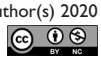
Original Article

VASCULAR MEDICINE

Vascular Medicine
2020, Vol. 25(5) 419–426



DOI: 10.1177/1358863X20926588
journals.sagepub.com/home/vmj


Endothelium function dependence of acute changes in pulse wave velocity and flow-mediated slowing

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Abstract

Flow-mediated slowing (FMS), defined as the minimum pulse wave velocity (PWV_{min}) during reactive hyperemia, is potentially a simple, user-objective test for examining endothelial function. The purpose of the current study was to determine the effects of a known endothelial dysfunction protocol on arm PWV and PWV_{min}. Complete data were successfully collected in 22 out of 23 healthy adults (23.8 years [SD 4.1], 16 F, 22.8 kg/m^2 [SD 2.8]). Local endothelial dysfunction was induced by increasing retrograde shear stress in the upper arm, through inflation of a distal (forearm) tourniquet to 75 mmHg, for 30 min. Pre- and post-endothelial dysfunction, PWV was measured followed by simultaneous assessment of PWV_{min} and flow-mediated dilation (FMD). PWV was measured between the upper arm and wrist using an oscillometric device, and brachial FMD using ultrasound. FMD (%) and PWV_{min} (m/s) were calculated as the maximum increase in diameter and minimum PWV during reactive hyperemia, respectively. Endothelial dysfunction resulted in a large effect size (ES) decrease in FMD ($\Delta = -3.10\%$; 95% CI: –4.15, –2.05; ES = −1.3), and a moderate increase in PWV ($\Delta = 0.38$ m/s; 95% CI: 0.07, 0.69; ES = 0.5) and PWV_{min} ($\Delta = 0.16$ m/s; 95% CI: 0.05, 0.28; ES = 0.6). There was a large intra-individual (pre- vs post-endothelial dysfunction) association between FMD and PWV_{min} ($r = -0.61$; 95% CI: –0.82, –0.24). In conclusion, acute change in PWV and PWV_{min} are at least partially driven by changes in endothelial function.

Keywords

arterial stiffness, endothelial dysfunction, flow-mediated dilation, measurement validity, retrograde shear stress

Introduction

Vascular homeostasis, and therefore cardiovascular disease risk, is partly governed by the vascular endothelium.[1,2] The gold-standard noninvasive approach for assessing endothelial function is the ultrasound-based flow-mediated dilation (FMD) test.[3,4] The FMD test measures an artery's vasodilatory response to increased shear stress, where increased shear stress is induced by reactive hyperemia following 5 min of downstream ischemia.[3,4] While the FMD test is highly valid, it is technically challenging, operator-dependent, and requires high levels of operator training.[3,4] A simpler, user-objective test would be of high value to both epidemiological and physiological investigations. One potential test is the novel flow-mediated slowing (FMS) technique.

FMS may be defined as the minimum pulse wave velocity (PWV_{min}) during reactive hyperemia, with a lower minimum thought to represent greater endothelial function.[5–9] PWV, which is the speed at which the forward pressure wave travels from a proximal to distal arterial segment, is considered the 'gold-standard' measure of arterial stiffness.[10,11] However, the assessment of PWV during reactive hyperemia can also be used to reflect endothelial function.[12–16] According to the Moens–Korteweg equation, PWV is directly proportional to the arterial wall width and Young's modulus, and inversely proportional to blood viscosity and vessel diameter.[17] Vessel diameter increases during FMD, due to endothelium-dependent vasodilation.[3,18] The endothelium-dependent increase in diameter would be expected to result in a reciprocal decline in PWV. However, while FMD and PWV are the most widely reported noninvasive assessments of endothelial function and arterial stiffness, respectively, FMS has garnered little

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attention.[5–9] Moreover, none of the known studies have investigated whether FMS is able to track acute changes in endothelial function.

Endothelial function naturally fluctuates throughout the day and is enhanced or impaired by external and internal factors which alter the local hemodynamic environment.[2,19–23] For example, while unidirectional shear stress enhances endothelial function,[19] oscillatory shear stress can impair endothelial function.[19–22] A model that we[20] and others[21,22] have used to increase retrograde, and subsequently oscillatory shear stress, and acutely impair endothelial function, is the inflation of a tourniquet to a low-moderate pressure (50–75 mmHg) distal to the arterial segment of interest for 20-30 min. The down-stream resistance, as the result of tourniquet inflation, increases retrograde and oscillatory shear stress. In turn, the oscillatory shear stress leads to diminished FMD (endothelial function).[21,22] This model can be used to confirm whether PWV and FMS are able to similarly track acute impairments in endothelial function. Therefore, the objective of the current study was to determine the effects of acute local endothelial dysfunction, induced by increasing retrograde shear stress for 30 min in the upper arm, on arm PWV and PWV_{min}. It was hypothesized that acute local endothelial dysfunction would increase PWV and PWV_{min}.

