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# **Validity of single-point assessments for determining leg pulse-wave velocity in sitting and supine positions.**

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- None of the authors has any conflicts of interest associated with this study.
- Raw data were generated at [UNC Chapel-Hill, USA]. Derived data supporting the findings of this study are available from the corresponding author [SF] on request.

## Summary

There has been a great deal of interest into the effects of prolonged sitting on lower limb vascular function. However, most studies use flow mediated dilation which is technically challenging. A simpler technique is pulse wave velocity (PWV) which can be estimated at any single arterial site of interest using a number of different calculations [Bramwell-hill ( $PWV_{BH}$ ),  $\beta$ -stiffness index ( $PWV_{\beta}$ ), and blood flow ( $PWV_{BF}$ )]. Findings from this technique would be better inferred if they compare to a standard criterion 2-point PWV assessment. The current study used ultrasound to determine which estimation of single-point PWV is most valid. The criterion was traditional ECG-gated 2-point (superficial femoral [SF]-posterior tibialis [PT]) PWV. Single-point estimates were calculated at the SF and PT arteries in both supine and seated positions. Single-point PWV was considered valid if the aSEE was  $<1.0\text{m}\cdot\text{s}$ . Findings show that for both postural positions, the absolute standard error of estimates (aSEE) criterion of  $<1.0\text{m}\cdot\text{s}$  was not achieved in either the PT or SF arteries using any of the single-point PWV calculations. However, single-point calculations consistently demonstrated the lowest error at the SF artery using  $PWV_{\beta}$  in both supine (SF aSEE = 1.7 vs. PT 2.7 $\text{m}\cdot\text{s}$ ) and seated (SF aSEE = 1.5 vs. PT 3.0 $\text{m}\cdot\text{s}$ ) positions. All single-point  $\Delta PWV$  (supine – seated) calculations were higher in sitting, with  $PWV_{\beta}$  having the closest agreement ( $\Delta SF$  aSEE 1.7 $\text{m}\cdot\text{s}$ ) to the 2-point criterion. Single-point PWV calculations do not directly reflect regional 2-point PWV. However, they are sensitive to change when moving from supine to seated positions.

## Key Words

Arterial Stiffness; Endothelial Function; Prolonged Sitting; Leg Vascular Function

## **Introduction**

Recently, there has been a great deal of interest in the effects of sedentary behaviour, particularly prolonged sitting, on cardiovascular health (McManus, *et al.* ; Morishima, *et al.* ; Restaino, *et al.* ; Restaino, *et al.* ; Thosar, *et al.* ; Vranish, *et al.*). Prolonged sitting has been shown to reduce lower limb vascular function (Restaino, Holwerda, Credeur, Fadel & Padilla ; Thosar, Bielko, Mather, Johnston & Wallace ; Thosar, *et al.*), specifically endothelial function (Morishima, Restaino, Walsh, Kanaley, Fadel & Padilla ; Restaino, Walsh, Morishima, Vranish, Martinez-Lemus, Fadel & Padilla). Endothelial function is typically determined using the flow mediated dilation technique (Stoner, *et al.* ; Stoner, *et al.*). However, this technique is time consuming, complicated, and has a high level of variability (Stoner, *et al.* ; Thijssen, *et al.*). As such, this technique has limited application for large-scale epidemiological studies. A viable alternative may be pulse wave velocity (PWV), as it is the gold-standard assessment for arterial stiffness (Jadhav & Kadam ; McEniery, *et al.* ; Naka, *et al.*), and a proxy for endothelial function (Stoner, *et al.*). PWV and thus arterial stiffness, is dependent on both vascular structure and function (McEniery, Wallace, Mackenzie, McDonnell, Newby, Cockcroft & Wilkinson ; Sun), and may change acutely due to a change in function caused by a perturbation such as prolonged sitting or a shift in posture. PWV can be assessed using oscillometric or ultrasound-based techniques. Whilst oscillometric devices are less time consuming, ultrasound assessments of PWV can provide greater diagnostic information. Recently authors have demonstrated that PWV significantly increases in response to 180 min of prolonged sitting (Credeur, *et al.*). However, when standing is used to interrupt or break-up prolonged sitting, PWV does not significantly increase (Barone Gibbs, *et al.*).

