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

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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}), β-stiffness index ($PWV_{ß}$), 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 <1.0m·s. Findings show that for both postural positions, the absolute standard error of estimates (aSEE) criterion of <1.0 m·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 $PWV_{ß}$ in both supine (SF aSEE = 1.7 vs. PT 2.7m·s) and seated (SF aSEE = 1.5 vs. PT 3.0m·s) positions. All single-point ΔPWV (supine – seated) calculations were higher in sitting, with $PWV_{ß}$ having the closest agreement (ΔSF aSEE 1.7m·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 ß-stiffness (PWV$_{ß}$), 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$_{ß}$, 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$_{ß,}$ 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 m·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. 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: $3.14 \times (\text{diameter}/2)^2 \times$ mean blood velocity x 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: $PWV_{BF,}$ $PWV_{BH,}$ and $PWV_{ß}$.

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

$$PWV_{ß} = \sqrt{(ß \cdot DBP)/(2p)}$$

Where; p is the blood density (1059 kg/m^3)(Harada, *et al.*) and *ß* is the *ß*-stiffness index, which is calculated using the formula:

$$ß = \ln(SBP/DBP)/[(Ds-Dd)/Dd]$$

where ln is the natural logarithm, SBP is systolic blood pressure, DBP is diastolic blood pressure, Ds is the lumen diameter during systole, and Dd 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^3)(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 ΔP 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 (Vulliémoz, *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 α 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 x $\sqrt{(1-r^2)}$ (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 ± 5.8 years; BMI: 24.7 ± 3.3 kg·m^2), 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 $PWV_ß$ 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.

-----------Insert Table 1 Near Here-----------

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 $PWV_ß$ has the closest agreement of all the equations in both the Δ SF and Δ 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).

-----------Insert Table 2 Near Here-----------

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 <1.0m·s for both aSSE and sSSE. The SF $PWV_{ß}$ 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 $PWV_{ß}$ 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_{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 $PWV_{ß}$ calculations, blood density is a component of the equation and we assumed this to be 1059 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, Stergiopulos & 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 $PWV_{ß}$ 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 $PWV_{ß}$ compared to the other calculations. This smaller error using $PWV_{ß}$ may be due to a greater dependency on pressure. Recently, our laboratory demonstrated that PWV_{BF}, PWV_{BH} and $PWV_{ß}$ 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 $PWV_{ß}$ 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 β-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 (≤ 1m·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 $PWV_{ß}$, 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, finding 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 be 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 (≥50 years) a reversed stiffness gradient occurred as the aorta became less compliant than the peripheral arteries (femoral-dosalis 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 a 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 $PWV_{ß}$ 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 $PWV_{ß}$ 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_{β} estimation is associated with the least error, and responds most similarly to a postural change.

Competing Interests

None of the authors on this manuscript have any conflicts of interest

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Barone Gibbs B, Kowalsky RJ, Perdomo SJ, Taormina JM, Balzer JR and Jakicic JM. Effect of alternating standing and sitting on blood pressure and pulse wave velocity during a simulated workday in adults with overweight/obesity. *J Hypertens* (2017); **35**: 2411-2418.

Butlin M, Qasem A, Battista F, Bozec E, McEniery CM, Millet-Amaury E, Pucci G, Wilkinson IB, Schillaci G and Boutouyrie P. Carotid-femoral pulse wave velocity assessment using novel cuff-based techniques: comparison with tonometric measurement. *J Hypertens* (2013); **31**: 2237-2243.

Credeur DP, Miller SM, Jones R, Stoner L, Dolbow DR, Fryer SM, Stone K and McCoy SM. Impact of Prolonged Sitting on Peripheral and Central Vascular Health. *The American journal of cardiology* (2019); **123**: 260-266.

Fraser CG. *Biological variation: from principles to practice* (2001). Amer. Assoc. for Clinical Chemistry.

Galis ZS and Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. *Circ Res* (2002); **90**: 251-262.

