Assessing Endothelial Function: Overview & Scientific Validation of Endo-PAT2000
Overview

For more than a decade Endothelial Dysfunction has been recognized by the medical community as the critical junction between risk factors and clinical disease. It is the earliest detectable stage of cardiovascular disease. Furthermore, it is treatable, and unlike the atherosclerotic plaque which it causes, is even reversible.

Endo-PAT2000 is the leading medical device for noninvasive endothelial function assessment. It is FDA-cleared, CE-marked and used in preeminent clinical institutions, research centers and Pharmaceutical clinical phase studies in over 40 countries. It is incorporated into numerous multi-center and population-based studies such as the Framingham Heart Study. Research using Endo-PAT has yielded more than 100 articles in peer-reviewed journals and abstracts. It is becoming widely recognized as the standard method for endothelial function assessment. Some of the features that make Endo-PAT appealing are its ease of use, user-independence and immediate, automatically calculated test results. It provides clinicians with a reliable and reproducible index of endothelial function in a 15-minute, office-based test.

Endo-PAT is based on noninvasive Peripheral Arterial Tone (PAT) signal technology described below. It measures endothelium-mediated changes in vascular tone using unique bio-sensors placed on the fingertips. These changes in arterial tone are elicited by creating a down-stream hyperemic response induced by a standard 5-minute occlusion of the brachial artery. Measurements from the contra-lateral arm are used to control for concurrent non-endothelial dependent changes in vascular tone. The automatically calculated result is an index of endothelial function.
The Test

Endo-PAT tests can be carried out in both the office and hospital settings, with patients positioned either sitting or supine. Endo-PAT bio-sensors are placed on the index fingers of both arms. The test takes 15 minutes to complete, is very easy to perform, and is both operator and interpreter independent. Thermo-neutral, quiet surroundings are recommended.

Endo-PAT quantifies the endothelium-mediated changes in vascular tone, elicited by a 5-minute occlusion of the brachial artery (using a standard blood pressure cuff). When the cuff is released, the surge of blood flow causes an endothelium-dependent Flow Mediated Dilatation (FMD). The dilatation, manifested as Reactive Hyperemia, is captured by Endo-PAT as an increase in the PAT Signal amplitude. A post-occlusion to pre-occlusion ratio is calculated by the Endo-PAT software, providing the EndoScore.

Automatic Analysis

Endo-PAT software is an integral part of the Endo-PAT system. It is straight-forward and easy to use. The software is used for both on-line data acquisition as well as off-line data analysis.

The online display allows real-time viewing of events as they occur. The signals are recorded on the computer for subsequent review and automatic analysis. Since analysis is performed by the software, inter- or intra-operator interpretation variability is avoided. Analyzed test results can be exported to an Excel spreadsheet that includes multiple study parameters, calculated variables, and measures of signal quality.

PAT Technology

Peripheral Arterial Tone (PAT) signal is a proprietary technology used for non-invasively measuring arterial tone changes in peripheral arterial beds\(^1\). The PAT Signal is measured from the fingertip by recording finger arterial pulsatile volume changes. Based on PAT Technology, the noninvasive Endo-PAT2000 system comprises a measurement apparatus that supports a pair of modified plethysmographic bio-sensors. The unique feature of the PAT bio-sensors is that they impart a uniform sub-diastolic pressure field to the distal two thirds of the fingers including their tips. Applying the pressure in this way is extremely important as it:

- Prevents distal venous blood pooling, that can induce a veno-arteriolar vasoconstriction reflex
- Unloads arterial wall tension, which generates a greater dynamic range of the measured PAT Signal
- Fixates the PAT bio-sensor to the finger, which reduces movement artifacts
Methodological Advantages

A. Simultaneous recording from both arms:

The subject serves as his/her own control: while endothelial function is tested in one arm, the contra-lateral arm is used to monitor systemic vascular changes (e.g., alterations in autonomic tone, transient environmental effects, etc.) that generally affect both arms. By measuring both arms, Endo-PAT2000 corrects for systemic changes that occur during the course of the test.

B. Assessment of occlusion and provocation quality:

The most common way of provoking the endothelium non-invasively is by induction of local ischemia in the arm for 5 minutes. The ischemia is achieved by inflating a blood-pressure cuff to a supra-systolic pressure, causing cessation of blood flow to the arm. In some cases complete occlusion is not achieved, allowing a residual passage of blood that perfuses the downstream tissues. This results in incomplete oxygen starvation necessary to elicit the full endothelial response. Endo-PAT2000 enables online detection of occlusion quality allowing the operator to respond by increasing cuff pressure.