Methods

This study is reported in accordance with CONSORT (Consolidated Standards of Reporting Trials) guidelines.[24] Ethical approval was obtained from the University of North Carolina at Chapel Hill Institutional Review Board, and all participants provided written informed consent prior to participating in the study.

Participants

Twenty-three young (18–35 years), healthy individuals were recruited from the host institution, a large state university. A healthy population sample was recruited to mitigate the risk of age or disease-related influences on the primary outcomes. Participants were excluded if they were pregnant, reported any known cardio-metabolic disorders, were taking medications known to affect cardiovascular function, or reported cigarette smoking.

Experimental design

This was a quasi-experimental, pretest–post-test design in which FMD and PWV_{min} were assessed pre- and post-acute local endothelial dysfunction. Following a familiarization visit, all measurements were collected on a single occasion in an environmentally controlled laboratory (average: 22°C, 50% humidity, 748 mmHg). Participants arrived between 0600 and 1000, having fasted for 12 h, consuming only water, and having refrained from supplement intake that morning. Additionally, participants were asked to avoid strenuous physical activity and alcohol for 24 h prior to experimentation. Following anthropometric assessments, participants were asked to rest quietly and supine for 20 min with both arms resting at right angles to the body and at heart level.[25] During the 20-min rest period a blood pressure cuff attached to the SphygmoCor XCEL (AtCor Medical, Sydney, Australia) device was fitted to the right upper arm to permit collection of control hemodynamic assessments. Primary outcome apparatus, including ultrasound (LOGIQ P6; GE Healthcare, Wauwatosa, WI, USA) for FMD and Vicorder (Arterial Stiffness Model; SMT Medical, Wuerzburg, Germany) for PWV/PWV_{min}, were positioned on the left arm (Figure 1). The quality of ultrasound and Vicorder pressure waveform signals were adjudicated, and adjustments were made if the signals were not optimal.

Following a 20-min rest, the baseline brachial diameter (D_{base}) and PWV were recorded. Subsequently, the maximum diameter (D_{max}) and PWV_{min} response to reactive hyperemia were simultaneously recorded, where reactive hyperemia was induced by inflating a pneumatic tourniquet (SC10; Hokanson, Bellevue, WA, USA) around the mid-forearm to 250 mmHg for 5 min. Following a 3-min recovery, endothelial dysfunction was induced by inflating the mid-forearm tourniquet to a pressure of 75 mmHg (Figure 1). This moderate pressure does not prevent arterial inflow, but does induce arterial wave reflections with resultant retrograde blood flow.[20,21,26,27] Following 30 min of increased retrograde shear stress, the tourniquet was slowly (over 30 s) deflated, and then all assessments were immediately repeated as described above.

Experimental measures

Ultrasound: Flow-mediated dilation and shear rate. A 11-2 MHz linear array probe (LOGIQ P6; GE Healthcare) was used to record brachial artery brightness-mode images and pulsed Doppler waveforms for the measurement of FMD and shear rate, respectively.[3] Using a custom-built probe-holding device, the ultrasound probe was fixed on the brachial artery 5–10 cm proximal to the antecubital fossa. The insonation angle was kept constant between 45° and 60° and the sample volume included most of the vessel. For the entire FMD procedure, video recordings were captured at 30 Hz using an external video capture system (AV.io HD Frame Grabber; Epiphan Video, Palo Alto, CA, USA). During the period of increased retrograde shear stress, one 30-s recording was captured to confirm increased retrograde shear rate. The captured videos were analyzed offline using specialized image analysis software (FMD Studio; Quipu srl, Pisa, Italy). The second-by-second diameter, antegrade velocity and retrograde velocity data were smoothed (3-s bins) and processed using custom-written Visual Basic code.[20,28] FMD (%) was calculated as $(D_{max} - D_{base})/D_{base} * 100\%$, where D_{base} is the mean diameter averaged over 2 min preceding the cuff inflation period, and D_{max} is the peak diameter response to reactive hyperemia (Figure 2). To estimate the FMD stimulus, shear rate area-under-the-curve (AUC) up to 40 s (Shear AUC) after cuff release was calculated.[28] Shear rate (s^{-1}) was calculated as 4 * mean velocity/diameter, and oscillatory index as retrograde shear rate/(antegrade shear rate + retrograde shear) * 100.[29]

