A Comparison of Flow-mediated Dilatation and Peripheral Artery Tonometry for Measurement of Endothelial Function in Healthy Individuals and Patients with Peripheral Arterial Disease

R.B. Allan a, C.L. Delaney a, M.D. Miller b, J.I. Spark a,*

a Department of Vascular Surgery, Flinders Medical Centre and Flinders University, Bedford Park, South Australia, Australia
b Department of Nutrition and Dietetics, Flinders University, Bedford Park, South Australia, Australia

WHAT THIS PAPER ADDS
Flow-mediated dilatation (FMD) and peripheral artery tonometry (PAT) are both methods for assessing vascular function that utilise the reactive hyperaemic response to transient occlusion. FMD measures predominantly nitric oxide mediated dilatation whereas PAT measures a more complex range of mechanisms. In this study there was a lack of correlation between the two methods and in addition there was no change in PAT for the subjects with probable endothelial dysfunction. This suggests that PAT is not a sensitive method for testing nitric-oxide dependent endothelial function and supports the concept that the two methods are measuring different mechanisms. These findings highlight the importance of choosing an assessment method appropriate for the function under investigation.

Objective: Flow-mediated dilatation (FMD) and peripheral artery tonometry (PAT) are commonly used methods for assessing endothelial function in a research setting but it is unclear how well they correlate. This study aimed to compare and correlate these methods in patients with peripheral arterial disease (PAD) and in healthy individuals.

Materials and methods: FMD and PAT measurements were obtained as samples of convenience from 26 patients with PAD and 25 healthy subjects. FMD was defined as the percentage increase in the brachial artery diameter after distal occlusion and PAT was measured using the reactive hyperaemia index (RHI).

Results: Patients with PAD had a significantly lower FMD than healthy subjects (2.43% vs. 5.80%, p < 0.001). No difference was found in RHI between the two groups. No correlation was found between the FMD and RHI in subjects with PAD (r = 0.284, p = 0.160), in healthy subjects (r = 0.153, p = 0.464) or when both groups were combined (r = 0.174, p = 0.22).

Conclusion: The lack of change in RHI in PAD patients suggests that PAT is not a sensitive measure of endothelial function. The lack of correlation suggests that FMD and PAT are not interchangeable. PAT should not be used as a substitute for FMD as a measure of endothelial function.

Crown Copyright © 2012 Published by Elsevier Ltd on behalf of European Society for Vascular Surgery. All rights reserved

Article history: Received 2 October 2012, Accepted 5 December 2012, Available online 12 January 2013

Keywords: Flow mediated dilatation, Peripheral artery tonometry, Endothelial function, Peripheral arterial disease

INTRODUCTION
The vascular endothelium provides an active biological interface between the blood and all other tissues and has a primary role in maintenance of the vascular environment through the release of agents that regulate vasomotor function, inflammatory responses and homeostasis in response to mechanical and hormonal stimuli.1,2 Nitric oxide (NO) is the most important of these agents and, as well as being a powerful vasodilator, inhibits inflammatory activity, vascular smooth muscle cell proliferation and platelet adhesion and aggregation.3 These properties result in NO having important anti-atherogenic functions.4 The endothelium is of great interest in the study and treatment of peripheral arterial disease (PAD) due to the central role that alterations in these functions play in the development of arterial disease.

Endothelial dysfunction refers to a loss of these functions with a switching of the endothelial cells to an
activated phenotype with a reduction in NO production. This results in a loss of vasodilatory ability and the promotion of thrombosis, inflammation and cellular proliferation. Endothelial dysfunction has been established as an important early event in the pathogenesis of atherosclerosis with changes in endothelial function occurring well before the onset of clinically apparent cardiovascular disease. Endothelial dysfunction has been shown to predict cardiovascular events and to correlate with known cardiovascular risk factors.

Many non-invasive methods for measuring endothelial function have been investigated with two of the most commonly used being flow mediated dilatation (FMD) and peripheral artery tonometry (PAT).