C. Large dynamic range of measurement:

The fingers have an inherently large ability to vary local vascular tone, enabling up to a hundred-fold change in blood flow. The pressurized PAT bio-sensors assure greater sensitivity to change, enhancing signal-to-noise ratio and accuracy.

D. Site of measurement:

The fingertips contain small conduit vessels as well as resistance vessels and highly regulated A-V shunts, reflecting a diversity of vascular beds. This further enhances the reliability of Endo-PAT.
Validation Studies

The essential validity of Endo-PAT2000 as a measure of endothelial function has been demonstrated in several independent key studies, at leading medical centers.

A. Endo-PAT correlates with assessment of coronary endothelial function

Endo-PAT provides high degrees of sensitivity and specificity when compared to the assessment of coronary artery endothelial function. Coronary endothelial function is quantified by measuring arterial diameter change and blood flow in response to graded intra-coronary infusion of Acetylcholine during angiography. In a study performed by Bonetti et al. at the Mayo Clinic, Rochester, MN\(^2,3\), a group of 94 subjects underwent angiographic assessment of coronary endothelial function and subsequent Endo-PAT tests. The results of this comparative study served as the basis for the FDA clearance of the Endo-PAT in the detection of coronary endothelial dysfunction. An EndoScore cut-off value of 1.67 provides a sensitivity of 82\% and a specificity of 77\% to diagnosing coronary endothelial function.

B. Endo-PAT measures a Nitric-Oxide (NO) mediated response

Nohria and Gerhard et al., at the Brigham & Women’s Hospital, Boston, demonstrated the central role for nitric oxide in the post-occlusion vasodilatory response measured by Endo-PAT\(^4\). The EndoScore was measured in a group of nineteen healthy volunteers, before and after intra-arterial infusion of L-NAME (a specific inhibitor of endothelial Nitric Oxide Synthase). Fifteen matched controls were infused with Saline or PhenylEphrine (an endothelium independent vasoconstrictor). The study showed that L-NAME blocked 46\% of the vasodilatory response (p=0.002). These results provide direct confirmation that Endo-PAT indeed measures a NO-mediated endothelial response.

C. Correlation between Endo-PAT and Brachial Artery Ultrasound (BAUS)

BAUS is a common research method for peripheral, noninvasive assessment of endothelial function. It differs from Endo-PAT in several ways. While the BAUS assesses a single conduit vessel, Endo-PAT measures several vascular beds, composed of small vessels and micro-circulation. Furthermore, Endo-PAT corrects for systemic changes by a simultaneous measurement from the (un-occluded) contra-lateral arm. With minimal training necessary, Endo-PAT is practically operator independent, while BAUS requires a trained ultrasound technician and is highly user-dependent in both data acquisition and analysis. Furthermore, the response measured with Endo-PAT has a much larger dynamic range (hundreds of \%) than the miniscule changes assessed by BAUS (around 10\% for a normal response).

Several studies have simultaneously measured Flow-Mediated Dilatation (FMD) with Endo-PAT and BAUS. Kuvin et al\(^5\). at the Tufts Medical Center, Boston, demonstrated a significant correlation between the two methods (r=0.55, p=0.0001) in a group of 89 adult patients suffering from chest pain. In another study by Kuvin et al\(^6\). 60 patients (32 with Coronary Artery Disease (CAD) and 28 without CAD) were studied simultaneously with both Endo-PAT and a portable ultrasound. A significant relationship was reported between FMD and the EndoScore in both the CAD and non-CAD populations (r=0.3; p<0.05, for both).
A correlation was also reported by Dhindsa et al.\textsuperscript{7} who found that the EndoScore was significantly and positively associated with BAUS ($r=0.47$, $p<0.01$) in 40 apparently healthy adults. Gurru et al\textsuperscript{8}, studied 246 individuals (3 groups: no vascular disease, Inflammatory Bowel Disease and CAD). BAUS and Endo-PAT were not correlated; however, Endo-PAT was significantly lower in the CAD group while the BAUS did not differentiate between the patient groups. These results are summarized in Table 1.

