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Review

Methodological considerations and practical recommendations for the application of peripheral arterial tonometry in children and adolescents

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ABSTRACT

Endothelial dysfunction is recognized as the primum movens in the development of atherosclerosis. Its crucial role in both cardiovascular morbidity and mortality has been confirmed. In the past, research was hampered by the invasive character of endothelial function assessment.

The development of non-invasive and feasible techniques to measure endothelial function has facilitated and promoted research in various adult and paediatric subpopulations. To avoid user dependence of flow-mediated dilation (FMD), which evaluates nitric oxide dependent vasodilation in large vessels, a semi-automated, method to assess peripheral microvascular function, called peripheral arterial tonometry (Endo-PAT®), was recently introduced. The number of studies using this technique in children and adolescents is rapidly increasing, yet there is no consensus with regard to either measuring protocol or data analysis of peripheral arterial tonometry in children and adolescents. Most paediatric studies simply applied measuring and analysing methodology established in adults, a simplification that may not be appropriate.

This paper provides a detailed description of endothelial function assessment using the Endo-PAT for researchers and clinicians. We discuss clinical and methodological considerations and point out the differences between children, adolescents and adults. Finally, the main aim of this paper is to provide recommendations for a standardised application of Endo-PAT in children and adolescents, as well as for population-specific data analysis methodology.

1. Introduction

Endothelial dysfunction is considered to be the earliest phase of atherosclerosis [1]. In an elegant and integrant consensus article by the working group on endothelin and endothelial factors of the European Society of Hypertension, Deanfield and colleagues set the clear definition of endothelial dysfunction as "an imbalance between vasodilating and vasoconstricting substances produced by (or acting on) endothelial cells" [2]. The endothelium normally tightly regulates vascular tone by coupling the production of vasodilating and vasoconstricting factors to the needs of a particular organ, in order to supply oxygen and nutrients. Nitric oxide (NO) has been identified as the main regulator of vascular smooth muscle cell relaxation, leading to vasodilation, but more actors have been identified [3].

Endothelial dysfunction is associated with standard and novel cardiovascular (CV) risk factors leading to atherosclerosis, namely hypertension [4], an adverse lipid profile [5], inflammation [6] and adipose tissue dysfunction [7–9]. Yet endothelial dysfunction is more than a mere bystander of CV risk, since endothelial dysfunction is an independent predictor of CV morbidity and mortality in both adults with and without coronary artery disease [10].

The development of tools to assess endothelial function has progressed rapidly. The first described method relied on the intra-coronary administration of vaso-active reagents such as acetylcholine [11], raising serious ethical concerns on its application on a broader scale. Soon a less invasive technique called FMD was developed [12] both for adults and children. This method only requires an ultrasound assessment of brachial artery diameter before and after raised shear stress (induced by in- and deflating a manometer cuff around the arm), which causes minimal discomfort for the participant. Considerable operator training is required to achieve reproducible results of

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FMD, which are still characterised by substantial inter-observer variability. Hence, a more automated and less operator-dependent method called peripheral arterial tonometry (Endo-PAT) was introduced by Itamar Ltd. (Caesarea, Israel) and their first marketed device was the Endo-PAT 2000.

2. Application of the Endo-PAT

The Endo-PAT comprises two pneumatic probes that register arterial pulse wave amplitudes (PWAs) and which are usually placed on both index fingers of both hands. The pneumatic probes basically resemble a thimble, with a rigid exterior and a soft internal membrane. Before measurements are started, the internal cuff is inflated to sub-diastolic pressure to prevent veno-arteriolar reflex vasoconstriction. The apparatus itself measures pressure changes in the closed loop circuit, which according to the ideal gas law result from volume differences due to finger arteriolar volume changes. The PWA is defined as the difference between the highest and lowest point of a pulse wave. After a 5 min baseline assessment, the brachial artery of the non-dominant arm is occluded by inflating a sphygmomanometer to suprasystolic pressure for 5 min until cuff release. The resulting hyperaemia is recorded during another 5 min. The provided software automatically calculates a reactive hyperaemia index (RHI). Equations commonly used for calculating the RHI are presented in the section on data analysis.