FMD is the most established and commonly used non-invasive method for assessing endothelial function. The FMD technique is based on the reactive hyperaemia phenomenon of increased arterial blood flow following a period of transient arterial occlusion. This increase in blood flow results in an increase in shear stress on the vessel wall and this induces the release of nitric oxide (NO) by endothelial cells, causing vasodilatation. This response is termed NO-mediated flow mediated dilatation and is typically measured in the brachial artery. It has become apparent that the position of the occlusive cuff has a significant effect on FMD. Proximal, upper arm, occlusion causes a greater dilatation but this is not solely a NO-mediated response. Distal, forearm, occlusion causes a lesser, but more specifically NO-mediated, dilatation and has recently become the recommended FMD method.

PAT is a more recently developed method that has been used to measure endothelial function. PAT uses pneumatic finger probes to measure digital arterial pulse wave amplitude when reactive hyperaemia is induced. Ease of use, standardised methodology and the availability of validated cut-off thresholds to predict future cardiovascular events have encouraged the use of PAT in studying endothelial function.

The degree to which the two tests correlate with one another is not clear and the use of different occlusion methods for FMD has confused the available evidence, with most studies investigating correlation using proximal occlusion FMD and fewer studies using the currently recommended technique of distal occlusion.

Comparison studies of PAT and distal occlusion FMD have investigated normal individuals or a community based population but no studies comparing PAT and FMD have been reported in a population with PAD.

Due to the importance of endothelial dysfunction in the development of PAD this study was performed to 1) identify whether a detectable difference exists in subjects with established PAD and in healthy individuals with respect to distal occlusion FMD and PAT and 2) assess whether there was a correlation between the two methods.

### MATERIALS AND METHODS

#### Participant selection

Data was collected from participants enrolled in two studies in which FMD and PAT were performed as tests of endothelial function as part of the investigation of specific interventions.

The first study was investigating the effects on endothelial function of moderate dose fish oil on healthy participants with a parent with PAD. The second study was investigating the effects of two different exercise regimes on participants with claudication. FMD and PAT tests were performed at baseline, at 6 weeks (for the fish oil study only) and at the 12 week conclusion of the studies. Inclusion and exclusion criteria for each study are presented in Table 1.

This analysis was limited to the baseline tests because of the non-independent nature of the follow-up measurements and the risk of confounding variables due to potential differing effects of the interventions on each test. The outcomes of the interventions are not reported as the studies are on-going and beyond the scope of this study.

Due to the operator dependent nature of FMD, test-retest reproducibility testing of pre-occlusion brachial artery diameter was performed. Brachial artery diameters were acquired from a group of healthy volunteers (n = 10) on two separate occasions, 48 h apart.

This study was approved by the Southern Adelaide Clinical Human Research Ethics Committee. All participants provided written informed consent for the measurement of FMD and PAT prior to commencement of data collection.

#### Peripheral arterial tonometry

Participants were instructed to fast for eight hours, refrain from caffeine, alcohol and tobacco and avoid exercise for eight hours prior to testing. PAT tests were performed using an EndoPAT device (Itamar Medical Ltd, Caesarea, Israel), following the manufacturer’s guidelines, in a quiet, dimmed and temperature controlled room. The occlusion cuff was placed above the elbow on the left arm and the finger tip plethysmography probes were placed on the index fingers of each hand. The pulse wave amplitude was continuously recorded by the device for the duration of the test. The test commenced with a 5 min baseline period, followed by 5 min with the cuff inflated to 250 mmHg to achieve total brachial artery occlusion and then a final 5 min period of reactive hyperaemia following the cuff release.

The 15 min recordings were then analysed using the device’s proprietary software and the reactive hyperaemia index (RHI) was calculated by the device using the method previously described by McCrea.