Pulse wave velocity and flow-mediated slowing. Simultaneous to FMD, the Vicorder device was used to measure PWV and

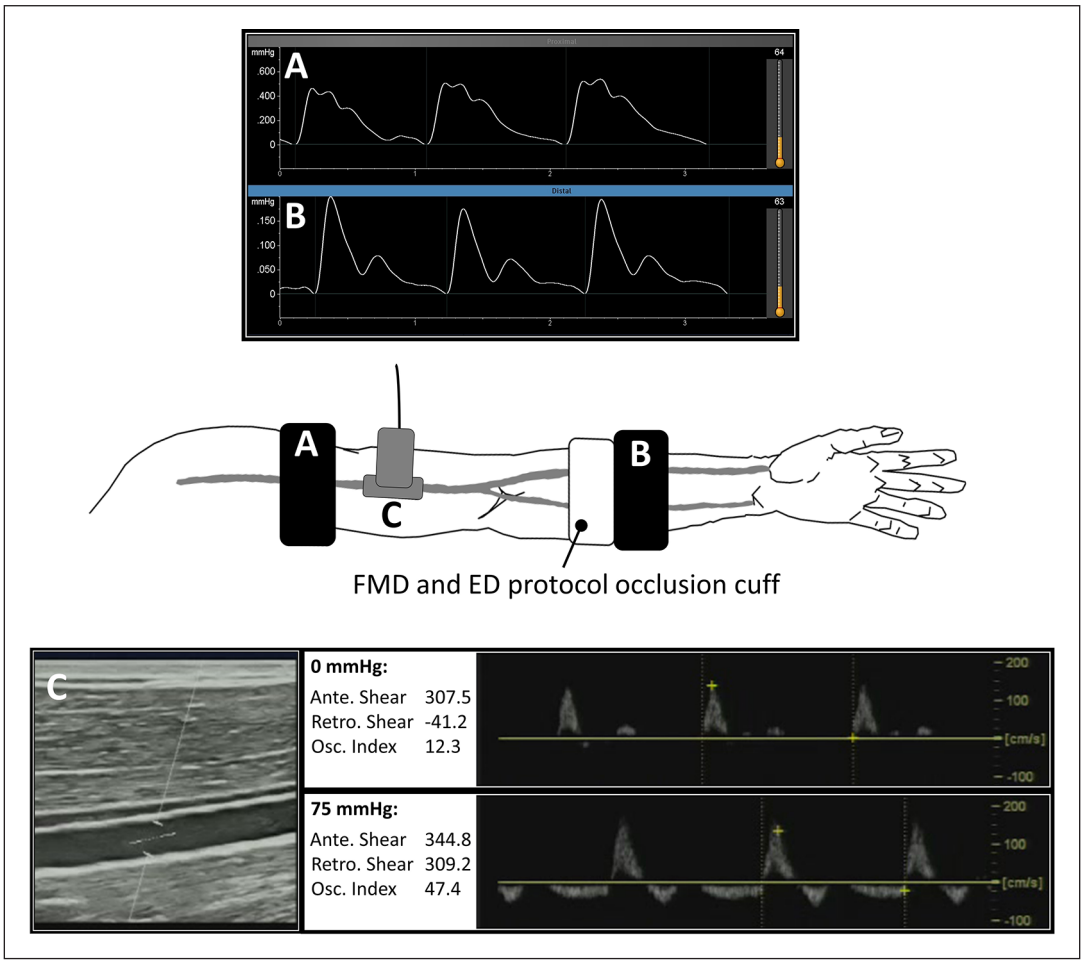

Figure 1. Experimental set-up.
To measure PWV at baseline, and then the minimum PWV during reactive hyperemia (FMS), arterial pressure waveforms were simultaneously captured at proximal (A) and distal (B) sites using an oscillometric device. Duplex Doppler ultrasound was used to measure brachial artery FMD, and to capture pulsed Doppler waveforms (C) at baseline (0 mmHg) and during the ED protocol (75 mmHg).
Ante, antegrade; ED, endothelial dysfunction; FMD, flow-mediated dilation; FMS, flow-mediated slowing; Osc, oscillatory; PWV, pulse wave velocity; Retro, retrograde.

