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# Validation of a new method for non-invasive assessment of vasomotor function

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# Validation of a new method for non-invasive assessment of vasomotor function

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## **Abstract**

**Background:** Reactive hyperaemia induces a slowing of pulse wave velocity (PWV) in conduit arteries of healthy subjects (flow-mediated slowing [FMS]). This could be an alternative method for assessing peripheral vasomotor function to the gold standard method of flow-mediated dilatation (FMD) a more expensive and technically demanding technique. We aimed to assess the reproducibility of FMS in healthy participants and to test its ability to detect differences in vasomotor function in patients with familial hypercholesterolaemia (FH) and post lipoprotein apheresis (LA) treatment.

**Methods:** 25 healthy participants were studied on two occasions to assess reproducibility of FMS. In a case control study of 22 patients with FH and matched healthy controls FMD and FMS were compared. An intervention study in 12 patients with FH looked at the impact of a single LA treatment on FMS assessed pre and post treatment.

**Results:** FMS demonstrated good reproducibility (coefficient of variation [CoV] 7.3%). Patients with FH had reduced FMS in comparison to matched healthy controls (C) (FMS<sub>%</sub> FH  $-15.13 \pm 5.04\%$  vs C  $-18.41 \pm 5.15\%$   $p=0.023$ ), with no difference in FMD<sub>%</sub> between the two groups. A single LA treatment significantly improved FMS (pre  $-18.81 \pm 9.84$  vs post  $-24.09 \pm 7.61\%$   $p=0.016$ ).

**Conclusions:** FMS is a reproducible technique, which is able to detect differences in vasomotor function both in a condition associated with endothelial dysfunction and following an acute intervention known to improve endothelial function. This simple technique has potential for accessible assessment of vasomotor function in clinical studies.

**Word count:** 245

**Keywords:** endothelium, vasodilatation, hyperaemia, pulse wave velocity

## Introduction

The current gold standard method for the non-invasive assessment of endothelial function uses ultrasound to measure flow-mediated dilatation (FMD) mediated by the release of endothelium derived vasodilators such as nitric oxide (NO). This technique, which has good reproducibility within expert vascular laboratories, requires high levels of operator training, is technically demanding and relatively expensive to set up. **It is possible to use FMD in large scale population studies,<sup>1,2</sup>** although, this can be expensive and logistically challenging. Other potentially simpler methods are available but their reproducibility and clinical relationships to endothelial function are less well defined and validated.<sup>3</sup> One new technique based on measuring changes in pulse wave velocity (PWV) in the brachio-radial arterial tract following a standard reactive hyperaemic (RH) stimulus was first demonstrated by Naka et al.<sup>4</sup> This method used pressure transducers, attached to non-occlusive cuffs positioned around the brachial and radial arteries, to measure PWV. RH was generated by a 5-minute period of forearm ischaemia via inflation of a cuff located at the wrist to 250 mmHg. The resultant reduction in vascular tone led to a flow-mediated slowing in PWV which could be measured, and the change from baseline calculated.

Development of the software and equipment for this system, now consisting of a small box and a laptop computer has made it highly portable. Thus, it potentially lends itself to use in the community and/or in larger studies where it is more challenging to assess FMD, whilst also favouring use by non-specialist investigators. However, applicability of a new method depends on its reliability, its ability to characterize the impact of disease and its progression on the vasculature and to evaluate the effect of interventions. We therefore aimed to assess the reproducibility of this technique and its ability to detect differences in vasomotor function in patients with familial hypercholesterolaemia (FH) and following an acute intervention (lipoprotein apheresis [LA]).

## **Methods**

All participants were treated in accordance with the Declaration of Helsinki and provided informed consent for the study. The South East Wales Research Ethics Committee Panel B (ref: 09WSE0238) approved the study.