#### Brachial artery ultrasound flow-mediated dilatation

FMD was performed and measured using techniques consistent with published guidelines. FMD testing was performed 15 min after the completion of the PAT test as this time interval between arm occlusion episodes has been...
shown to have no effect on FMD results. All tests were performed with a SonoSite M-Turbo ultrasound system (SonoSite, Inc, Bothel, USA) with a 6–13 MHz broadband linear array transducer. All tests were performed by two operators experienced in the technique (RA and CD). With the participant supine, the right arm was placed in a supporting cradle, a blood pressure cuff placed around the forearm and the ultrasound transducer was placed on the arm proximal to the elbow with the aid of a sterotactic stand. A 3 lead ECG was connected to the ultrasound machine to enable display of the cardiac cycle.

A 30 s baseline period of scanning of the brachial artery was recorded prior to cuff inflation. The cuff was then inflated to 250 mmHg for 5 min to achieve total brachial artery occlusion. Recording recommenced at 15 s post occlusion and continued for three minutes.

The pre and post occlusion cine clips were then transferred to a computer for measurement by automated edge detection software (Brachial Artery Analyser, MIA-LLC, Coralville, USA). All measurements were obtained during diastole. The point of maximum dilatation was identified and the maximum diameter was obtained by averaging 5 images from consecutive cardiac cycles. The FMD was calculated using the equation:

\[
\text{FMD} = \frac{\text{[peak diameter} - \text{baseline diameter]}}{\text{baseline diameter}} \times 100
\]

**Statistical analyses**

Data were analysed using the SPSS for Windows statistical package version 19 (SPSS Inc, Chicago, IL, USA).

Age, gender, body-mass index (BMI), FMD and RHI were presented for each sample.

Student’s t-test was performed to assess for significant differences in either FMD or RHI between the healthy and PAD samples.

Correlation between FMD and RHI was assessed using Pearson correlation coefficient and illustrated using scatter plots. Correlation between FMD and RHI was tested for each sample and for all participants grouped as one sample. Reproducibility of the pre-occlusion brachial artery diameter measurement was assessed by calculating the intra-class correlation coefficient (ICC) for the first and second measurements in the group of healthy volunteers.

All tests were two-tailed and the level of statistical significance was set at \( p < 0.05 \).

**RESULTS**

The sample populations consisted of 25 healthy subjects in the fish oil intervention study and 26 participants with PAD in the exercise intervention study.

The age, gender and BMI, FMD% and RHI are presented in Table 2 for each group. While BMI was similar between the two groups, age and gender characteristics were significantly different with the PAD population being older and predominantly male, matching the known characteristics of patients with PAD. No subjects were on nitrate-based medications. None of the healthy subjects were on statins while all subjects with PAD were on statins. A box plot (Fig. 1A) of RHI for both groups demonstrated similar distributions and t-testing revealed no difference in RHI between the healthy and PAD groups (\( p = 0.944 \)). The box plot for FMD (Fig. 1B) suggested that the FMD for the PAD group was lower than that found in the healthy subjects and this was confirmed by a significant difference in the t-test (\( p < 0.001 \)).

---

**Table 1.** Inclusion and exclusion criteria for participants in the intervention studies used as sample groups.

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
<th>Fish oil intervention on healthy participants</th>
<th>Exercise intervention on patients with PAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent with PAD</td>
<td>Radiographic evidence of infra-inguinal PAD with history consistent with intermittent claudication</td>
<td></td>
</tr>
</tbody>
</table>

| Exclusion criteria | Clinical history of coronary, cerebral or peripheral arterial disease or ankle-brachial pressure index <0.9 |
|--------------------| Lower limb ischaemic rest pain or tissue loss |
|                    | Vasoactive medication known to impact on endothelial function |
|                    | Seafood allergy |
|                    | Taking therapeutic doses of fish oil |
|                    | Inability to communicate effectively to give consent |
|                    | Treatment doses of anti-thrombotic agents |
|                    | Dual anti-platelet therapy |
|                    | Diagnosis of a bleeding disorder |

<table>
<thead>
<tr>
<th>Diagnosis of a bleeding disorder</th>
</tr>
</thead>
</table>

---

| Age, gender, body-mass index (BMI), FMD and RHI were presented for each sample. |