PWV_{min}. PWV (m/s) was calculated by dividing arterial path length by the pulse transit time between a proximal (upper arm) and distal (wrist) arterial segment. Specifically, path length was directly measured, using a custom device to bypass body contours, between the mid-point of the two cuffs. To measure pulse transit time, the cuffs were simultaneously inflated to a low (~50 mmHg) pressure, and a proprietary algorithm was used to automatically calculate the time between the foot of the proximal pressure waveform to the foot of the distal pressure waveform. As part of the FMS test, PWV was recorded continuously for 2 min prior to inflation of the forearm tourniquet used to induce reactive hyperemia (Figure 2). This 2-min recording was averaged to derive PWV. Subsequently, PWV_{min} was captured by continuously recording PWV during reactive hyperemia, and averaging PWV over 3.5-s cycles. PWV_{min} was also expressed as the change relative to baseline (PWV_{min}%), and the integrated 30 (PWV_{30}), 60 (PWV_{60}), 90 (PWV_{90}), and 120-s (PWV_{120}) response relative to baseline.

Peripheral and central hemodynamics. The SphygmoCor XCEL (AtCor Medical) was used to measure peripheral and central BP as well as arterial wave reflection. An appropriately sized cuff was placed around the upper portion of the right arm. Each measurement cycle lasts ~60 s. This includes inflating the cuff to measure brachial systolic (SBP) and diastolic (DBP) blood pressure, and then re-inflating 5 s later to 10 mmHg below DBP to acquire a volumetric displacement signal for 10 s.[30] The brachial waveforms are calibrated using the cuff-measured SBP and DBP, and mean arterial pressure (MAP) is derived by integrating the AUC. A corresponding aortic pressure waveform is generated using a validated proprietary transfer function and calibrated using DBP and MAP.[30] The aortic waveform is used to derive central SBP (cSBP), aortic backward pressure (Pb), and aortic forward pressure (Pf). The Pf and Pb wave pressures were determined by assuming a triangular flow wave.[31] This method creates a triangular-shaped flow wave by matching the start, peak, and end of the flow wave to the timings of the foot, inflection point, and incisura of the aortic pressure wave, respectively.

Sample size

Sample size calculations were made using GPower 3.1. A prior study reported that the endothelial dysfunction

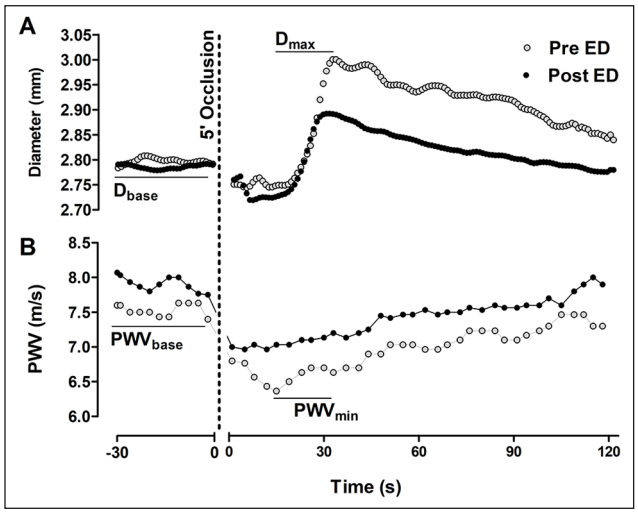

Figure 2. Example traces of FMD (A) and FMS (B) data recorded before and after the ED protocol.
(A) Diameters were recorded at 30 Hz for 2 min at baseline, during the 5 min of occlusion, and for 3 min following occlusion release. The diameters were analyzed offline using specialized image analysis software. FMD was calculated as $(D_{max} - D_{base})/D_{base} * 100$. (B) PWV was automatically recorded every 3.5 s by the Vicorder device. For PWV_{base}, PWV was recorded continuously for 2 min and then averaged. For PWV_{min}, the minimum PWV following occlusion release was automatically detected and then manually confirmed.
D_{base}, baseline brachial diameter; D_{max}, maximum brachial diameter during reactive hyperemia *r*; ED, endothelial dysfunction; FMD, flow-mediated dilation; FMS, flow-mediated slowing; PWV, pulse wave velocity; PWV_{base}, PWV at baseline; PWV_{min}, minimum PWV during reactive hyperemia.

protocol used in the current study decreased FMD% from 7.2% (SD: 3.0) to 4.2% (SD: 3.4), with an effect size (ES) of 1.0.[21] Using a more conservative effect size of 0.5, a Type I error of 5% and Type II error of 20%, 21 subjects were required. This number was inflated to 23 to account for the potential for missing data.