*Study 1* Reproducibility: 25 healthy adults aged 21 to 37 years were studied to assess the reproducibility of changes in PWV for flow-mediated slowing (FMS). All subjects were non-smokers and attended having fasted and avoided caffeine for at least four hours. The study was carried out in a temperature-controlled laboratory at the Wales Heart Research Institute. Participants attended on two occasions at the same time of day. FMS and FMD were assessed concurrently

*Study 2* FH versus controls: 22 patients aged 28 to 65 years with FH (defined by the Simon Broome criteria) and 22 age and gender matched controls were studied.<sup>5</sup> All subjects were non-smokers and attended having fasted and avoided caffeine for at least four hours. FH patients receiving vasoactive medications were asked to withhold these on the morning of the study. The study was carried out in a temperature-controlled laboratory at the Wales Heart Research Institute. FMS and FMD were assessed concurrently

*Study 3* Effect of lipoprotein apheresis (LA) in FH on FMS: 12 patients aged 41 to 76 years undergoing LA treatment were assessed to see whether a change in FMS was detectable following a single apheresis session. Patients were non-smokers and attended for LA as clinically scheduled, having fasted and avoided caffeine for at least four hours. Those taking vasoactive medications were asked to withhold these on the day of study. The study took place in a temperature controlled laboratory at the Lipid Unit at University Hospital Llandough. LA treatment was undertaken using three systems according to patient tolerability and clinical requirements, polyacrylate whole blood adsorption (DALI® n = 8), whole blood

dextran sulfate adsorption (n = 1), or plasma dextran sulfate adsorption (n = 3), as previously described.<sup>6</sup>

FMD was not assessed concomitantly in study 3 due to time, equipment and space limitations

#### *Vascular function assessment*

*Flow-mediated slowing.* FMS was assessed using a Vicorder device (Skidmore medical, Bristol, UK).

Participants rested supine on a bed, with their right arm positioned 70-80° to their body. Two Hokanson SC5 cuffs were placed around the upper arm and just above the wrist. A third cuff was positioned around the forearm just below the medial epicondyle and used to occlude the forearm for 5 minutes (see appendix 1). The distance between the two PWV cuffs was measured mid cuff to mid cuff and entered into the Vicorder. Baseline PWV was recorded every 15-20 seconds for 5 minutes by inflating the cuffs to 65mmHg and deflating after several seconds, once a PWV reading was registered. Following a 5-minute cuff occlusion at 250mmHg PWV was recorded for a further 10 minutes. PWV readings from the first five minutes were averaged for baseline PWV. The lowest PWV measured within two and a half minutes of release of the occlusion cuff was taken for calculating the maximum change in PWV. In addition, the PWV at 1 min post hyperaemia was also noted. Change in PWV was calculated as absolute change, (minimum PWV- baseline PWV) or percentage change ([absolute change/ baseline PWV] x100).

*Flow-mediated dilatation.* FMD was assessed using high resolution ultrasound (ALOKA prosound 5500) according to a standard protocol. A detailed description is in appendix 1 but briefly, dilatation of the right brachial artery during RH was assessed following a 5 minute forearm cuff occlusion. FMD was expressed as maximal change in diameter post-RH expressed both as percentage and absolute changes. Changes in



blood flow were also measured and RH was expressed as a percentage change between baseline and peak velocity time integral (VTI).

### *Statistical analysis*

Data are presented as mean  $\pm$  SD. Within method reproducibility is expressed as the percentage of the coefficient of variation [(SD of the paired differences/the overall mean)  $\sqrt{2}$ ]. Data are also presented as Bland-Altman plots. Relationships between FMS and FMD responses were explored using Pearson and Spearman correlations having used the Shapiro-Wilk test to check for normality. Independent samples t-test or the independent samples Mann-Whitney U test were used to assess differences in responses between FH and matched controls. Paired t-tests or related samples Wilcoxon-signed rank test were used to compare responses before and after LA. All tests were two-sided and p-values of  $<0.05$  were viewed as significant. SPSS version 20 was used for the statistical analysis.

## **Results**

### *Study 1 Reproducibility study*

Baseline VTI and reactive hyperaemia were similar on the two visits (BL VTI V1  $0.045 \pm 0.016\text{m}$  & V2  $0.042 \pm 0.016\text{m}$   $p=0.34$ , RH% V1  $715 \pm 187\%$  & V2  $752 \pm 229\%$   $p=0.42$ ).

Baseline PWV was similar between the two visits and highly reproducible (Table 1). Maximal change in PWV following release of the occlusion cuff had a coefficient of variation (CoV) below 10%, whereas, change in PWV measured at 1 minute post cuff release had CoVs closer to 20% whether expressed as percentage or absolute change. Interestingly, the CoV for FMS was smaller than the CoV for FMD assessed concurrently in the same arm.

Bland-Altman plots also show good agreement between the two visits for baseline PWV, minimum PWV, absolute change and percentage change in PWV (Figure 1).

