



This is a peer-reviewed, post-print (final draft post-refereeing) version of the following published document, © 2018 Elsevier Inc. and is licensed under Creative Commons: Attribution-Noncommercial-No Derivative Works 4.0 license:

Credeur, Daniel, Miller, Sabina M, Jones, Raymond, Stoner, Lee, Dolbow, David R, Fryer, Simon M ORCID logoORCID: <https://orcid.org/0000-0003-0376-0104>, Stone, Keeron J ORCID logoORCID: <https://orcid.org/0000-0001-6572-7874> and McCoy, Stephanie M (2019) Impact of Prolonged Sitting on Peripheral and Central Vascular Health. American Journal of Cardiology, 123 (2). pp. 260-266. doi:10.1016/j.amjcard.2018.10.014

Official URL: <https://doi.org/10.1016/j.amjcard.2018.10.014>
DOI: <http://dx.doi.org/10.1016/j.amjcard.2018.10.014>
EPrint URI: <https://eprints.glos.ac.uk/id/eprint/6202>

Disclaimer

The University of Gloucestershire has obtained warranties from all depositors as to their title in the material deposited and as to their right to deposit such material.

The University of Gloucestershire makes no representation or warranties of commercial utility, title, or fitness for a particular purpose or any other warranty, express or implied in respect of any material deposited.

The University of Gloucestershire makes no representation that the use of the materials will not infringe any patent, copyright, trademark or other property or proprietary rights.

The University of Gloucestershire accepts no liability for any infringement of intellectual property rights in any material deposited but will remove such material from public view pending investigation in the event of an allegation of any such infringement.

PLEASE SCROLL DOWN FOR TEXT.

Impact of Prolonged Sitting on Peripheral and Central Vascular Health

Daniel P. Credeur, PhD, Sabina M. Miller, MS, Raymond Jones, MS, Lee Stoner, PhD, MPH,
David R. Dolbow, DPT, PhD, Simon M. Fryer, PhD, Keeron Stone, MS, and Stephanie M. McCoy, PhD, MPH.

Abstract

Prolonged, uninterrupted sitting negatively impacts markers of peripheral vascular health, particularly, vasodilatory function of leg arteries. Whether sitting can similarly impact measures of central vascular health, as well as overall leg vasoreactivity (i.e., vaso-dilatory and vasoconstrictor function) remains unknown. To address this, measurements were made in relatively healthy participants (i.e., free of overt disease; $n = 20$, age = 26 ± 7 ; body mass index = 30 ± 7 kg/m²; 7 female) pre, during and post 3 hours of uninterrupted sitting. Measures of central vascular health included arterial wave reflection (augmentation index and Reflection Magnitude — RM%) and aortic vascular stiffness (aortic pulse wave velocity). Local vasoreactivity of the distal, posterior tibial artery was measured using flow-mediated dilation—FMD, coupled with low-flow mediated constriction, and microvascular function was assessed through the total hyperemic blood velocity (area-under-curve) response during FMD. After sitting, there was a significant increase in aortic pulse wave velocity (pre sit = 5.7 ± 0.3 vs post sit = 6.1 ± 0.3 m/s; $p = 0.009$, $d = 0.36$), whereas, augmentation index decreased (pre sit = 13 ± 3 vs post sit = $3 \pm 1\%$; $p < 0.001$, $d = 0.71$). Albeit a moderate effect for decrease, RM % was not significantly altered during sitting ($p = 0.13$, $d = 0.3$). Vasodilatory (i.e., FMD pre sit = 0.5 ± 0.04 vs post sit = -0.3 ± 0.04 mm; $p = 0.014$, $d = 0.29$) and microvascular function (i.e., Microvascular area — under — curve: pre sit = $2,196 \pm 333$ vs $1,571 \pm 172$ AU; $p = 0.003$, $d = 0.31$) decreased, but vasoconstrictor function (low-flow mediated constriction; $p = 0.85$, $d = 0.005$) was unaffected by sitting. In conclusion, these data demonstrate that a prolonged bout of uninterrupted sitting negatively impacts markers of peripheral and central vascular health in relatively healthy adults. © 2018 Elsevier Inc. All rights reserved. (Am J Cardiol 2018; 00:1–7)