The conventional assessment techniques for determining PWV are undertaken using 2-point measurements, or they can be estimated at any arterial site of interest using single-point

calculations. When estimating these single-point PWV calculations, multiple equations can be used (Van Bortel, *et al.*). Common calculations of PWV at arterial sites include a derivative of  $\beta$ -stiffness ( $PWV_{\beta}$ ), the Bramwell-Hill equation ( $PWV_{BH}$ ), compliance coefficient, distensibility coefficient, and an estimation based on local changes in blood-flow ( $PWV_{BF}$ ) (Lim, *et al.*; Van Bortel, Laurent, Boutouyrie, Chowienczyk, Cruickshank, De Backer, Filipovsky, Huybrechts, Mattace-Raso & Protogerou). Given that a change in posture has been shown to alter central and peripheral blood pressure (Zieff, *et al.*), focusing on single-point calculations which have been shown to be the least pressure dependent would be beneficial. Zieff, Heffernan, Stone, Fryer, Credeur, Hanson, Faulkner and Stoner (previously found that  $PWV_{\beta}$ ,  $PWV_{BH}$ , and  $PWV_{BF}$  were the least blood pressure dependent. However, no known study has compared any single-point calculations of PWV to the criterion 2-point PWV in supine and seated positions. If the single-point estimate aligns with the 2-point and responds similarly to a perturbation such as moving between different postural positions, then inferences would be simpler and more time efficient.

The current study sought to determine the validity (accuracy) of different single-point calculations of PWV ( $PWV_{\beta}$ ,  $PWV_{BH}$ ,  $PWV_{BF}$ ) obtained using B-mode ultrasound in supine and seated positions by comparing to a criterion, conventional 2-point PWV assessment; superficial femoral (SF) to posterior tibial (PT). The accuracy of single-point PWV will be considered acceptable if the absolute standard error of estimates (aSEE) and the standardized error of estimates (sSEE) is  $< 1.0 \text{ m}\cdot\text{s}$  (Wilkinson, *et al.*) and the standardized indicator of error is moderate (0.6 – 1.2) or better (Hopkins).

## **Method**

This observation study is reported in accordance with STROBE (Strengthening the reporting of Observational Studies in Epidemiology) guidelines (Von Elm, *et al.*)

### *Participants*

Thirty-two young healthy participants (50% females) volunteered to take part in the current study. For this initial study, healthy young volunteers were recruited to minimize any potential effects of age or disease on the data. Participants were excluded if they smoked, reported any known cardio-metabolic disorders, or were taking any medication known to affect cardiovascular function. Ethical approval, which adhered to the standards of the journal, and the Helsinki Declaration (Puri, *et al.*), was granted from the University of North Carolina prior to any recruitment or data collection. All participants provided written informed consent prior to taking part in the study.

### *Experimental design*

Prior to the study, all participants were familiarized with the experimental procedures. Following this, all participants attended a single session (between 0700 and 1000) in the laboratory following an overnight fast, and consuming only water. Participants were asked to avoid any strenuous activity and alcohol consumption for 24hrs prior to their visit. Participants were randomly allocated into two groups, one group (n=16) initially rested (20 min) in a supine position and the other group (n=16) initially rested (20 min) in a seated position. Randomization was conducted using the software [www.randomizer.org](http://www.randomizer.org). An experienced single operator used an electrocardiogram (ECG)-gated ultrasound to capture 3 x 10 s images of the PT and SF arteries. Immediately following the ultrasound measurements blood pressure was recorded in triplicate on the left arm (SphygmoCor Xcel, AtCor). The closest two blood

pressure measurements were averaged to provide a single value. Participants were then transferred to the alternate posture (either seated or supine) where they rested for a further 10 minutes. Again, three 10 s images of the PT and SF arteries were captured, followed by three blood pressure measurement. One regional (criterion 2-point) and three site-specific (single-point) measures of PWV were then determined using three different equations (each equation is described in detail later in the manuscript). Our laboratory has previously reported within and between-day reliability data for the three single-point PWV measures (Zieff, Heffernan, Stone, Fryer, Credeur, Hanson, Faulkner & Stoner).