Except for an association between max FMS<sub>ABS</sub> and FMD<sub>ABS</sub> at visit 2 ( $r=-0.45$   $p<0.05$ ) there was no consistent correlation between FMS and FMD assessed concurrently.

### *Study 2 Familial Hypercholesterolaemia vs controls*

Baseline PWV was similar between the two groups (C  $8.2 \pm 0.9$ m/s vs FH  $7.7 \pm 0.8$ m/s  $p=0.12$ ). FMS was greater in the healthy controls than in the FH patients, whether expressed as a percentage or absolute change in PWV post reactive hyperaemia (FMS% C  $-18.4 \pm 5.2\%$  vs FH  $-15.1 \pm 5.0\%$   $p=0.023$  & FMS<sub>ABS</sub> C  $-1.5 \pm 0.5$ m/s vs FH  $-1.2 \pm 0.5$ m/s  $p=0.023$ ) (Figure 2a).

There was no difference between the baseline arterial diameter for the two groups (FH  $3.59 \pm 0.60$ mm vs C  $3.56 \pm 0.61$ mm  $p=0.86$ ). Baseline velocity time integral and reactive hyperaemia were similar between the two groups (baseline VTI FH  $0.045 \pm 0.010$ m vs C  $0.042 \pm 0.015$ m  $p=0.54$  & RH% FH  $796 \pm 159\%$  vs C  $816 \pm 234\%$   $p=0.74$ ). FMD was not significantly different between FH patients and the age and gender matched controls when assessed concurrently with FMS in the same arm (Figure 2b).

### *Study 3 Effect of lipoprotein apheresis on FMS in patients with familial hypercholesterolaemia*

LA significantly reduced total cholesterol (pre  $6.05 \pm 1.57$ mmol/L, post  $2.72 \pm 0.84$ mmol/L  $p<0.001$ ), LDL (pre  $4.08 \pm 1.44$ mmol/L, post  $1.37 \pm 0.65$ mmol/L  $p=0.002$ ), HDL (pre  $1.15 \pm 0.47$ mmol/L, post  $0.94 \pm 0.46$ mmol/L  $p=0.002$ ) and triglycerides (pre  $1.77 \pm 0.57$ mmol/L, post  $0.89 \pm 0.34$ mmol/L  $p<0.001$ ). Baseline PWV did not change following LA (pre  $8.8 \pm 1.2$ m/s vs post  $9.2 \pm 1.2$ m/s,  $p=0.19$ ).

FMS was greater after LA when expressed both as a percentage or absolute change in PWV (FMS<sub>%</sub> pre  $-18.8 \pm 9.8\%$  vs post  $-24.1 \pm 7.6\%$   $p=0.016$  & FMS<sub>ABS</sub> pre  $-1.7 \pm 0.9\text{m/s}$  vs post  $-2.2 \pm 0.9\text{m/s}$   $p=0.015$ ).

## Discussion

In this study we have demonstrated that FMS is a reproducible method for assessing vasomotor function, can detect vasomotor dysfunction in patients with familial hypercholesterolaemia and can also detect an improvement in vascular function following a single lipoprotein apheresis treatment.

The technique of assessing changes in PWV following reactive hyperaemia was initially developed by Naka et al.<sup>4</sup> They found that PWV was decreased after a 5-minute forearm occlusion period. They also demonstrated the influence of NO on PWV by showing that PWV increased concomitantly with a decrease in brachial artery diameter when the nitric oxide synthase inhibitor L-N<sup>G</sup>-monomethyl Arginine citrate (L-NMMA) was infused into the arm, whereas stimulation of NO release by acetylcholine caused a decrease in PWV whilst brachial artery diameter increased. This suggests that this technique is a potential marker of brachial artery endothelial function, in keeping with findings from studies demonstrating reduction of FMD following L-NMMA infusion, despite maintained reactive hyperaemia.<sup>7, 8</sup>

Technical development of the system used for assessing PWV has made it very portable, which together with its ease of use suggests considerable potential for its application beyond the specialist vascular physiology research laboratory setting, where use of the current gold standard method of FMD, although possible, is considerably more challenging. Although the Vicorder method of assessing PWV has been shown to be reproducible, there has been little information on the reproducibility of using this device for assessing dynamic vasomotor function.<sup>9</sup> Before endorsing any new method for wider

application, it is not only important for it to be technically and practically feasible but it is also essential to understand the reproducibility/variability of the method in the investigators' hands. We therefore undertook this study to demonstrate the reproducibility of the method for assessing vasomotor function. Notably, in our preliminary work using the method of Naka et al, we were unable to detect a significant fall in PWV with the RH stimulus provoked by positioning the occlusion cuff at the wrist<sup>4</sup>. We therefore repositioned the occlusion cuff to just below the medial epicondyle to increase the ischaemic territory, thus, provoking a greater hyperaemic stimulus. This produced a readily detectable reduction in PWV post-RH.