Introduction

Recent studies have sought to characterize the cardiovascular health response to periods of prolonged sitting; however, a majority has focused largely on peripheral artery vasodilatory function.^{1, 2} While a reduction in vasodilatory function after sitting is clinically meaningful, examining vasoconstrictor function could compliment this measurement, thus, providing an assessment of overall vasoreactivity.^{3, 4} Further, few studies have reported on the central hemodynamic response to prolonged sitting, and of these, peripheral blood pressures were the primary outcome.^{5, 6} Although it has been established the accumulation of sitting bouts and resultant long-term exposure to sedentariness can increase central cardiovascular burden,⁷ it is unclear whether an acute period of sitting could initiate this process. As such, the purpose of this study was to determine whether a single bout of prolonged, uninterrupted sitting (3 hours) affects markers of both peripheral (i.e., vasodilatory and vasoconstrictor function) and central vascular health (i.e., aortic: pressures, wave reflection, and vascular stiffness). Using a pre–post design, we

hypothesized prolonged sitting would reduce overall leg vasoreactivity but increase central vascular stiffness and arterial wave reflection in relatively healthy adults

Methods

All procedures and protocols conformed to the *Declaration of Helsinki* and were reviewed and approved by the University of Southern Mississippi Institutional Review Board (Protocol Approval #16061301). Twenty volunteers were recruited. Inclusion criteria consisted of: men and women (18 to 55 years), free of any diagnosed cardiovascular, metabolic or neurological diseases, nonsmokers, asymptomatic (i.e., resting blood pressure <140/90, and no signs of arrhythmias), and not pregnant. To account for potential influences of hormonal status on study outcomes, premenopausal women were studied during the early follicular phase of their menstrual cycle or during placebo phase of oral contraceptive use.

Each experiment lasted ~5 hours. Participants arrived in the morning (8:00 to 11:00 am) at least 2-hours fasted, having refrained from caffeine for 12-hours as well as alcohol and strenuous physical activity for 24-hours. After providing written informed consent, participants completed a medical health history form followed by assessments of height and weight. Participants positioned themselves supine on the exam table and were instrumented for experimental measurements (Figure 1 for protocol timeline). After 15 minutes of quiet rest, baseline (pre-sit) testing commenced. After baseline, participants positioned themselves in a chair next to the exam table where they remained for 3 hours. While seated, participants were allowed to perform standard tasks (e.g., read a book, work on a laptop), but were asked to refrain from boisterous activities (e.g., listening to heavy music). In addition, participants were allowed to move their upper-body but not their legs, that is, fidgeting.⁸ To ensure protocol compliance, two research assistants observed the participants during sitting. At 10 minutes, cardiovascular measures (ECG, pulse wave analysis and shear rate) were performed, and repeated every hour of sitting. After 180-minutes, participants were hoisted through a mechanical lift (Invacare Reliant 450, Invacare, Elyria, Ohio) and positioned supine on the exam table, followed by postsitting assessments. After study completion, each participant was given a triaxial accelerometer (Actigraph, wGT3X-BT, Pensacola, FL) to wear for 7 days to determine physical activity status.

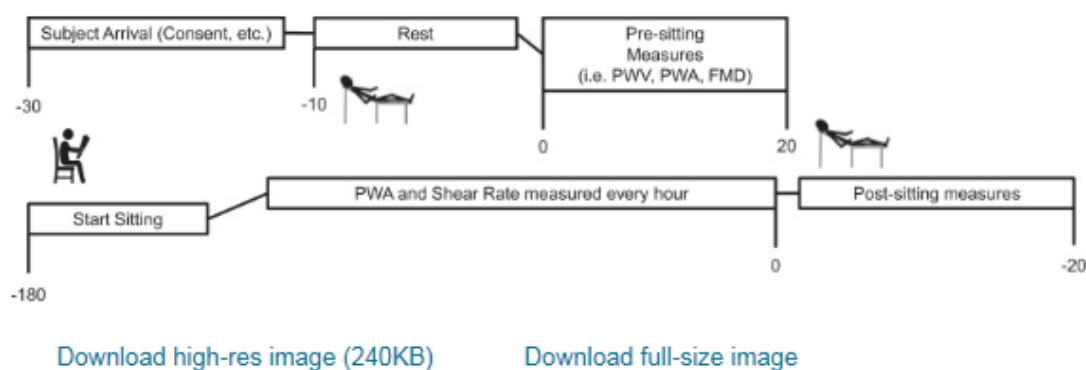


Figure 1. Protocol timeline for experimental measurements.