### *Measurement sites*

Prior to assessing 2-point and single-point PWV in both postural positions, suitable sites for monitoring the SF and PT arteries were identified. Both the SF and PT measurement sites were located and marked in the supine position. For the SF assessments, the bifurcation between the SF and the common femoral artery was visualized and the top edge of the ultrasound probe was re-positioned to directly cross the bifurcation. For the PT assessments, the mid-point of the ultrasound probe was placed approximately 2 cm proximal to the medial malleolus.

### *Ultrasound*

A single trained ultrasound operator with extensive experience collected all measurements using an ultrasound device equipped with an 11-2 mHz linear array probe (GE Healthcare, Wauwatosa, USA) to sequentially scan and obtain ECG-gated pulse-wave Doppler waveforms at the SF and PT arteries. It was ensured that the vessel clearly was extended across the entire (unzoomed) imaging plane to minimize the risk of skewing the vessel walls. Ultrasound global (acoustic output, gain, dynamic range, gamma and rejection) and probe-dependent (zoom factor, edge enhancement, frame averaging and target frame rate) settings were standardized.

Three 10-second videos of the ultrasound and gated ECG readings were recorded at each site using external video capturing software (AV.io HD Frame Grabber, Epiphan Video, CA). A fourth brightness-mode-only recording was made in which the isonation angle was perpendicular to the vessel wall to ensure an optimal diameter measurement. During each 10s video capture, participants were instructed to hold their breath wherever they were in their breathing cycle (without having a large inhalation) in order to control cyclical variation and ensure optimal image quality.

### *Data Analysis*

The 10s video clips were analyzed offline using automated edge-detecting software (FMD Studio, Quipu, Italy). Custom written Excel Visual Basic code was used to fit peaks and troughs to the diameter waveforms in order to calculate diastolic, systolic, and mean diameters. Blood flow was calculated from continuous diameter and mean blood velocity recordings using the equation:  $3.14 \times (\text{diameter}/2)^2 \times \text{mean blood velocity} \times 60$ .

Ultrasound images showing the gated ECG trace and the velocity profiles were analysed offline using ImageJ (<https://imagej.nih.gov/ij/>, National Institutes of Health, Bethesda, USA) (Schneider, *et al.*) by a single blinded operator. In brief, following a scaled calibration of a known distance, the interval between the r-wave of the QRS complex and the foot of the systolic upstroke in the Doppler spectral envelope was measured, and averaged over at least five consecutive cardiac cycles for each video. Subsequently the data from the closest two videos were averaged to give a single value.

### *2-point calculation of pulse wave velocity*



The 2-point PWV measurements were made at both the SF and PT arteries in both the supine and seated positions. To determine 2-point PWV, the pulse transit time (PTT) was defined as the difference between the intervals of time measured at each arterial segment (SF-PT, PTT). Arterial path length was estimated by measuring the linear distance from the mid-point of probe at the SF to the mid-point of the probe at the PT (SF-PT  $D$ ). 2-point PWV was then calculated as:

$$\text{2-point PWV} = \text{SF-PT } D / \text{SF-PT PTT}.$$

#### *Single-point calculations of Pulse Wave Velocity*

Single point PWV measurements were made at both the SF and PT arteries in both the supine and seated positions. For each artery and in each position, three calculations were made:  $\text{PWV}_{\text{BF}}$ ,  $\text{PWV}_{\text{BH}}$ , and  $\text{PWV}_{\beta}$ .

- (1) The  $\beta$ -stiffness derivative method utilizes the  $\beta$ -stiffness index to estimate PWV. The  $\beta$ -stiffness index is based on changes in pressure and diameter and can be described as:

$$\text{PWV}_{\beta} = \sqrt{(\beta \cdot \text{DBP}) / (2p)}$$

Where;  $p$  is the blood density ( $1059 \text{ kg/m}^3$ ) (Harada, *et al.*) and  $\beta$  is the  $\beta$ -stiffness index,

which is calculated using the formula:

$$\beta = \ln(\text{SBP}/\text{DBP}) / [(D_s - D_d) / D_d]$$

where  $\ln$  is the natural logarithm, SBP is systolic blood pressure, DBP is diastolic blood pressure,  $D_s$  is the lumen diameter during systole, and  $D_d$  is the lumen diameter during diastole (Kawasaki, *et al.*).

