such as thrombotic events.

Ultrasound assessment of endothelial function

The long preclinical phase of atherosclerotic diseases provides a window for noninvasive assessment of subclinical atherosclerosis before ischemic symptoms appear. Lack of accurate diagnostic tests of preclinical atherosclerosis, however, has hampered the ability to detect and monitor early atherosclerosis. The development of diagnostic tests to detect subclinical disease could facilitate the early diagnosis and management of high-risk individuals in childhood. These tests should be noninvasive, safe, accurate, reproducible, and relatively inexpensive to allow for the clinical testing of apparently healthy individuals for risk stratification purposes. The measurement of arterial endothelial function might be a useful test, as endothelial health reflects the propensity of the vascular tree to develop early atherosclerosis.

Experimental studies performed in the early 1970s demonstrated in vivo that local increase in blood flow causes arterial dilatation (Lie et al 1970). Later this finding was corroborated by human studies showing flow-mediated dilatation (FMD) of the brachial artery (Sinoway et al 1989). Joannides and co-workers (1995) discovered that FMD, induced by reactive hyperemia, was dependent on intact endothelial layer and was mediated by NO. Many studies during recent years have utilized this physiologic phenomenon to study vascular endothelial function by measuring changes in the diameter of the conduit arteries (such as the brachial or femoral) using noninvasive ultrasound (Raitakari and Celermajer 2000). The ultrasound measurement of endothelial function in children was first described by Celermajer and co-workers in 1992 (Celermajer et al 1992). The test is based on the measurement of the brachial artery diameter at baseline and after an increase in blood flow caused by inflating and then deflating a forearm blood pressure cuff. In this test, the proportional increase in luminal diameter induced by hyperemia is calculated and used as a marker of systemic endothelial function. The FMD response is traditionally measured at 60s post-occlusion (Celermajer et al 1992). Later studies have demonstrated that the arterial dilatation response to ischemia-induced reactive hyperemia is mediated through increased release of endothelial nitric oxide (NO), when arterial occlusion times not greater than 5 minutes are used (Mullen et al 2001). Sørensen and coworkers (1995) have previously studied the accuracy and reproducibility of the brachial ultrasound method and shown that although the day-to-day within-subject variation in FMD is rather substantial, the long-term within-subject variation during weeks and months is acceptable and the measurement of FMD is reproducible. In recent studies, the day-to-day within-subject variation has varied greatly, with coefficients of variation ranging between 9% and 50% (De Roos et al 2003; Järvisalo et al 2003; Malik et al 2004). Much of the long-term variation in FMD measurements is due to physiological fluctuation in endothelial function and not measurement error, as the long-term reproducibility of brachial artery baseline diameter measurements is excellent (Sørensen et al 1995). Individual ultrasound laboratories should, however, commit to repetitive testing of measurement reproducibility and quality control, and the training of ultrasound technicians should be performed with great care. Computer software has been developed for automated analysis of brachial ultrasound image sequences. These automated methods provide a decreased analysis bias, increased reproducibility, and improved measurement accuracy. Consequently, inter- and intraobserver as well as interinstitution variability are lower (Herrington et al 2001; Woodman et al 2001; Sonka et al 2002).

There are currently few large-scale studies published on FMD in children. In the largest population-based FMD study comprising 333 British children, the mean FMD at 60s post-occlusion was 4.7 ± 4.3% (Leeson et al 2001), whereas in a sample of 105 healthy Finnish children, the mean maximal FMD was 7.7 ± 4% (Järvisalo et al 2002). In a large sample of 4040 subjects aged 14–98 years, the mean FMD was 4.4 ± 0.9% (Herrington et al 2001), and in 2109 Finnish 24- to 39-year-old adults, the mean FMD was 7.0 ± 4.0% for men and 8.8 ± 4.6% for women (Juonala 2005). Therefore, there is still controversy on the normal values for FMD in children and adults, although some previous papers have defined endothelial dysfunction as FMD less than 3% (Adams et al 1996; Järvisalo, Raitakari, et al 2004).

An international task force of experts in the field has recently stated its recommendations on the execution of the ultrasound test for endothelial function (Corretti et al 2002). By using this test, endothelial dysfunction has been demonstrated to associate with several risk factors in adults, such as high serum low-density lipoprotein (LDL) and total cholesterol (Celermajer et al 1992), serum high-density lipoprotein (HDL) cholesterol (Toikka et al 1999), cigarette

smoking (Celermajer, Sørensen, Georgakopoulos, et al 1993; Raitakari et al 1999), hypertension (Muiesan et al 1999), diabetes (Clarkson et al 1996), markers of oxidized LDL (Heitzer et al 1996), visceral obesity (Hashimoto et al 1998), mental stress (Spieker et al 2002), and family history of premature myocardial infarction (Gaeta et al 2000). The brachial artery dilator response to increased shear stress has been shown to correlate significantly with invasive testing of brachial (Irace et al 2001) and coronary endothelial function (Anderson et al 1995), as well as with the extent and severity of coronary atherosclerosis (Neunteufl et al 1997) and carotid artery intima-media thickness (Gaeta et al 2000). Furthermore, recent studies have shown that brachial artery endothelial dysfunction is an independent predictor of the occurrence of cardiovascular events in adults (Perticone et al 2001; Gokce et al 2003).