Statistical analysis

Statistical analyses were performed using Jamovi (2019, Version 1.0.1) and the rmcorr (repeated measures correlation) package for R (R Foundation for Statistical Computing, Vienna, Austria).[32] Only participants who had complete data for the primary outcomes were included in the analyses. The significance level was set a priori for all statistical procedures at $\alpha = 0.05$. Raw data are presented as mean (SD) and mixed model data are presented as mean (95% CI). The corresponding author (LS) had full access to the data in the study and was responsible for the integrity of the data set and the data analysis.

The effect of endothelial dysfunction on PWV, PWV_{min}, and FMD were tested using paired Student's *t*-tests. Additionally, linear mixed models (random intercept, fixed slope) were used to adjust FMD for within-subject changes in shear AUC, and to adjust PWV and PWV_{min} for within-subject changes in MAP. Linear mixed models were also used to assess the effect of condition (baseline, during cuff inflation (endothelial dysfunction protocol), post-cuff inflation) on hemodynamic data. When the condition effect was significant, during cuff inflation and post-cuff inflation were compared to baseline, with Bonferroni adjustments. Intra-individual (within-subject) associations between FMD with PWV and PWV_{min} were analyzed using rmcorr. The rmcorr statistical technique determines the overall within-individual relationship among paired measures assessed on two or more occasions.[32]

For paired *t*-tests and linear mixed models, ES were calculated as Cohen's *d* where $\leqslant 0.2$ was defined as trivial, 0.2–0.3 as small, 0.4–0.8 as moderate, and $\geqslant 0.8$ as large. For the mixed models, Cohen's *d* was calculated as the effect of condition (β) from linear mixed models divided by the baseline SD. For rmcorr, *r* value estimates of 0.1, 0.3, and 0.5 were defined as small, medium, and large, respectively.[33]

Results

Participants

Complete data were collected on 22 of the 23 participants (23.8 years [SD 4.1], 73% F, 22.8 kg/m^2 [SD 2.8]). One participant (20 years, F, 20.8 kg/m^2) was excluded from data analysis due to inadequate image quality (i.e. the ultrasound image analysis software inadequately traced the vessel walls). The PWV data for this participant were similar to the group mean (PWV 8.6 vs 8.5 m/s and PWV_{min} 7.1 vs 6.9 m/s, respectively).

Hemodynamic data

Hemodynamic/control data are presented in Table 1. Across conditions (baseline, during cuff inflation, post-cuff inflation), the brachial diameter did not significantly change ($p = 0.720$). There was a significant change in the shear rate parameters, including antegrade shear rate ($p < 0.001$), retrograde shear rate ($p < 0.001$), and oscillatory index ($p < 0.001$). Compared to baseline, each of the shear rate parameters significantly increased during partial occlusion ($p < 0.001$ for all measures) but were not different to baseline post-occlusion ($p = 1.000$ for all measures).

There was a significant change in MAP ($p < 0.001$), DBP ($p < 0.001$), and SBP ($p < 0.001$). However, there was a non-significant change in central hemodynamic parameters, including cSBP ($p = 0.142$), Pb ($p = 0.401$), and Pf ($p = 0.736$).

Flow-Mediated Dilation (FMD)

Example data from one subject are shown in Figure 2, and the primary study outcomes are reported in Table 2. Following the 30-min endothelial dysfunction protocol, there was a large significant decrease ($\Delta = -3.10$; 95% CI: –4.15, –2.05; ES = −1.3) in FMD. There was a moderate decrease in shear AUC ($\Delta = -4124$; 95% CI: –6923, –1326; ES = −0.7). After adjusting FMD for the shear AUC stimulus, the decrease in FMD remained large (ES = −2.4, $p < 0.001$).

Pulse Wave Velocity (PWV)

Following the 30-min endothelial dysfunction protocol, there was a moderate significant increase ($\Delta = 0.38$; 95%

Table 1. Hemodynamic measures ($n = 22$).