(2) The Bramwell-Hill equation theoretically relates PWV, distensibility and pulse pressure using the following mathematical model:

$$PWV_{BH} = \sqrt{\left(\frac{A}{p}\right)\left(\frac{1}{CC}\right)}$$

Where A is the lumen area, p is the blood density (1059 kg/m<sup>3</sup>)(Harada, Okada, Niki, Chang & Sugawara), and CC is the compliance coefficient (Van Bortel, *et al.*), which is calculated using the formula:

$$CC = (2D \cdot \Delta D + D^2)/(4 \cdot \Delta P)$$

where D is the lumen diameter and  $\Delta P$  is the pulse pressure (SBP-DBP)(Van Bortel, Duprez, Starmans-Kool, Safar, Giannattasio, Cockcroft, Kaiser & Thuillez).

(3) For the blood flow (BF) method, PWV is estimated as the ratio between the change in BF and the change in cross-sectional area during the reflection-free (early systolic wave) period of the cardiac cycle:

$$PWV_{BF} = (\Delta V / \Delta A)$$

Where V is blood volume and A is the lumen area (Vulli moz, *et al.*).

### *Sample Size*

Using a clinically meaningful mean difference of 1.0 m/s (Wilkinson, McEniery, Schillaci, Boutouyrie, Segers, Donald & Chowienczyk) and a typical PWV error of 1.27 m/s (Butlin, *et al.*), with the maximum chances of a type 1 error set at 5% (i.e. very unlikely), and a Type II error of 20% (unlikely), the approximate number of participants required is 27 (Hopkins, *et*

*al.*). To account for unknown sources of variation, loss of data, and to ensure an equitable randomization, the sample size was inflated to 32.

### *Statistical Analysis*

Statistical analyses were performed using Statistical Package for Social Sciences version 24 (SPSS, Inc., Chicago, Illinois). All data are reported as means and standard deviation (SD) unless otherwise stated. The  $\alpha$  was set at  $p < 0.05$  (two tailed). Two measures of validity were used to determine agreement between test and criterion devices: i) aSEE, and ii) sSEE. The aSEE was calculated as:  $aSEE = SD \times \sqrt{(1-r^2)}$  (Fraser ; Townsend, *et al.*), whereby SD is the SD of the criterion measure and  $r$  is the Pearson product-moment correlation between single-point and the 2-point criterion PW. The sSEE was calculated by dividing aSEE by the SD of the criterion, whereby  $< 0.20$  is considered a trivial difference, 0.2-0.6 small, 0.6-1.2 moderate, 1.2-2.0 large and  $> 2.0$  very large difference (Fraser). Relative standard of error (RSE) was also calculated by dividing the aSEE by the PWV mean and multiplying it by 100.

## **Results**

Thirty-two healthy participants (50% female) were recruited with 31 participants' (age:  $25.7 \pm 5.8$  years; BMI:  $24.7 \pm 3.3$  kg·m<sup>2</sup>), data being used in all analyses. One female participant who did not notably differ from the other participants in anyway was excluded, as the PT artery images could not be analysed.

For both supine and seated positions, the aSEE target of  $< 1.0$  m·s was not achieved for the single-point assessments of PWV in either the PT or SF arteries during both supine and seated positions (Table 1). The most accurate single point measures were found using the calculation

for  $PWV_{\beta}$  in both the SF and PT arteries during both supine (SF aSEE = 1.7 and PT 2.7 m·s) and seated (SF aSEE = 1.5 and PT 3.0 m·s) postural positions. Whereas the least accurate calculation was that based on BF ( $PWV_{BF}$ ) in both the SF and PT arteries during both supine (SF aSEE = 5.5 and PT 6.1 m·s) and seated (SF aSEE = 3.4 and PT 5.6 m·s) positions.