**Table 2.** Characteristics of the sample groups.

<table>
<thead>
<tr>
<th></th>
<th>Healthy subjects (( n = 25 ))</th>
<th>Subjects with peripheral arterial disease (( n = 26 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (range) years</td>
<td>44.96 (17–67)</td>
<td>72.61 (58–90)</td>
</tr>
<tr>
<td>Gender: male:female</td>
<td>11:14</td>
<td>19.7</td>
</tr>
<tr>
<td>Mean BMI (range)</td>
<td>29.2 (21.5–40.2)</td>
<td>29.4 (23.1–43.6)</td>
</tr>
<tr>
<td>FMD% (SD)</td>
<td>5.80 (3.57)</td>
<td>2.43 (2.47)</td>
</tr>
<tr>
<td>RHI (SD)</td>
<td>1.81 (0.55)</td>
<td>1.83 (0.83)</td>
</tr>
</tbody>
</table>

BMI: body mass index, FMD: flow-mediated dilatation, RHI: reactive hyperaemia index, SD: standard deviation.
Correlation of FMD and RHI

Scatterplots of FMD and RHI for both the healthy group and the PAD group (Fig. 2A and B) suggested no evidence of correlation and this was confirmed by the Pearson correlation coefficients of $r = 0.153 \ (p = 0.464)$ for the healthy group and $r = 0.284 \ (p = 0.160)$ for the PAD group. A scatterplot of FMD and RHI for a sample combining both groups also suggested no evidence of correlation (Fig. 2C), with a correlation coefficient of 0.174 ($p = 0.22$) confirming no significant correlation between the FMD and RHI.

Reproducibility testing

The mean pre-occlusion diameter of the brachial artery was 3.885 mm for the first measurement and 3.931 mm for the second, giving a mean difference of 1.18%. The ICC of 0.989 ($p < 0.001$) indicated excellent agreement between first and second measurements.

DISCUSSION

This is the first study to compare the performance of FMD and PAT in healthy individuals and subjects with established PAD and the first to investigate whether FMD and PAT correlate in subjects with PAD. In the present study no correlation was found between FMD and PAT in either the
healthy or PAD groups, or when the two groups were combined into one sample. The evidence base regarding correlation of FMD and PAT is small and contradictory. Of the nine studies identified that reported the results of correlation testing between FMD and PAT, six reported weak to moderate ($r = 0.17 \pm 0.57$) but significant correlation while three reported no significant correlation. Technical and sample group differences need to be considered when interpreting the results of the present study to those of the available literature.

The position of the occlusion cuff is an important technical difference that has been shown to have a marked effect on the percentage dilatation obtained with FMD. Previous studies use either occlusion proximal to the transducer in the upper arm or distal occlusion in the forearm. Occlusion proximal to the transducer has been shown to cause a greater degree of vessel dilatation with FMD. This has been attributed to non-NO-mediated factors contributing to the reactive hyperaemia response with ischaemia induced vasodilatory metabolites and a loss of myogenic tone being proposed as likely mechanisms. This phenomenon has led to recommendations that FMD should be performed with occlusion at the forearm to ensure that the measured response is primarily NO-mediated in origin. This also has the effect of reducing the magnitude of the dilatation observed with FMD, an important factor when comparing results from different studies. By its nature PAT requires a proximal occlusion and this may be one of the reasons why non-NO-mediated mechanisms appear to contribute the hyperaemic response measured by PAT. It would be reasonable to expect that proximal occlusion FMD might correlate better with PAT than distal FMD. The present study, and previous studies of correlation, would tend to support this conclusion with five of the six studies that used proximal occlusion FMD demonstrating a correlation with PAT while three of four studies (if the present study is included) that used distal forearm occlusion finding no evidence of correlation. Although recent guidelines have recommended that the distal occlusion technique should be used to measure NO-mediated endothelial function, the FMD literature remains a mixture of both techniques, necessitating care when reviewing the conclusions of studies involving FMD.