Harada A, Okada T, Niki K, Chang D and Sugawara M. On-line noninvasive one-point measurements of pulse wave velocity. *Heart Vessels* (2002); **17**: 61-68.

Hickson SS, Nichols WW, McDonnell BJ, Cockcroft JR, Wilkinson IB and McEniery CM. Influence of the central-to-peripheral arterial stiffness gradient on the timing and amplitude of wave reflections. *Hypertens Res* (2016); **39**: 723.

Hopkins W, Marshall S, Batterham A and Hanin J. Progressive statistics for studies in sports medicine and exercise science. *Med Sci Sports and Ex* (2009); **41**: 3.

Hopkins WG. Measures of reliability in sports medicine and science. *Sports Med* (2000); **30**: 1-15.

Jadhav UM and Kadam N. Non-invasive assessment of arterial stiffness by pulse-wave velocity correlates with endothelial dysfunction. *Indian Heart J* (2005); **57**: 226-232.

Kawasaki T, Sasayama S, Yagi S-I, Asakawa T and Hirai T. Non-invasive assessment of the age related changes in stiffness of major branches of the human arteries. *Cardiovasc Res* (1987); **21**: 678-687.

Lim J, Pearman M, Park W, Alkatan M, Machin DR and Tanaka H. Impact of blood pressure perturbations on arterial stiffness. *American Journal of Physiology-Heart and Circulatory Physiology* (2015).

Lim J, Pearman M, Park W, Alkatan M and Tanaka H. Interrelationships among various measures of central artery stiffness. *Am J Hypertens* (2016); **29**: 1024-1028.

McEniery CM, Wallace S, Mackenzie IS, McDonnell B, Newby DE, Cockcroft JR and Wilkinson IB. Endothelial function is associated with pulse pressure, pulse wave velocity, and augmentation index in healthy humans. *Hypertens* (2006); **48**: 602-608.

McManus AM, Ainslie PN, Green DJ, Simair RG, Smith K and Lewis N. Impact of prolonged sitting on vascular function in young girls. *Exp Physiol* (2015); **100**: 1379-1387.

Morishima T, Restaino RM, Walsh LK, Kanaley JA, Fadel PJ and Padilla J. Prolonged sitting-induced leg endothelial dysfunction is prevented by fidgeting. *Am J Physiol Heart Circ Physiol* (2016); **311**: H177-H182.

Naka KK, Tweddel AC, Doshi SN, Goodfellow J and Henderson AH. Flow-mediated changes in pulse wave velocity: a new clinical measure of endothelial function. *Eur Heart J* (2005); **27**: 302-309.

Puri K, Suresh K, Gogtay N and Thatte U. Declaration of Helsinki, 2008: implications for stakeholders in research. *J Postgrad Med* (2009); **55**: 131.

Restaino RM, Holwerda SW, Credeur DP, Fadel PJ and Padilla J. Impact of prolonged sitting on lower and upper limb micro-and macrovascular dilator function. *Exp Physiol* (2015); **100**: 829-838.

Restaino RM, Walsh LK, Morishima T, Vranish JR, Martinez-Lemus LA, Fadel PJ and Padilla J. Endothelial dysfunction following prolonged sitting is mediated by a reduction in shear stress. *Am J Physiol Heart Circ Physiol* (2016); **310**: H648-H653.

Schneider CA, Rasband WS and Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* (2012); **9**: 671.

Schroeder EC, Rosenberg AJ, Hilgenkamp TI, White DW, Baynard T and Fernhall B. Effect of upper body position on arterial stiffness: influence of hydrostatic pressure and autonomic function. *J Hypertens* (2017); **35**: 2454-2461.

Stone K, Fryer S, Kelsch E, Burnet K, Zieff G, Faulkner J, Credeur D, Lambrick D, Hanson ED and Stoner L. Validity and reliability of lower-limb pulse-wave velocity assessments using an oscillometric technique. *Exp Physiol* (2019); **104**: 765-774.