<table>
<thead>
<tr>
<th>Group (ref)</th>
<th>N</th>
<th>Population</th>
<th>$r$</th>
<th>$p$</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kuvin et al.\textsuperscript{5}</td>
<td>89</td>
<td>Chest pain</td>
<td>0.55</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Kuvin et al.\textsuperscript{6}</td>
<td>60</td>
<td>CAD(+) and CAD(-)</td>
<td>0.3</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Dhindsa et al.\textsuperscript{7}</td>
<td>40</td>
<td>Apparently healthy</td>
<td>0.47</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Gurru et al.\textsuperscript{8}</td>
<td>246</td>
<td>Apparently healthy, IBD and CAD(+)</td>
<td>--</td>
<td></td>
<td>Only EndoScore is significantly lower in CAD group</td>
</tr>
<tr>
<td>Erbs et al.\textsuperscript{9}</td>
<td>15</td>
<td>Obese adolescents</td>
<td>0.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### D. Endo-PAT reproducibility

Several studies demonstrated good reproducibility of Endo-PAT. These results are in the upper range or even above the published reproducibility of BAUS assessment of FMD, when operated by a qualified BAUS sonographer. Table 2 provides a summary of the key findings.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Cohort</th>
<th>Time interval</th>
<th>Statistical Parameter</th>
<th>Result</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reisner et al.\textsuperscript{10}</td>
<td>113</td>
<td>Adult volunteers</td>
<td>24 hours</td>
<td>ICC*</td>
<td>0.56</td>
<td>Classification of normal vs. dysfunction maintained in 75% of males and 70% of females between days ($p&lt;0.01$)</td>
</tr>
<tr>
<td>Selamet Tierney et al.\textsuperscript{11}</td>
<td>30</td>
<td>Young adult volunteers</td>
<td>1 to 7 days</td>
<td>ICC*</td>
<td>0.78</td>
<td>($p&lt;0.001$)</td>
</tr>
<tr>
<td>Tomfohr et al.\textsuperscript{12}</td>
<td>12</td>
<td>Young adult volunteers</td>
<td>1 to 7 days</td>
<td>ICC*</td>
<td>0.73</td>
<td>($p&lt;0.001$)</td>
</tr>
<tr>
<td>JT Kuvin – Tufts Medical Center</td>
<td>47</td>
<td>Adults with chest pain</td>
<td>24 hours</td>
<td>ICC*</td>
<td>0.59</td>
<td>Part of FDA submission - unpublished data</td>
</tr>
<tr>
<td>Haller et al.\textsuperscript{13}</td>
<td>44</td>
<td>Type 1 Diabetes adolescents</td>
<td>4 weeks</td>
<td>Coefficient of variation</td>
<td>14.8%</td>
<td></td>
</tr>
</tbody>
</table>

* ICC - Intra-Class Correlation

### E. EndoScore as a predictor of Cardiovascular (CV) outcome

Rubinshtein et al.\textsuperscript{14} assessed the incremental value of the EndoScore to the Framingham Risk Score (FRS) in a cohort of 270 outpatients. Major Adverse Cardiovascular Events (MACE) that
are cardiac death, myocardial infarction, revascularization or cardiac hospitalization, were recorded over an average follow-up period of 5.8 years. The rate of MACE in patients who tested positive for endothelial dysfunction was 39% vs. normal endothelial function 25% (p=0.024). The study showed that patients at low FRS risk but with Endothelial Dysfunction were at a higher actual risk of future CV events than patients with high FRS but normal Endothelial Function. Furthermore, Endothelial Dysfunction was found to be an independent risk factor for future MACE on multivariate analysis (p=0.002) (see figure 2).

**F. Correlation of EndoScore to traditional CV risk factors**

Since 2003 the Framingham Heart Study has included endothelial function measurements with Endo-PAT. All three study cohorts (the original study population, the Offspring and the 3rd generation cohort) have been tested with Endo-PAT, totaling over 5,000 subjects. A cross-sectional analysis of 1,957 3rd Generation subjects was published in Circulation (May 2008) by Hamburg et al.\(^1\). The study demonstrated a significant inverse relation between EndoScore and multiple CV risk factors, including: male sex, body mass index, total/HDL cholesterol, diabetes, smoking and lipid-lowering treatment.