	Baseline		Cuff		Post		$P_{Overall}$	P_{Cuff}	P_{Post}
	Mean	(SD)	Mean	(SD)	Mean	(SD)			
Diameter (mm)	3.01	(0.41)	3.03	(0.45)	3.01	(0.44)	0.720		
Ante shear	186.3	(54.6)	305	(81.1)	175	(51.0)	< 0.001	< 0.001	0.508
Retro shear	–37.4	(27.4)	–201	(90.3)	–38.3	(30.3)	< 0.001	< 0.001	0.952
Osc index	17.6	(13.4)	38.4	(10.4)	18.7	(13.3)	< 0.001	< 0.001	0.749
MAP (mmHg)	78.3	(8.90)	79.5	(7.68)	82.3	(9.16)	< 0.001	0.226	< 0.001
DBP (mmHg)	66.1	(7.02)	68.0	(7.47)	70.1	(7.48)	< 0.001	0.022	< 0.001
SBP (mmHg)	112.3	(10.4)	112.9	(9.8)	115.7	(9.4)	0.008	0.602	0.004
cSBP (mmHg)	98.7	(9.4)	97.9	(8.6)	100.3	(9.4)	0.142		
Pb (mmHg)	11.8	(2.38)	11.3	(2.07)	11.5	(1.87)	0.401		
Pf (mmHg)	25.3	(4.16)	24.9	(3.98)	25.4	(4.41)	0.736		

Ante, antegrade; cSBP, central systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; Osc, oscillatory; Pb, aortic backward pressure; Pf, aortic forward pressure; Retro, retrograde; SBP, systolic blood pressure.

Table 2. Primary outcomes pre- and post-acute endothelial dysfunction ($n = 22$).

	Baseline		Post		p	ES
	Mean	(SD)	Mean	(SD)		
Shear AUC	30,426	(9080)	26,302	(7647)	0.006	–0.7
FMD (%)	6.17	(2.00)	3.07	(2.40)	< 0.001	–1.3
PWV (m/s)	8.47	(1.17)	8.85	(1.17)	0.020	0.5
PWV_{min} (m/s)	6.87	(0.67)	7.04	(0.63)	0.009	0.6
PWV_{min} (%)	18.3	(6.51)	19.6	(5.62)	0.396	0.2
PWV_{30} (%)	15.9	(6.42)	15.9	(6.05)	0.995	0.0
PWV_{60} (%)	13.9	(6.39)	13.1	(6.01)	0.590	–0.2
PWV_{90} (%)	12.3	(6.42)	11.2	(5.87)	0.474	–0.2
PWV_{120} (%)	11.2	(6.47)	9.89	(5.74)	0.388	–0.2

AUC, area-under-the-curve; ES, effect size (Cohen's *d*); FMD, flow-mediated dilation; PWV, pulse wave velocity; PWV_{min}, minimum pulse wave velocity; $PWV_{30–120}$, PWV integrated to 30–120 s during reactive hyperemia relative to baseline.

CI: 0.07, 0.69; ES = 0.5) in PWV. After adjusting for MAP, the effect remained moderate (ES = 0.5, $p < 0.022$). As reported in Figure 3A, there was a moderate but inconclusive intra-individual association between FMD and PWV ($r = −0.3$; 95% CI: –0.67, 0.12; $p = 0.121$).

Flow-Mediated Slowing (FMS)

Following the 30-min endothelial dysfunction protocol, there was a moderate significant increase ($\Delta = 0.16$; 95% CI: 0.05, 0.28; ES = 0.6) in PWV_{min}. After adjusting for MAP, the effect remained moderate (ES = 0.6, $p < 0.009$). There was a large intra-individual association between FMD and PWV_{min} ($r = −0.61$; 95% CI: –0.82, –0.24; $p = 0.002$) (Figure 3B). The endothelial dysfunction protocol had an inconclusive effect on the remainder of the FMS parameters (Table 2), with non-significant intra-individual associations with FMD ($p = 0.295$–0.991).