**Table 1.** Comparison of single-point assessments of pulse wave velocity against the criterion 2-point assessment using three different equations in both supine and seated postural positions.

PWV Measure	Single-point		Comparison: 2-point vs. Single-point				
	PWV (m·s)		$\Delta$ 2P - 1P (m·s)		aSEE	sSEE	RSE
	X	(SD)	X	(SD)	(m·s)		%
<b>Supine Position</b>							
<b>Criterion 2-Point</b>	9.8	1.2	-				
$\beta$ -stiffness SF	8.0	1.8	1.8	1.9	1.7	1.5	17.8
B-Hill SF	9.2	2.1	0.6	2.1	2.0	1.7	20.6
Blood Flow SF	9.7	5.5	0.1	5.6	5.5	4.7	56.0
$\beta$ -stiffness PT	9.9	2.9	-0.1	2.8	2.7	2.4	28.0
B-Hill PT	11.4	3.4	-1.6	3.2	3.2	2.8	33.1
Blood Flow PT	9.7	6.5	0.1	6.2	6.1	5.3	62.5
<b>Seated Position</b>							
<b>Criterion 2-Point</b>	12.9	1.9	-				
$\beta$ -stiffness SF	9.2	1.5	3.7	2.3	1.5	0.8	11.6
B-Hill SF	10.4	1.6	2.5	2.4	1.6	0.8	12.6
Blood Flow SF	9.9	3.4	3.0	4.1	3.4	1.8	26.5
$\beta$ -stiffness PT	11.2	3.1	1.7	3.9	3.0	1.6	23.4
B-Hill PT	12.7	3.5	0.2	4.2	3.4	1.8	26.6
Blood Flow PT	8.9	5.6	4.0	6.2	5.6	2.9	43.3

PWV= pulse wave velocity; SF= Superficial Femoral; PT= Posterior Tibialis; B-Hill= Bramwell Hill;  $\beta$ -beta; X= mean; aSSE= absolute standard error of estimates; sSSE= standard indicator of error; RSE%= relative standard error; m·s= meters per second; 1P= Single-point assessment; 2P= criterion two-point assessment;  $\Delta$ = delta score.

Data presented in Table 2 shows that single-point measures are all sensitive to a perturbation, as all PWV calculations increased from supine to seated positions. Similar to the direct comparisons in Table 1, Table 2 shows that  $PWV_{\beta}$  has the closest agreement of all the equations in both the  $\Delta$  SF and  $\Delta$  PT arteries (SF aSSE 1.7, PT aSSE 4.2 m·s), as well as the smallest differences (SF sSEE = 1.1 and PT sSEE = 2.8).

**Table 2.** Delta ( $\Delta$ ) of single-point assessments derived from PWV in seated vs. supine, showing direction of change due to postural alterations.

PWV Measure	2-Point ( $\Delta$ m·s)		Single-point ( $\Delta$ m·s)		Mean Diff (m·s).		aSEE	sSSE	RSE
	X	(SD)	X	(SD)	X	(SD)			
$\Delta$ PWV( $\beta$ )SF	3.1	(1.5)	1.2	(1.7)	1.9	(2.2)	1.7	1.1	54.0
$\Delta$ PWV(BH)SF	3.1	(1.5)	1.2	(1.9)	1.9	(2.3)	1.9	1.3	61.8
$\Delta$ PWV(BF)SF	3.1	(1.5)	0.2	(5.0)	2.9	(5.4)	5.0	3.4	161.3
$\Delta$ PWV( $\beta$ )PT	3.1	(1.5)	1.3	(4.3)	1.8	(4.9)	4.2	2.8	134.6
$\Delta$ PWV(BH)PT	3.1	(1.5)	1.3	(4.9)	1.8	(5.4)	4.8	3.2	153.3
$\Delta$ PWV(BF)PT	3.1	(1.5)	-0.8	(7.7)	3.9	(8.0)	7.6	5.1	245.8

PWV= pulse wave velocity; SF= Superficial Femoral; PT= Posterior Tibialis; B-Hill= Bramwell Hill;  $\beta$ -beta; X= mean; aSEE= absolute standard error of estimates; sSSE= standard indicator of error; RSE%= relative standard error; 1P= Single-point assessment; 2P= criterion two-point assessment;  $\Delta$ = delta score; m·s= meters per second.