A publication from the KORA/Monica cohort\(^6\) reported a significant inverse correlation of the EndoScore with age, BMI, waist circumference, systolic and diastolic blood pressures, Total/HDL Cholesterol ratio, Triglycerides and fasting and 2 hour glucose. HDL Cholesterol was positively correlated to the EndoScore.

Bonetti et al\(^2\) reported significant relationships between EndoScore index and obesity and HDL levels. Kuvin et al. found that EndoScore inversely correlated with the number of cardiovascular risk factors\(^3\). In another study by Kuvin et al. an inverse correlation was shown between EndoScore and the number of cardiovascular risk factors ($r =0.3$, $P<0.002$)\(^4\). EndoScore was lower in patients with hypertension, hyperlipidemia, tobacco use, and a family history of CAD.
G. Endo-PAT: separation of clinically distinct populations in case/control studies

The discriminative ability of Endo-PAT between degrees of known CVD risk has been evaluated according to the number of cardiovascular risk factors, the results of myocardial perfusion imaging, or by assessing CAD patients vs. apparently healthy controls.

Subjects, divided into 4 groups:
1. 12 healthy volunteers
2. 39 patients with chest pain and normal coronary endothelial function
3. 55 patients with chest pain and coronary endothelial dysfunction
4. 12 patients with advanced CAD

This study demonstrated that EndoScore is similarly and significantly attenuated in patients with early and advanced CAD (groups 3 and 4 above) compared with healthy individuals or subjects with a healthy coronary endothelium (groups 1 and 2 above; see figure 3). A significant separation between CAD patients and controls was also shown by Kuvin et al. who observed a significantly lower EndoScore in CAD(+) subjects compared to CAD(-) (p<0.05).

In another study by Kuvin et al. the EndoScore was assessed in 68 patients with chest pain, who performed exercise Myocardial Perfusion Imaging (SPECT Sestamibi). The index was significantly lower in those with positive exercise myocardial perfusion, indicative of ischemic heart disease.

Robertsson et al. studied 133 patients referred for myocardial perfusion imaging (MPI). EndoScore was significantly lower in the group with perfusion defects than in the group without perfusion defects (p=0.003). Furthermore, EndoScore was significantly lower in the group with reversible perfusion defects than in the group without reversible defects (p=0.01). In a multivariate analysis model, adjusting for age, gender, BMI and diastolic blood pressure, the EndoScore was the only independent predictor of reversible perfusion defects (p<0.05).

Endothelial dysfunction is believed to be a pan-systemic disease associated with numerous disease states. The EndoScore was shown to separate cases from controls in various disease populations including: Type 1 and 2 diabetes, and glucose intolerance, Poly Cystic Ovary Syndrome, Pre-Eclamptic Toxemia, Inflammatory Bowel Disease, Systemic Lupus Erythematosus, mood disorders, Pulmonary HTN, and Obstructive Sleep Apnea.

H. Endo-PAT: Response to treatment

Endothelial Dysfunction has been shown to respond well to treatment. Broadly, treatment options fall into 3 main categories:
1. Lifestyle modification (including dietary changes, exercise etc)
2. Drugs - through pleiotropic effects, (e.g. Statins), or directly, (e.g., Tetra-Hydro Biopterin, L-Arginine)
3. Treatment of co-morbidities (e.g., glycemic control for diabetics)

Several Endo-PAT studies have demonstrated improvement in endothelial function as a result of a variety of clinical interventions. These are collated in Table 3.
Table 3: Endo-PAT studies demonstrating improvement in endothelial function

<table>
<thead>
<tr>
<th>Category</th>
<th>Intervention</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lifestyle modification</td>
<td>Smoking cessation</td>
<td>Komatsu et al.32</td>
</tr>
<tr>
<td>Diet changes</td>
<td>Flavonoids</td>
<td>Schroeter et al.33, Fisher et al.34, Barringer et al.35, Fisher et al.36, Hollenberg et al.37</td>
</tr>
<tr>
<td></td>
<td>Omega 3</td>
<td>Dangardt et al.38</td>
</tr>
<tr>
<td></td>
<td>Low carb/fat diet</td>
<td>Davis et al.39</td>
</tr>
<tr>
<td></td>
<td>Conjugated Linoleic Acid</td>
<td>Fielitz et al.40</td>
</tr>
<tr>
<td>Devices for co-morbidity</td>
<td>EECP</td>
<td>Bonetti et al.41</td>
</tr>
<tr>
<td></td>
<td>Oral Appliances</td>
<td>Ithzhaki et al.42, Pillar43</td>
</tr>
<tr>
<td></td>
<td>CPAP</td>
<td>Ithzhaki et al.44, Morgenthaler et al.45</td>
</tr>
<tr>
<td>Drugs</td>
<td>PDE5-I</td>
<td>Prince et al.47, Aversa et al.48</td>
</tr>
<tr>
<td></td>
<td>BH4</td>
<td>Hsu et al.46</td>
</tr>
<tr>
<td></td>
<td>l-Arginine</td>
<td>Yeo et al.49,50</td>
</tr>
<tr>
<td></td>
<td>Eplerenone</td>
<td>Thum et al.51</td>
</tr>
<tr>
<td></td>
<td>Clopidogrel</td>
<td>Luu et al.52</td>
</tr>
</tbody>
</table>