The ultrasound assessment of systemic arterial endothelial function is usually performed in the brachial artery, because of a more suitable luminal diameter compared with the radial artery and because it is more easily examined than the femoral artery. In pre-pubertal children, the measurements can, however, be reliably performed in the femoral artery in addition to the brachial artery. It is known that brachial artery diameter is inversely associated with FMD (Järvisalo et al 2000). Arteries less than 2.5 mm in diameter (mostly seen in children) present a potential limitation to the ultrasound technique as the risk for "off-center" imaging, and thereby, inaccurate diameter measurements increases as the diameter becomes smaller (Corretti et al 1995; Stadler et al 1996).

For reliable assessment of small changes in luminal diameter, ultrasound transducer frequencies equal to or greater than 7 MHz are required to achieve a satisfactory resolution. The noninvasive nature of the brachial artery ultrasound test has made it possible to assess systemic endothelial function in children and adolescents with or without cardiovascular risk factors. Studies in children have demonstrated that coronary risk factors may lead to attenuated endothelial function arising in childhood.

The brachial artery ultrasound test for endothelial function usually includes administration of sublingual nitrates to examine the vasodilating effect of an exogenous source of NO. Nitric oxide acts directly at the level of the arterial smooth muscle cells and produces an endothelium-independent dilatation response. Nitrate-mediated dilatation (NMD) therefore serves as a control test for the FMD measurement to ensure that a decreased FMD capacity observed is truly a consequence of endothelial dysfunction, and not a reflection of underlying smooth muscle dysfunction. Recent evidence indicates that in addition to influencing endothelial function, atherosclerosis may also induce changes in arterial dilatation responses to exogenous NO (Adams et al 1998; Raitakari et al 2001). Adult patients with coronary artery disease show impaired brachial NMD compared with healthy controls (Raitakari et al 2001). Attenuated NMD is associated with serum cholesterol concentration independent of endothelial function in apparently healthy adults (Adams et al 1998). In most previous studies in children, the NMD responses have actually been mildly reduced in high-risk individuals although the difference has not reached statistical significance, possibly due to limited sample size (Celermajer et al 1992; Celermajer, Sørensen, Ryalls, et al 1993; Singh et al 2003). We have recently shown that impaired NMD is closely associated with low FMD, increased oxidized LDL and higher intima-media thickness in healthy and high-risk children (Järvisalo, Lehtimäki, et al 2004). These findings suggest that decreased dilatation response to exogenous NO donors occurs in children at increased risk for atherosclerosis.

Hypercholesterolemia and endothelial function in children

Familial hypercholesterolemia (FH) is a common autosomal dominant inherited disorder of the LDL cholesterol metabolism in which elimination of LDL cholesterol is impaired as a result of defects in the LDL receptor (Goldstein and Brown 1973). Subjects with untreated heterozygous FH develop coronary heart disease (CHD) 15–20 years earlier than healthy subjects. Children with heterozygous FH show impaired FMD at the age of 6–7 years (Sørensen et al 1994; de Jongh, Lilien, Bakker, et al 2002). In one study, a positive family history for cardiovascular disease was shown to carry additional risk in children with FH (de Jongh, Lilien, Bakker, et al 2002). In children with FH, the correlation between serum LDL concentration and FMD has been shown in one study (Aggoun et al 2000), but not all (Sørensen et al 1994; Mietus-Snyder and Malloy 1998). However, in a relatively large group of healthy normocholesterolemic children, LDL cholesterol concentration was inversely correlated with FMD responses (Järvisalo et al 2002). In the study by Sørensen et al (1994), serum Lp(a) level was inversely associated with FMD in FH children independent of serum cholesterol. FMD has also been shown to be impaired in children with familial combined hyperlipidemia, another common form of hereditary dyslipidemia (Mietus-Snyder and Malloy 1998).