Heart rate (HR) and R-R variability (HRV) were monitored continuously, but recorded for 2 minutes during each time point through surface electrocardiography (Powerlab Bioamp, AD-Instruments, Dunedin, Otago). Central

hemodynamics and aortic vascular stiffness were measured through automated sphygmomanometry and applanation tonometry, respectively (SphygmoCor XCEL, AtCor Medical, Itasca, Illinois). Two measures were taken during each time point and averaged. Carotid-femoral pulse transit time (Δt in seconds) was determined through tonometry over the left common carotid artery, coupled with cuff oscillometry over the left upper-thigh. Prior to this, distance measures (m) were obtained between the carotid pulse site and sternal notch ($L1$), and sternal notch to proximal edge of the thigh cuff ($L2$). Along with Δt , distance was applied to the following equation: aortic pulse wave velocity (aPWV) = $Length (L2 - L1)/\Delta t$. To note, aPWV data were not collected in 3 participants due to limitations in cuff size (i.e., limb circumference exceeded optimal range of cuff for an accurate measurement).

All vascular function assessments were performed pre- and postsitting (supine) by the same sonographer using the flow-mediated dilation (FMD) technique⁹ with a duplex Doppler-ultrasound (Logiq P5; GE Medical systems, Milwaukee, WI). To do this, posterior tibial artery images were obtained ~5 cm superior to the calcaneus and ~1 cm posterior to the medial malleolus on the left ankle with a 11-MHz linear array transducer, as previously performed.¹⁰ Blood velocity signals were obtained using the same probe in pulse wave mode, at a frequency of 5 MHz and insonation angle of 60°. Using video capture (El Gato; San Francisco, CA), 2 minutes of baseline diameter and blood velocity data were recorded, after which a pneumatic cuff (Hokanson) positioned ~1 cm distal from the fibular head was inflated (220 mm Hg) for 5 minutes. Video capture resumed through cuff occlusion and for 3 minutes post cuff-release. Doppler-ultrasound analog signals were video outsourced (30 Hz) and stored as AVI files for later processing. During sitting, diameter and blood velocity signals were also obtained (1 minute recordings) after 10 minutes, and every hour of sitting. Venous pooling was estimated from calf circumference measures over the course of sitting. To do this, a tape measure was positioned over the largest portion of the right calf.

Data analysis

Both HR and HRV data were analyzed using LabChart software (AD-Instruments, Colorado Spring, CO). For HRV, both time [e.g., standard deviation of R-R intervals (SDNN) and root mean square of successive differences] and frequency domain indices [e.g., high frequency (HF) and low frequency (LF), and LF/HF ratio measured through power spectral analysis] were averaged over each testing period (2-minutes) to provide an index of overall autonomic balance (i.e., sympathetic and vagal input to HR).¹¹ To determine physical activity status (i.e., active vs inactive), bouts of moderate (3-6 METs) and vigorous activity (>6 METs) were based off the Freedson 1998 cut points which are 2,020 and 5,999 counts, respectively, consistent with NHANES physical activity data reduction.¹²

Oscillometric brachial pressure waveforms were analyzed by the XCEL device using a validated transfer function¹³ to derive the following: central systolic blood pressure, diastolic, pulse pressure, mean arterial pressure—MAP, and augmentation pressure—AP. The AP is maximum systolic pressure minus the pressure at the inflection point. This value is then expressed as a percentage of total PP and is considered augmentation index (Aix). The generalized aortic pressure waveform is then decomposed into its forward—Pf and backward—Pb components.¹⁴ The reflection magnitude (RM%) is then calculated as $Pb/Pf \times 100$. Day-to-day reliability for Aix ($n = 9$) and aPWV ($n = 8$) performed in our laboratory yielded intraclass correlation coefficients of 0.88 and 0.81, respectively.