In addition to variation resulting from the method of occlusion, there are also differences in the physiology of the vascular beds being tested that need to be considered. It has been shown that there are differences in the response of resistive and conduit vessels to reactive hyperaemia. PAT measures the response to reactive hyperaemia in the digits, a vasculature bed which is a combination of macro and micro-circulation with a significant role played by arteriovenous anastomoses which are regulated primarily by the sympathetic nervous system. NO makes only a partial contribution to reactive hyperaemia with PAT suggesting that the sympathetic nervous system plays a significant role in this response. In addition skin reactive hyperaemia has been shown to be independent of NO mediation. FMD measures the hyperaemic response in a large conduit vessel with stimulus for dilatation being the increased shear stress observed on release of the occlusion cuff. This dilatation, in contrast to PAT, has been shown to be predominantly NO-mediated.

Because endothelial dysfunction is a precursor to the development of atherosclerosis, it would be expected that measures of endothelial function would be reduced in subjects with PAD, and this is supported by observational evidence. Endothelial function has also been demonstrated to reduce with age. The results of FMD testing in the present study support this by demonstrating significantly reduced FMD in subjects with PAD, who were also older, compared with healthy individuals. The surprising result from this study is that there is no difference in RHI between healthy subjects and those with PAD. This has not been previously reported and supports the theory that PAT measures a more complex range of factors, with NO-mediated effects being less predominant. It may be that there is preservation of sympathetic and myogenic responses to ischaemia in patients with PAD and that PAT may be less sensitive as a measure of NO-mediated endothelial function.

Operator dependent measurement variability is unlikely to be a factor in these findings. Reproducibility testing demonstrated very good repeatability for the measurement of the brachial artery and the EndoPAT machine uses an automated software algorithm that calculates the RHI, removing operator variables. The study methodology controlled for subject preparation and timing, ensuring that FMD and PAT were performed under the same conditions.

A further source of variability could be vasoactive medications. None of these patients were taking nitrate based pharmacotherapy, while all the subjects with PAD, but none of the healthy subjects, were on statins, which are known to improve endothelial function. FMD and PAT results may have been improved by statin therapy but the results for the PAD group were still significantly lower than that found in the healthy group.

A limitation of the present study is that FMD and RHI results from subjects with PAD were compared to a group of healthy individuals that were not age and gender matched. Age has been shown to have a significant negative correlation with endothelial function and it is likely that some of the reduction in FMD found in the PAD sample may be due to the effects of age. This limitation does not do alter the significance of the lack of difference in RHI between the healthy and PAD groups. The subjects with PAD clearly have reduced endothelial function and the inability of PAT to identify this is important regardless of the specific cause of the endothelial dysfunction.

Another potential limitation may be that the subjects were obtained from pre-existing interventional studies rather than being specifically recruited for this study, however the specifics of these interventions would have had no effect on the analysis of the FMD and PAT results as only the baseline measurements were used. Despite extensive study, endothelial function testing remains a research technology. It has been shown to be a valid
tool for research at a group level but the high variability reported at an individual level\textsuperscript{37,38} has limited the translation of these methods to clinical practice.

This study showed no correlation between FMD and PAT, in both healthy subjects and those with peripheral arterial disease. This is most likely due to differences between the methods in cuff position relative to measurement site and circulatory physiology of the vascular beds. The lack of standardisation of cuff position in FMD is an important consideration when reviewing the literature of endothelial function assessment.

Furthermore, the lack of change in hyperaemic response measured by PAT in older subjects with PAD suggests that it is not a sensitive method for measuring NO-mediated endothelial function.

Whilst PAT, due to its ease of use and simplicity, is an attractive option as a research tool, caution is required when considering it as a substitute for FMD as a measure of endothelial function, particular if NO-mediated endothelial function is the phenomena under investigation.

ACKNOWLEDGEMENTS
None.

CONFLICT OF INTEREST
None.

FUNDING
None.

REFERENCES
1 Vita JA, Hamburg NM. Does endothelial dysfunction contribute to the clinical status of patients with peripheral arterial disease? Can J Cardiol 2010;26:45A–50A.


38 Anderson TJ. Arterial stiffness or endothelial dysfunction as a surrogate marker of vascular risk. Can J Cardiol 2006;22(Supplement B):72B–80B.