## Discussion

With the recent interest into the detrimental effects of sedentary behaviours such as prolonged sitting on vascular health (Thosar, Bielko, Mather, Johnston & Wallace ; Vranish, Young, Kaur, Patik, Padilla & Fadel), there is a need to develop simple, time efficient, mechanistic tools to enable researchers and clinicians to determine arterial health in different postural positions. The current study demonstrates that single-point estimates at the SF and PT arteries did not meet the validity criteria set at  $<1.0\text{m}\cdot\text{s}$  for both aSSE and sSSE. The SF PWV $_{\beta}$  had the closest agreement to the criterion 2-point PWV in both supine (sSSE = 1.5; aSSE = 1.7 m·s) and seated (sSSE = 0.8; aSSE = 1.5 m·s) positions. Additionally, the SF PWV $_{\beta}$  estimate was most closely aligned with the criterion 2-point when a change in posture occurred (seated vs. supine), showing only a moderate (Hopkins) difference (sSSE = 1.1; aSSE = 1.7 m·s). Conversely, the PWV $_{\text{BF}}$  calculation had the least agreement with the 2-point criterion in both supine and seated positions.

### *Study limitations and strengths*

In order to better contextualize the present findings, several limitations and strengths should be considered. Firstly, we used only young health individuals and so the findings cannot be applied to older or diseased populations. Secondly, for both the  $PWV_{BH}$  and  $PWV_{\beta}$  calculations, blood density is a component of the equation and we assumed this to be  $1059 \text{ kg/m}^3$  based on the work by (Harada, Okada, Niki, Chang & Sugawara). Given that the current study sample consisted of young, healthy individuals, and the nature of the research question is within-subjects based, the constant is likely an accurate representation in both postural positions. Third, single-point ultrasound-based methods for measuring arterial stiffness assumes that early systole is unidirectional and reflectionless, which is important because the pressure and flow waves are likely congruous during this period (Townsend, Wilkinson, Schiffrin, Avolio, Chirinos, Cockcroft, Heffernan, Lakatta, McEniery & Mitchell). There is strong evidence to show that the early systolic period of the pressure wave is indeed reflectionless (Vulliémoz, Stergiopoulos & Meuli). Fourth, given the relatively small diameter and anatomical location of the PT, collecting clear diameter and flow measurements was difficult. However, given the sonographer had over 18-years' experience determining vascular measurements using ultrasound, we are confident that data is truly representative. However, to ensure accuracy, all measures were averaged over at least five consecutive cardiac cycles, and subsequently averaged to give a single value.

#### *Comparison with previous studies*

As far as the authors are aware, no previous study has directly compared single-point calculations of PWV with a criterion regional 2-point PWV assessment; further there has been no comparison in response to a postural change. In the current study, the  $PWV_{\beta}$  equation appears to be the most robust when comparing single-point calculations with the criterion 2-point PWV. Irrespective of arterial location (SF and PT), or postural position, Tables 1 and 2

show that the aSEE, sSEE and RSE% are consistently smaller for  $PWV_{\beta}$  compared to the other calculations. This smaller error using  $PWV_{\beta}$  may be due to a greater dependency on pressure. Recently, our laboratory demonstrated that  $PWV_{BF}$ ,  $PWV_{BH}$  and  $PWV_{\beta}$  are all pressure dependent (Zieff, Heffernan, Stone, Fryer, Credeur, Hanson, Faulkner & Stoner). However, it may be that although all are pressure dependent, these different stiffness calculations may not be equally as dependent on pressure as each other. For example, it may be that  $PWV_{BF}$  is less pressure dependant, as blood pressure is not part of the equation, and thus it will likely have a smaller influence on the calculation. Whereas, previously it has been reported that  $PWV_{\beta}$  is more heavily dependent on pressure (Lim, *et al.* ; Schroeder, *et al.* ; Tanaka), and thus the calculation may well be more affected, particularly during a postural change.