In addition to the aforementioned studies, Endo-PAT has been employed in numerous clinical and basic science protocols (several in press or in preparation). The inherent ease of use, short learning curve, scientific validation, standardization and objectivity make Endo-PAT2000 an excellent method for both the office-based clinical setting and for large-scale diagnostics. Some prominent large scale studies which use Endo-PAT are:

- **Gutenberg Heart Study (formerly PREVENT-IT)** – Johannes Gutenberg University, in Mainz, Germany, is a highly advanced epidemiological study of cardiovascular risk factors in a non selected adult population of over 17,000 participants. The aim of the study is the development of a score for cardiovascular risk stratification, taking into account subclinical disease, protein patterns and genetic variability.

- **Gene Bank at Emory University**, Atlanta, GA, aims to establish a large database of cardiac catheterization and heart failure patients from the Emory Health System. Each individual undergoes advanced clinical tests and genetic analysis. From this cohort, 5,000 patients will be tested with Endo-PAT.

- **META-Health at Emory & Morehouse Universities**, Atlanta, GA. The goal of the study is to assess ethnic differences between African-Americans and whites in obesity-related cardiovascular disease and discovering new intervention strategies. The aim is to recruit a cohort of 1,000 individuals between the ages 30-65 years.

- **University of Pittsburgh Medical Center (UPMC)** – “The Role of Arterial Endothelial Dysfunction in Racial Disparities of Cardiovascular Disease”, part of Heart SCORE (Heart Strategies Concentrating On Risk Evaluation) study. A community based, outcome study, with a cohort of 2,000 subjects, half Caucasians and half African-Americans.

- **Jackson Heart Study** is a large scale, epidemiological study located in the Delta of the Mississippi where cardiovascular mortality is the highest in the US. Endo-PAT has been incorporated in the study since 2007.

- **KORA - Cooperative Health Research, Augsburg, Germany**, formerly WHO MONICA study, is a regional research platform for population-based surveys and subsequent follow-up studies. Endo-PAT is used in a subset of over 1,000 patients.
References


19. Mahmud FH, Earing MG, Lee RA, Lteif AN, Driscoll DJ, Lerman A. Altered Endothelial Function in Asymptomatic Male Adolescents with Type 1 Diabetes. Congenital Heart Disease 2006; 1:98-103


43. Pillar G. Oral appliance improves Sleep Apnea and Endothelial Function. OTO 2007

44. Itzhaki S, Pillar G, Lavie P, Lavie L. Endothelial function of patients with Obstructive Sleep Apnea improves following 3 months on CPAP. SLEEP 2006; (Suppl.):A164

45. Morgenthaler T, Leerman A. Endothelial Dysfunction Assessed By Peripheral Arterial Tonometry In Obstructive Sleep Apnea Patients Improves With CPAP Therapy. SLEEP 2005; 28 (Suppl.):A177


51. Thun T, Schmitter K, Fracaroliro D, Jakob M, Werthmann R, Bunemann M, Ertl G, Bauersachs J. Deleterious effects of aldosterone on human endothelial progenitor cells are protein kinase A-mediated and can be prevented by mineralocorticoid receptor blockade. EHJ 2007; 28 (Suppl.):478

52. - Luu L, Willoughby SR, Cameron JD, Nelson AJ, Worthley SG, Worthley MI. One Week of Clopidogrel Improves Endothelial Function in Subjects with Stable Coronary Artery Disease: A Randomized Control Study. ACC 2009