Posterior tibial artery diameters and blood velocities were analyzed off-line by one operator separate from the sonographer using an automated edge-detecting software (FMD Studio, QUIPU; Pisa, Italy). Data were consolidated into 1-second bins and processed through a custom calculation macro (visual basic; Microsoft Excel).³ Diameter and blood velocity data from the 2-minute baseline recording were averaged ($Diam_{rest}$), and the largest diameter after cuff release during the FMD was considered peak ($Diam_{peak}$), and used to calculate FMD in both absolute (mm) and relative (%) terms; i.e., FMD change = $(Diam_{peak} - Diam_{rest})/Diam_{rest} \times 100$. Shear rate (s^{-1}) was calculated as $4 \times \text{mean blood velocity}/\text{diameter}$. To determine the FMD stimulus, shear rate area-under-curve (AUC) up to 40 seconds (Shear AUC-40) after cuff release was calculated.¹⁵ The oscillatory shear index (OSI) was also determined: $OSI = \text{retrograde shear rate}/(\text{antegrade shear rate} + \text{retrograde shear}) \times 100$.^{10,16} Microvascular function was estimated from the hyperemic blood velocity (cm/s) AUC, that is, microvascular AUC, from cuff release to the point of return to baseline level, as previously performed.²

Low-flow mediated constriction (L-FMC) was calculated and combined with

FMD (FMD+L-FMC) to estimate overall vasoreactivity.³ L-FMC was defined as the nadir diameter value ($Diam_{nadir}$) obtained during final 30 seconds of occlusion during FMD, and expressed in absolute (mm) terms: $L-FMC = Diam_{rest} - Diam_{nadir}$. Resting arterial tone (%), which represents the baseline diameter expressed as a percentage of the vasoactive range was calculated: $Tone\% = (Diam_{peak} - Diam_{rest})/(Diam_{peak} - Diam_{nadir}) \times 100$.³ Day-to-day reliability ($n = 5$) for FMD and L-FMC performed in our laboratory yielded intraclass correlation coefficients of 0.819 and 0.870, respectively.

Sample size calculations were based on aPWV. While the effects of prolonged sitting on central hemodynamics have not been investigated, previous reports indicate that prolonged sitting reduces leg vasodilatory function ~57% to 80%.^{2, 8} Based on an aPWV = 6.6 m/s, which is expected for healthy adults <30 years,¹⁷ a 57% decrease would be 3.8 m/s. Presently, we opted to sample based on a more conservative change score of 1 m/s, which would be considered a large, and clinically meaningful change.¹⁸ Using a magnitude-based inference to estimate sample size, approximately $n = 12$ participants were required to address our hypotheses with ample power and effect. To substantiate comparisons, we chose to recruit a sample of $n = 20$.

To determine the impact of prolonged sitting (10 to 180 minutes) on central hemodynamics, one-way repeated measures ANOVAs were performed, with Bonferroni selected as the post-hoc test. Central hemodynamic variables (central diastolic BP, PP, MAP, and Pf) were not normally distributed (by way of Shapiro-Wilk tests) and analyzed with Friedman repeated measures ANOVA on ranks, with Tukey's pairwise multiple comparison selected as the post-hoc test. Paired t tests were performed to determine differences (central hemodynamics, aPWV, and vasoreactivity) between pre- vs postsitting time points. To account for differences in resting posterior tibial artery diameter, allometrically scaled FMD was examined and analyzed using a general linear model with diameter change expressed in the natural log ($\ln Diam_{peak} - \ln Diam_{rest}$), sitting time point (pre vs post) set as the fixed factor, and $\ln Diam_{rest}$ as the covariate.¹⁹ Effect sizes were calculated pairwise and reported using Cohen's d , where <0.20 is considered to be a small, >0.20 to <0.50 a moderate, and >0.60 a large effect. Statistical analyses were performed by way of Sigma-Plot

(Version 12, San Jose, CA) and IBM SPSS Statistics (Version 23, Armonk, NY). Significance was set a priori at $p < 0.05$, and moderate-large effect sizes were considered meaningful. Data are presented as mean \pm standard error.

Results

Participants were 26 ± 7 years and had a body mass index of 30 ± 7 kg/m². Within this cohort, 13 were males, and 7 females. In addition, 13 met federal physical activity recommendations, and 7 did not.²⁰ All central cardiovascular hemodynamic data, main effects (seated time points), and pairwise comparisons (pre vs post sitting time points) are reported in Table 1. From pre–post sitting time points there was no change in MAP ($p > 0.05$) (Figure 2), but a significant decrease in resting HR, AP, and AIx ($p < 0.05$) (Figure 2). There was no significant change noted for Pf pre–post sitting ($p > 0.05$), but a moderate, yet nonsignificant decrease in Pb and RM% (Figure 2). There was a moderate, statistically significant increase in aPWV (presit = 5.7 ± 1 vs postsit = 6.1 ± 1.1 m/s, $p = 0.009$, $d = 0.36$) (Figure 3). To explore the potential interaction of biologic sex, a secondary sensitivity analysis was performed on aPWV, and revealed that males exhibited an increase in aortic stiffening after prolonged sitting ($n = 11$; presit = 6.2 ± 0.2 vs postsit = 6.6 ± 0.2 m/s, $p = 0.004$), a response that was not observed in females ($n = 6$; presit = 4.9 ± 0.3 vs postsit = 5.0 ± 0.4 m/s, $p = 0.6$). Overall, there was no significant influence of physical activity status on central hemodynamics or vascular function in response to the sitting intervention ($p > 0.05$). Due to sample limitations, race/ethnic-based differences were not explored.