FMS was shown to be very reproducible for both maximal percentage and absolute changes in PWV (CoV 7.3% & 8.2% respectively). Measures of maximal change in FMS were more reproducible than readings taken at 1-minute time point post cuff release (CoV 17.2%). The reproducibility of this FMS method was superior to that of FMD acquired simultaneously using ultrasound in our study. Despite a very good coefficient of variation for measurement of baseline diameter (CoV 2.2%), FMD had relatively poor reproducibility when expressed as both absolute and percentage change in diameter (CoV 28.1% & 26.6% respectively). Importantly, this is probably due to concurrent assessment of FMS and FMD in the same arm with resultant movement of the artery and surrounding muscles caused by the inflation and deflation of the cuffs used for assessment of FMS limiting the accuracy of measurement of arterial diameter by ultrasound during hyperaemia. In another study, Donald et al, showed a CoV of 7.1% for FMD which is comparable to our FMS method.<sup>3</sup> In this study FMD had superior reproducibility to other non-invasive methods of assessing endothelial function (changes in pulse wave analysis and pulse contour analysis following inhalation of salbutamol). In our study, FMS also had smaller coefficients of variation than these alternative methods (pulse wave analysis CoV 11.5%) and pulse contour analysis CoV 18.2%), demonstrating its potential for use as a measure of vasomotor function.<sup>3</sup>

Another non-invasive method for assessment of vasomotor function is reactive hyperaemia pulse amplitude tonometry (RH-PAT). This simple technique measures changes in fingertip pulse amplitude following a hyperaemic stimulus, giving a reproducible measure of microvascular function in part mediated by NO.<sup>10, 11</sup> RH-PAT is associated with FMD although heterogeneity between results has been noted.<sup>12, 13</sup> As FMS is a measure of conduit-vessel function we might expect some differences between FMS and RH-PAT which together could provide easily accessible and complementary insights into vascular status.

Previous studies have assessed the reproducibility of the change in PWV following a reactive hyperaemic stimulus and have demonstrated good CoV%.<sup>14, 15</sup> However, these studies have used different methods for assessing PWV (applanation tonometry and mechanotransducers) and have measured PWV from the carotid to the radial arteries.<sup>14, 15</sup> Additionally, there are differences between these studies in the positioning of the occlusion cuff; Kamran et al used an upper arm occlusion cuff placement, whereas Graf et al place the occluding cuff on the forearm, as did we.<sup>14, 15</sup> Therefore, ours is the first study to look at the reproducibility of a cuff-based method for assessing the change in PWV in the brachial artery in response to hyperaemia. The lack of association between FMD and FMS parameters agrees with previous findings by Dhindsa et al and could indicate different influences of mechanical and physiological changes on diameter and stiffness of the vessels walls.<sup>16</sup>

We found that FMS could identify vasomotor dysfunction in a group of FH patients compared with matched controls, consistent with previous work demonstrating endothelial dysfunction in adults and children with dyslipidaemia.<sup>17, 18</sup> That a difference was not seen with FMD could be due to confounding mechanical effects of concurrently assessing PWV during cuff inflation with lower reproducibility of FMD in this setting impairing detection of differences between groups. Alternative reasons could be that

the two techniques assess different pathways, and possibly differential confounding influences of disease processes and/or treatments.<sup>19</sup>

LA has previously been shown to improve endothelial function in both the microvasculature and coronary circulation.<sup>20,21</sup> Our study is the first to demonstrate an improvement in peripheral conduit artery vasomotor function and is consistent with these previous findings. Although Stadler et al found no change in FMD post LA in 6 patients; their study was probably underpowered to detect a modest difference.<sup>22</sup> Due to time and space constraints we were unable to evaluate FMD concurrently in this protocol.