Diabetes and endothelial dysfunction in children

Type 1 diabetes is an important risk factor for cardiovascular events. Individuals with diabetes have a two- to fourfold increased risk of developing atherosclerotic diseases, which is inadequately explained by differences in the levels of traditional vascular risk factors (Pyörälä et al 1987). Wiltshire et al (2002) studied FMD in 36 diabetic children with a mean age of 14 years and a mean duration of diabetes of under 6 years. These diabetic children without diabetic complications had attenuated endothelial function compared with controls. In their study, diabetic endothelial dysfunction was correlated with red cell folate concentration. Disease duration and glycemic control, however, were not associated with endothelial dysfunction in these children (Wiltshire et al 2002). Previously, Donaghue and co-workers (1997) demonstrated in a study of 20 diabetic adolescents that young diabetics with clinical complications had decreased endothelial and smooth muscle function compared with healthy controls. We have recently shown that endothelial dysfunction is commonly found in young children with type 1 diabetes, and that those diabetic children affected by impaired FMD are at risk of having increased carotid artery wall thickness, which is a structural marker of early atherosclerosis (Järvisalo Raitikari, Toikka, et al 2004). These findings suggest that endothelial dysfunction in children with type 1 diabetes may predispose them to the development of early atherosclerosis.

Other risk factors associated with endothelial dysfunction in childhood

Recent studies have associated childhood obesity, a condition with steeply increasing prevalence, with endothelial dysfunction (Woo et al 2004). The effect of low birth weight on later endothelial dysfunction has been the topic of several studies as it has been associated with increased risk for CHD. Singhal and co-workers (2001) studied prematurely born children and found no differences in endothelial function compared with control children. In another small-scale study, low birth weight was associated with endothelial dysfunction in healthy 9-year-old children (Martin et al 2000), and there are data indicating that low birth weight may influence endothelial function even in adulthood (Leeson et al 2001). Also, rarer conditions such as chronic renal failure (Kari et al 1997), homozygous homocystinuria (Celermajer, Sørensen, Ryalls, et al 1993), HIV infection (Bonnet et al 2004), lupus erythematosus (Soep et al 2004), and Henoch-Schonlein purpura (Kurotobi et al 2004) have been associated with impaired FMD in children. Gaeta et al (2000) showed that the young adult off-spring of patients who had experienced premature myocardial infarction have attenuated FMD compared with matched control subjects. Conversely, endothelial function has been shown to be directly correlated with habitual physical activity in 5- to 10-year-old children (Abbott et al 2002). So far no studies have associated childhood hypertension with endothelial dysfunction (Leeson et al 1997; Järvisalo et al 2002).

Is childhood endothelial dysfunction reversible?

Childhood endothelial function has been shown to be potentially reversible by the use of combined tocopherol and ascorbic acid antioxidant therapy in hypercholesterolemic chidren (Mietus-Snyder and Malloy 1998; Engler et al 2003). In a recent study, de Jongh and co-workers demonstrated in a randomized, placebo-controlled setting that early simvastatin therapy of 10–40 mg daily restores endothelial function in 28 weeks in 9- to 18-year-old children with heterozygous familial hypercholesterolemia (de Jongh, Lilien, op't Roodt, et al 2002). No improvement in FMD was, however, observed with plant sterol therapy in FH children despite a contemporaneous decrease in serum LDL concentration (de Jongh et al 2003). Folic acid therapy has lately been shown to improve endothelial function in children with chronic renal failure (Bennett-Richards et al 2002) and in children with type 1 diabetes (Pena et al 2004). Several recent studies have demonstrated that obesity-related endothelial dysfunction can be ameliorated by increasing exercise and improving dietary habits (Watts et al 2004a, 2004b; Woo et al 2004). Recent findings have led to attempts to develop drugs directly aimed to treat and reverse the arterial wall pathology instead of merely diminishing risk factor load. For some drugs, such as the statin group, it seems that in addition to the lowering of serum cholesterol levels they carry pleiotropic effects including direct beneficial effects on the endothelium. In children, the data on effects of intervention strategies on arterial indices are currently very limited, although available evidence supports the positive effects of statin (de Jongh, Lilien, op't Roodt, et al 2002) and antioxidant (Mietus-Snyder and Malloy 1998) therapies on endothelial function in hypercholesterolemic children.

Clinical implications of endothelial dysfunction in children

Atherosclerosis is a chronic progressive condition that originates in childhood and is accelerated in the presence of cardiovascular risk factors. Risk factors for atherosclerosis show remarkable tracking and clustering, therefore early interventions in young subjects with increased risk factor load are invaluable and constitute the basis of primary prevention of coronary artery disease. The long asymptomatic phase of atherosclerotic diseases provides an opportunity for early primary prevention measures when the subjects that benefit the most from these interventions are carefully identified.

The development of imaging methods for the non-invasive assessment of endothelial function in children carries remarkable potential for clinical risk stratification. Ultrasonic assessment of arterial endothelial function meets the requirements for safety, price, and availability, and today the techniques and imaging methods have been refined to create reproducible, reliable results. No data are yet available on the predictive value of childhood endothelial function on cardiovascular prognosis but the data in adults are convincing.

The ultrasound method for assessment of endothelial function might prove valuable in determining the true benefits of early pharmaceutical risk factor management in high-risk children. However, further longitudinal studies are needed before these techniques may be accepted for tools in routine clinical practice as there are currently no data on whether an improvement in endothelial function during childhood will translate into decreased risk of future cardiovascular disease.

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