Table 1. Central cardiovascular hemodynamic response to prolonged sitting

	Pre sit	Seated (minutes)				Post sit	p value, Effect (Pre vs Post sit)	p value (Seated)
		10	60	120	180			
Heart rate (bpm)	65±2	69±2	69±2	66±3	67±3	60±2	<0.001, 0.56	0.013
Mean arterial pressure (mm Hg)	91±3	93±3	95±3	94±3	96±2	90±2	0.6, 0.09	0.36
Central systolic blood pressure (mm Hg)	113±3	112±3	113±3	113±3	115±3	113±3	0.75, 0.03	0.17
Central diastolic blood pressure (mm Hg)	76±2	81±2	83±2	82±3	83±2	77±2	0.88, 0.09	0.25
Central pulse pressure (mm Hg)	37±1	31±1	30±1	32±1	32±1	36±2	0.54, 0.19	0.63
Forward pressure component (mm Hg)	30±1	26±1	26±1	28±1	28±1	29±1	0.62, 0.19	0.44
Backward pressure component (mm Hg)	15±1	13±1	12±1	13±1	13±1	13±1	0.19, 0.4	0.44
Reflection magnitude (%)	50±2	49±2	47±1	47±1	49±2	47±2	0.13, 0.3	0.35
Augmentation pressure (mm Hg)	5±1	3±1	2±1	2±1	2±1	1±1	<0.001, 0.69	0.25
Augmentation index (%)	13±3	8±4	5±3	5±3	3±3	3±1	<0.001, 0.71	0.092

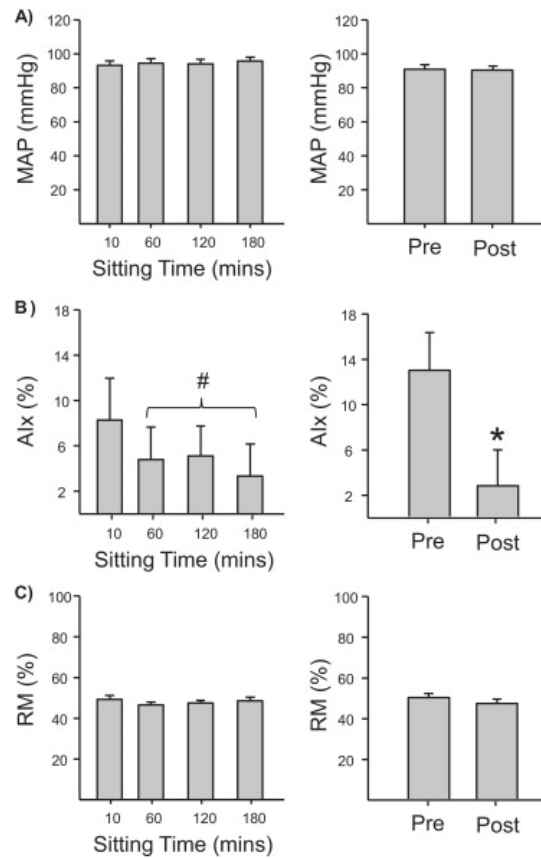


Figure 2. Mean summary data for mean arterial pressure (MAP) (**Panel A**), augmentation index (Alx) (**Panel B**), and reflection magnitude (RM%) (**Panel C**) in response to prolonged sitting. The left side of panels depicts the responses during sitting (i.e., 10, 60, 120, and 180 minutes), while the right side of panels shows data pre–post sitting when measured supine. *Denotes $p < 0.05$ versus Pre Sit; # $p < 0.05$ versus 10 minutes sitting.