There are some limitations with this study. We did not compare the responses of the two techniques to an independent NO-donor such as glyceryl trinitrate (GTN). However, Naka et al had previously demonstrated that the decrease in PWV in response to GTN administration was similar between patients with coronary heart disease and normal subjects.<sup>4</sup> The main aim of this study was to compare FMD with FMS as a practical measure of vasomotor function in response to hyperaemia, which is more consistently associated with cardiovascular pathophysiology and response to prognostically beneficial therapies than endothelium-independent vasodilator responses to NO donors.<sup>23</sup>

Repositioning the occluding cuff proximally to the lower measurement cuff may influence the mechanisms underpinning the changes in vascular tone, as has been demonstrated in FMD studies using different occlusion cuff positions.<sup>7</sup> This could mean that whilst FMD is predominantly mediated by NO, other factors may contribute to the FMS phenomenon. Assessing FMS following an infusion of L-NMMA could allow determination of the extent to which the observed reduction in PWV in response to hyperaemia is NO-mediated.

A limitation of the FMS technique itself is the lack of assessment of blood flow. However, we know from previous studies that there are rarely significant differences in RH between groups with or

without risk factors for arterial disease, as we found in study 2, or following a short-medium term intervention. However, this might not be the case in those with more advanced diabetes and cardiovascular disease. Therefore, the FMS technique is probably less appropriate for detailed mechanistic evaluation of vascular function, especially in groups with advanced diseases. As a technique, FMS could be useful as a global indicator of vasomotor function suitable for evaluation of large-scale populations and early stage disease cohorts. Its practical ease and low cost, with consequent potential scalability, could more than make up for the lack of RH-data. Smaller or nested studies using FMD or even invasive arterial physiological assessment, within such studies could be included to determine in more detail any influence of hyperaemia and potential underlying mechanistic pathways, should this be of interest.

Finally, the patients in the apheresis study were treated with three different methods for lipoprotein apheresis. Due to the limited number of patients available, it was not possible to investigate here whether these specific methodologies might have a differential impact on vascular function.

In conclusion FMS is a reproducible method for the assessment of vasomotor function and is sensitive enough to detect differences in vascular function in disease and following an intervention. This simple and reliable technique is a potentially cheaper and more accessible means to assess vasomotor function. It should lend itself well to large population and field studies, including use beyond the specialist vascular laboratory. It is not intended as a replacement for FMD or other established methods but to provide clinically relevant and complementary information regarding vascular function.

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## Legends

**Figure 1.** Bland-Altman plots for baseline PWV, minimum PWV post cuff release, absolute change in FMS and percentage change in FMS.

**Figure 2.** Result for FMS (a) and FMD (b) expressed as a percentage change, comparing patients with FH with age and gender matched healthy controls. Results are mean  $\pm$  SEM.

**Table 1.** Summary of FMS and FMD data for visits 1 and 2

	<b>Visit 1</b>	<b>Visit 2</b>	<b>p-value</b>	<b>CoV%</b>
<b>Baseline PWV (m/s)</b>	7.64 ± 1.05	7.53 ± 0.91	0.37	3.3
<b>Max FMS<sub>ABS</sub> (m/s)</b>	-1.60 ± 0.58	-1.60 ± 0.42	0.99	8.2
<b>Max FMS<sub>%</sub> (%)</b>	-20.66 ± 6.02	-21.12 ± 4.32	0.55	7.3
<b>1 min FMS<sub>ABS</sub> (m/s)</b>	-1.19 ± 0.59	-1.27 ± 0.43	0.47	18.4
<b>1 min FMS<sub>%</sub> (%)</b>	-15.22 ± 6.33	-16.95 ± 5.35	0.25	17.2
<b>Baseline Diameter (mm)</b>	3.85 ± 0.72	3.84 ± 0.71	0.71	2.2
<b>FMD<sub>ABS</sub> (mm)</b>	0.16 ± 0.12	0.17 ± 0.11	0.58	28.1
<b>FMD<sub>%</sub> (%)</b>	4.31 ± 3.36	4.55 ± 3.14	0.55	26.6

CoV%, coefficient of variation; PWV, pulse wave velocity; Max, maximum change in PWV post occlusion cuff release FMS<sub>ABS</sub>, flow-mediated slowing expressed as absolute change in pre and post occlusion PWV; FMS<sub>%</sub>, flow-mediated slowing expressed as percentage change; 1 min, change in PWV 1 minute post occlusion cuff release; FMD<sub>ABS</sub>, flow-mediated dilatation expressed as absolute change in pre and post occlusion artery diameter; FMD<sub>%</sub>, flow-mediated dilatation of the brachial artery expressed as a percentage change.