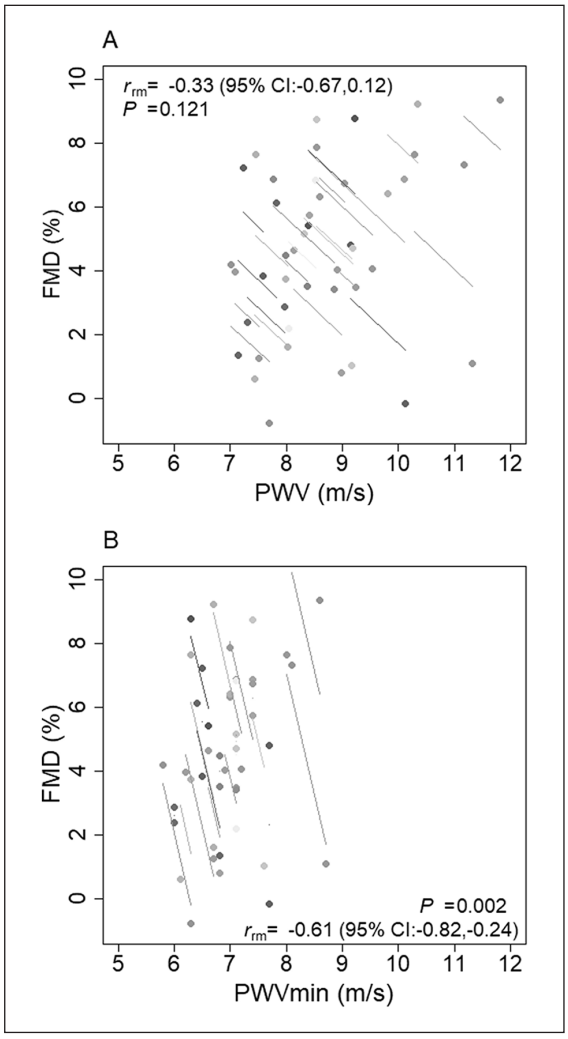

Figure 3. Intra-individual (pre- vs post-endothelial dysfunction) associations between FMD with PWV (A) and PWV_{min} (B) ($n = 22$ repeated measures).
FMD, flow-mediated dilation; PWV, pulse wave velocity; PWV_{min}, minimum pulse wave velocity.

Discussion

The objective of the current study was to determine the effects of acute endothelial dysfunction on PWV and PWV_{min}. Acute endothelial dysfunction, localized to the arm, resulted in a moderate and significant increase in arm PWV ($\Delta = 0.38$ m/s) and PWV_{min} ($\Delta = 0.16$ m/s). These

findings suggest that acute changes in PWV and PWV_{min} are at least partly driven by endothelial function.

Comparison to literature: PWV

In support of our hypothesis, the endothelial dysfunction protocol increased PWV by 0.38 m/s. We did not measure molecules associated with endothelial function in this study and can only speculate with respect to mechanisms. That being said, the increase in PWV following a known endothelial dysfunction protocol is in line with a number of studies reporting that infusion of a nitric oxide (NO) synthesis inhibitor, N^G-monomethyl-L-arginine (L-NMMA) or N^G-nitro-L-arginine methyl ester (L-NAME), decreases compliance and increases PWV.[12–15] Conversely, infusion of glyceryl trinitrate (nitroglycerin), which is metabolized to NO within the vascular wall, increases arterial compliance and decreases PWV.[12–14,16] While NO is not the only molecule regulating endothelial function,[18] these previous findings do support a direct association between endothelial function and arterial mechanical function. The current study extends these previous findings by demonstrating that non-specific local endothelial dysfunction increases arterial stiffness.

In the current study, we did see a 5.0 mmHg increase in MAP. MAP is a known determinant of PWV[11] and could have driven the PWV response. Stewart et al.[16] infused L-NMMA into a small ($n = 8$) sample of men and found that carotid–femoral PWV increased by 0.7 m/s. The authors argued that this response may have been driven by the 7.8 mmHg increase in MAP. In support, infusion of norepinephrine and dobutamine resulted in similar increases in PWV and MAP to that observed with L-NMMA infusion. However, association is not causation and the authors did not manipulate NO while controlling for MAP, nor did they statistically control for changes in MAP. The authors did test the association between MAP and PWV using ordinary least squares regression, but they used aggregate data and included the four conditions in the same model. This would have violated the assumption of statistical independence of errors, and could have resulted in an erroneous conclusion.[32] The findings of Stewart et al.[16] are contrary to Wilkinson et al.,[13] who used an ovine hind-limb model and reported that glyceryl trinitrate decreased PWV while L-NMMA increased PWV, and that these changes occurred independent of MAP. In the current study, we did statistically adjust for within-subject changes in MAP, and there remained a moderate ES increase in PWV. The intra-individual association between FMD and PWV was inconclusive, but we were not statistically powered to assess this association and the lack of association may have been driven by the restricted range of change values.[34]