The measuring site is always located distally to the occlusion (contrary to FMD measurements where the echo probe can be placed either proximally or distally to the cuff). Unlike FMD measured at the brachial artery, hyperaemia of the fingertip microvasculature is not entirely caused by NO. Nohria et al. demonstrated that only 50% of the digital hyperaemia was blocked when a nitric oxide synthase specific blocker (L-NAME) was infused in the brachial artery prior to Endo-PAT measurement [13]. Other factors are known to be involved in the reactive hyperaemia [14–16].

Nevertheless a correlation between microvascular function at the fingertip and invasively measured coronary endothelial function has been found [17] as well as between the former and macrovascular endothelial function measured by FMD [18–20]. On the other hand, in a large study in 1843 mainly healthy adults, the Framingham reactive hyperaemic index (F-RHI, for explanation see Data analysis section) did not correlate with FMD [21], suggesting that the differences in micro- and macrovascular reactivity may be population-specific. Interestingly, however, the added predictive value of the F-RHI beyond the traditional Framingham risk score was established in adults with unexplained chest pain [22].

The Endo-PAT measurement causes hardly any pain, and even in children high reproducibility can be achieved [23]. While the Endo-PAT method lends itself for studies in children due to its nearly painless application and reproducible results, there are several pitfalls that have to be considered and appropriately addressed when using Endo-PAT in paediatric studies. The following text provides a comprehensive overview on practical and methodological problems concerning the setting up and conductance of studies using the Endo-PAT to quantify endothelial function in children and adolescents.

3. Factors influencing endothelial function

Endothelial dysfunction has been described by Bonetti et al. as the "risk of the risk factors" [24] combining the effects of traditional and non-traditional CV risk factors as well as a genetic predisposition and local factors (e.g. shear stress). Yet this integrative capacity also increases complexity, since there are many confounding factors which should be taken into account (Table 1). In a research setting, it is imperative to recognise and identify these interfering factors and to carefully select appropriate control groups.

3.1. Diet

Dietary components can have beneficial as well as detrimental effects on endothelial function. For the sake of standardisation and to avoid confounding, endothelial function tests need to be performed in fasting conditions. Even a single high fat meal can negatively influence endothelial function [25], likewise for the quite popular energy drinks [26]. On the other hand, antioxidant vitamins like vitamin C and vitamin E, available over-the-counter, have a positive effect on endothelial function and cause a short-lived but significant improvement [27]. Additionally, the beneficial effects of flavonoid containing products like chocolate and tea on endothelial function have recently gained the attention of the scientific community [28].

3.2. Smoking

Both active and passive smoking are considered an independent risk factor for CV disease and their detrimental effects on endothelial function come as no surprise [29,30]. Ideally, children or adolescents with active tobacco use are excluded, or they need to refrain from smoking at least 4 to 6 h before testing [31]. Exposition to passive smoking [32] is more difficult to identify and control for.

Table 1
Factors influencing endothelial function.

Factors		Effect on endothelial function	Possible solutions
Dietary components	High fat meals	↓	Measurements in fasting conditions, assess eating habits using questionnaires
	Vitamin C	↑	
	Flavonoids	↑	
Smoking	Active and passive smoking	↓	Exclude active smokers, if possible or assess (parental) smoking habits
Medication	Beta-mimetics	↑	Exclude patients using these drugs, or postpone intake of drugs
	Insulin/metformin	↑	
	Growth hormone therapy	↑	
Hormonal influence	Menstrual cycle	~	Note phase of cycle
	Pubertal development	↑	Note pubertal stage
Timing of measurement	Time of day	Diurnal variation	Perform test between 8 and 12 pm
	Season	Seasonal variation	Include all participants in the same season or equally distributed throughout the year
	Mild infections	↓	Postpone the test for at least 2 weeks
	Blood sampling	↓	30 min prior to test
Stress		↓	Carefully explain the test, avoid vocal interaction
Blood pressure hyperreactivity		↑	Consider cold pressor test
Environmental factors	Skin temperature	Inverse correlation	Allow sufficient acclimatisation time
	Room temperature	Inverse correlation	Temperature controlled room (21–24 °C)
Exercise	Acute exercise bout	↓–↑	Ask not to engage in maximal exercise 24 h prior to test
	Exercise training	?	Use questionnaires to get an idea of exercise training