Stoner L, Erickson ML, Young JM, Fryer S, Sabatier MJ, Faulkner J, Lambrick DM and McCully KK. There's more to flow-mediated dilation than nitric oxide. *J Atheroscler Thromb* (2012); **19**: 589-600.

Stoner L, Faulkner J, Fryer S and Lambrick DM. Does Circumferential Stress Help to Explain Flow-Mediated Dilation? *Ultrasound Quarterly* (2013); **29**: 103-110.

Stoner L, Tarrant MA, Fryer S and Faulkner J. How should flow-mediated dilation be normalized to its stimulus? *Clin Physiol Funct Imaging* (2013); **33**: 75-78.

Stoner L, Young JM and Fryer S. Assessments of arterial stiffness and endothelial function using pulse wave analysis. *Int J Vasc Med* (2012); **2012**.

Sun Z. Aging, arterial stiffness, and hypertension. *Hypertens* (2015); **65**: 252-256.

Tanaka H. Various indices of arterial stiffness: are they closely related or distinctly different? *Pulse* (2017); **5**: 1-6.

Thijssen DH, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, Parker B, Widlansky ME, Tschakovsky ME and Green DJ. Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. *Am J Physiol Heart Circ Physiol* (2010); **300**: H2-H12.

Thosar SS, Bielko SL, Mather KJ, Johnston JD and Wallace JP. Effect of prolonged sitting and breaks in sitting time on endothelial function. *Med Sci Sports and Ex* (2015); **4**: 843-849.

Thosar SS, Bielko SL, Wiggins CC and Wallace JP. Differences in brachial and femoral artery responses to prolonged sitting. *Cardiovasc Ultrasound* (2014); **12**: 50.

Townsend RR, Wilkinson IB, Schiffrin EL, Avolio AP, Chirinos JA, Cockcroft JR, Heffernan KS, Lakatta EG, McEniery CM and Mitchell GF. Recommendations for improving and standardizing vascular research on arterial stiffness: a scientific statement from the American Heart Association. *Hypertens* (2015); **66**: 698-722.

Van Bortel LM, Duprez D, Starmans-Kool MJ, Safar ME, Giannattasio C, Cockcroft J, Kaiser DR and Thuillez C. Clinical applications of arterial stiffness, Task Force III: recommendations for user procedures. *Am J Hypertens* (2002); **15**: 445-452.

Van Bortel LM, Laurent S, Boutouyrie P, Chowienczyk P, Cruickshank J, De Backer T, Filipovsky J, Huybrechts S, Mattace-Raso FU and Protogerou AD. Expert consensus document on the measurement of aortic stiffness in daily practice using carotid-femoral pulse wave velocity. *J Hypertens* (2012); **30**: 445-448.

Von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC and Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies. *Int J Surg* (2014); **12**: 1495-1499.

Vranish JR, Young BE, Kaur J, Patik JC, Padilla J and Fadel PJ. Influence of sex on microvascular and macrovascular responses to prolonged sitting. *Am J Physiol Heart Circ Physiol* (2017); **312**: H800-H805.

Vulliémoz S, Stergiopulos N and Meuli R. Estimation of local aortic elastic properties with MRI. *Magnetic Resonance in Medicine: An Official Journal of the International Society for Magnetic Resonance in Medicine* (2002); **47**: 649-654.
Wilkinson IB, McEniery CM, Schillaci G, Boutouyrie P, Segers P, Donald A and Chowienczyk PJ. ARTERY Society guidelines for validation of non-invasive haemodynamic measurement devices: Part 1, arterial pulse wave velocity. *Artery Research* (2010); **4**: 34-40.
Zieff GH, Heffernan K, Stone K, Fryer S, Credeur D, Hanson ED, Faulkner J and Stoner L. The pressure-dependency of local measures of arterial stiffness. *J Hypertens* (2018).
Zieman SJ, Melenovsky V and Kass DA. Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arterioscler Thromb Vasc Biol* (2005); **25**: 932-943.