As previously mentioned, it would appear that single-point PWV in the SF artery when calculated using  $\beta$ -stiffness is associated with the least error (aSSE & sSSE Table 1). However, it is important to note that neither the SF nor PT arteries track perfectly. Mechanistically, the SF and PT might not have met validity criteria ( $\leq 1m \cdot s$ ) perfectly because a 2-point assessment of PWV tracks across two different arteries, and thus represents a measure of regional stiffness. The PT artery is a more muscular artery as it sits further down the vascular tree, and so it would likely have a reduced compliance compared to the SF artery (Zieman, *et al.*). In addition, the PT artery includes the more tortuous knee, which would likely cause disruption to both flow and diameters, and this could be further compounded during sitting as lower limb blood pooling would likely occur, potentially impacting local haemodynamics (Stone, *et al.*). This disruption in blood flow may explain why  $PWV_{BF}$  has the greatest error and the largest mean difference compared to the 2-point criterion in both seated and supine positions. This in turn may explain why  $PWV_{BF}$  at the PT is not higher, as would be expected, than that at the SF (Table 1). Given that arterial stiffness is not uniform throughout the vascular tree and is often considered

'patchy' (Galis & Khatri), the use of a single-point calculation using  $PWV_{\beta}$ , may be better used to provide additional important information about the effects of prolonged sitting on site specific vascular function. However, further investigation into this to ensure validity is warranted.

As previously mentioned, findings from the current study suggest that single-point calculations should not be used as a direct proxy for 2-point PWV. However, whilst an additional measure is not time efficient, single-point calculations might help scientists in further understanding the effects of sedentary behaviour. For example, understanding the association between single-point and 2-point leg PWV may be of use when investigating the long-term effects of sedentary behaviour and cardiovascular health, as the gradient of central and peripheral arterial stiffness changes with age (Hickson, *et al.*). Hickson, Nichols, McDonnell, Cockcroft, Wilkinson and McEniery (found that with aging ( $\geq 50$  years) a reversed stiffness gradient occurred as the aorta became less compliant than the peripheral arteries (femoral-dorsalis pedis). This reversal was associated with an increased reflection site distance and a paradoxical increase in augmentation pressure and augmentation index. As such, gaining a greater understanding of the interactions at several single peripheral arterial sites (permitting the identification of arterial stiffness gradient), as well as the interactions between central and peripheral arterial sites, maybe important in understanding physiological mechanisms, and developing new diagnostic tools to aid with identifying cardiovascular disease risk.

### *Clinical perspectives and future directions*

With the recent increased interest into the effects of sedentary behaviours on cardiometabolic and cardiovascular health (Thosar, Bielko, Mather, Johnston & Wallace ; Vranish, Young,



Kaur, Patik, Padilla & Fadel), there remains a need to find new time efficient measures to determine key markers of health such as PWV in different postural positions. The current study suggests that single-point SF  $PWV_{\beta}$  may provide alternate additional information to the use of a conventional regional 2-point PWV assessment. The authors recognise that given the moderate aSEE and sSEE, single-point and 2-point PWV cannot be directly compared. However, SF  $PWV_{\beta}$  does make for the closest comparison, and is the one that changes most similarly with a perturbation. Whilst a single-point assessment requires half the time of a 2-point assessment for both the measurement and the analysis, this information should be used as complimentary to the existing 2-point measure of PWV, but should not be used instead of. As such, future research should try and determine more accurate single-point PWV calculations. Valid and reliable single-point measures of PWV would be time efficient and as such be of benefit to large scale epidemiological studies. Additionally, investigating time efficient measures using ultrasound techniques is important as these devices provide more diagnostic information compared to quicker osscliometric devices.

### *Conclusion*

In brief, the purpose of the current study was to determine the validity of different calculations of single-point PWV compared to the criterion 2-point regional assessment in both seated and supine positions. Neither the SF or PT artery met the validity criteria of 1.0 m·s. However, findings suggest that the SF artery most closely aligns to the criterion 2-point assessment, and the  $PWV_{\beta}$  estimation is associated with the least error, and responds most similarly to a postural change.

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