Comparison to literature: FMS

We found that the endothelial dysfunction protocol had an inconclusive effect on each of the PWV_{min}% calculations (response relative to baseline). However, there was a moderate significant increase ($\Delta = 0.16$ m/s) in the raw FMS signal (i.e. PWV_{min}). As such, while a limited number of studies have reported that PWV_{min}% is reduced in those with cardiovascular complications,[5–7] and that PWV_{min}% is associated with cardiovascular risk factors,[9] these findings may not be attributable to endothelial function. Alternatively, the lack of effect of endothelial dysfunction on PWV_{min}% in the current study may be attributable to a shifting numerator. Baseline PWV did increase by 0.38 m/s, which may have masked any change in the denominator, PWV_{min}. The PWV_{min} did increase during reactive hyperemia following endothelial dysfunction, which is consistent with the reduced peak arterial diameter and the assumption made by the Moens–Korteweg equation.[17] As such, at least for studies investigating acute change in endothelial function, the raw PWV_{min} signal may be superior to PWV_{min}%. The use of PWV_{min} is supported by the large intra-individual association between FMD and PWV ($r = -0.61$).

Limitations and strengths

This study had several considerations, which were considered when designing the study. First, a healthy population sample was recruited to mitigate the risk of age or disease-related influences on the primary outcomes. While our sample population did permit optimal signal-to-noise-ratio, future studies with older and clinical populations are now required to better generalize the findings. Second, we were not sufficiently powered to stratify our sample by sex. Further investigation is required to ascertain whether endothelial dysfunction similarly affects PWV and PWV_{min} in men and women. Third, the study was powered to estimate the effect of the endothelial dysfunction protocol on PWV_{min}, not to determine associations between PWV and PWV_{min} with FMD. As such, the association data should not be directly used to adjudicate the hypothesis. Lastly, FMD was assessed simultaneously to PWV_{min}, meaning the upper arm and wrist cuffs were inflated to 50 mmHg throughout the FMD test. Pilot testing indicated that 50 mmHg was the minimum inflation pressure for permitting quality pressure waveforms. We did consider conducting FMD and FMS measurements on separate arms, or on separate days, but both alternative approaches would have introduced error variance. Rather, we completed both tests simultaneously, under the assumption that partial occlusion would have similarly affected both tests. Nonetheless, we cannot discount that partial occlusion did introduce some noise, particularly to the FMD data.

There were several strengths of the current study. First, we used the standard FMD test to confirm endothelial dysfunction. Second, we non-specifically induced local endothelial dysfunction. While previous studies have demonstrated an association between PWV and endothelial function by targeting NO bioavailability,[12–16] it should be acknowledged that NO is not the only molecule regulating endothelial function.[18]

Implications

Findings from this study indicate that PWV_{min} and PWV are viable options for measuring acute changes in endothelial

function. These options are attractive alternatives to existing methods, including FMD, because they are user-objective, automated, and likely more precise (reliable) than FMD. For example, one study compared the reliability of brachial FMD to brachial–radial PWV_{min}% in a group of 22 healthy participants (28–65 years old), reporting a coefficient of variation (CV) of 7.3% for PWV_{min}% and 26.6% for FMD.[7] The same study reported a CV of 3.3% for PWV, and, while PWV_{min} was not reported, the CV for the absolute change in PWV (PWV_{min} – baseline PWV) was 8.2%. The reported reliability of FMD and PWV are in accordance with the available literature.[35–37] To confirm viability, additional investigation is warranted to: (i) estimate the precision of PWV_{min}; (ii) confirm whether PWV and PWV_{min} decrease to a greater extent following a perturbation known to improve endothelial function; (iii) confirm whether an increase or decrease in PWV and PWV_{min} corresponds with changes in the balance agonist and antagonistic molecules known to regulate endothelial function (e.g. NO and endothelin-1); (iv) use a technique which does not require partial occlusion to measure PWV (e.g. tonometry of photoplethysmography) to determine whether partial occlusion during reactive hyperemia modifies the PWV_{min} response; and (v) compare PWV_{min} and FMD in terms of the ability to discriminate between those with and without known cardiovascular disease.

Conclusions

The objective of the current study was to determine the effects of acute local endothelial dysfunction, induced by increasing retrograde shear stress in the arm for 30 min, on arm PWV and PWV_{min}. Endothelial dysfunction resulted in a moderate significant increase in PWV and PWV_{min}. These findings indicate that PWV and PWV_{min} are viable, user-objective, and automated tools for monitoring acute changes in endothelial function.

Acknowledgements

The authors would like to thank Arnd Warmuth, SMT Medical, for providing technical insights.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

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