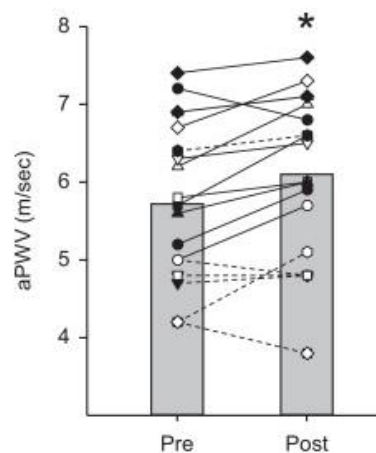


Figure 3. Summary data for aortic stiffness (aPWV) response to prolonged sitting. Individual data points and group average pre–post sitting are shown. Female participant ($n = 6$) responses are indicated by dashed lines. *Denotes $p < 0.05$ versus Pre Sit.

For HRV time domain data, there was a significant increase in SDNN (presit = 70 ± 33 vs postsit = 80 ± 39 ms, $p = 0.033$, $d = 0.27$) and root mean square of successive differences (presit = 68 ± 46 vs postsit = 85 ± 57 ms, $p = 0.003$,

$d = 0.35$). For frequency domain, there was a decrease in LF (presit = 35 ± 17 vs postsit = 27 ± 12 AU, $p = 0.019$, $d = 0.56$), but no change noted for HF ($p = 0.115$, $d = 0.4$), LF/HF ratio ($p = 0.145$, $d = 0.33$) and total power ($p = 0.18$, $d = 0.37$).

All vascular data, main effects, and pairwise comparisons are reported in Table 2. Over the course of sitting (10 to 180 minutes), there was a significant reduction in resting posterior tibial artery diameter, mean shear rate, and OSI ($p < 0.05$). After sitting (pre–post), there was a significant decrease in posterior tibial artery FMD (mm), allometrically scaled FMD (Figure 4), and overall vasoreactivity (FMD+L-FMC) ($p < 0.05$), but no change occurring for FMD% or L-FMC (mm) ($p > 0.05$). There was a nonsignificant trend for a reduction in Tone% ($p = 0.083$, $d = 0.2$) and the FMD stimulus (Shear AUC-40: $p = 0.073$, $d = 0.21$). Total blood velocity AUC during reactive hyperemia (i.e., Microvascular AUC) significantly decreased after sitting ($p < 0.05$) (Figure 4). Finally, sitting resulted in a small, but significant increase in calf circumference (10 minutes = 40 ± 1 vs 180 minutes sitting = 42 ± 1 cm, $p < 0.001$, $d = 0.1$).

Table 2. Peripheral vascular response to prolonged sitting

	Seated (minutes)						p value, effect	p val
	Pre sit					Post sit	(Pre vs post sit)	(Seat post sit)
	Supine	10	60	120	180	Supine		
Resting diameter (mm)	2.3±0.1	2±0.1	1.9±0.1	1.9±0.1	1.9±0.2	1.9±0.1	0.051, 0.23	<0.00
Mean shear rate (s ⁻¹)	122±14	147±18	122±10	118±10	121±9	100±8	<0.001, 0.16	<0.00
Oscillatory shear index (%)	23±2	14±2	14±2	12±2	12±2	20±2.2	<0.001, 0.1	<0.00
Nadir diameter (mm)	1.9±0.1	-	-	-	-	1.6±0.1	0.029, 0.27	-
Peak diameter (mm)	2.7±0.1	-	-	-	-	2.3±0.1	0.005, 0.34	-
Time-to-peak diameter (s)	83±8	-	-	-	-	88±8	0.71, 0.04	-
Shear stimulus (AUC)	20695±2123	-	-	-	-	15533±1830	0.073, 0.21	-
Microvascular function (AUC)	2196±333	-	-	-	-	1157±172	0.003, 0.31	-
Flow mediated dilation (mm)	0.5±0.04	-	-	-	-	0.3±0.04	0.014, 0.29	-
Low-flow mediated constriction (mm)	0.39±0.11	-	-	-	-	0.39±0.1	0.853, 0.005	-
Flow mediated dilation (%)	22±3	-	-	-	-	17±3	0.163, 0.17	-
Vasoreactivity (FMD+L-FMC) (mm)	0.9±0.1	-	-	-	-	0.7±0.1	0.048, 0.2	-
Tone (%)	61±7	-	-	-	-	46±8	0.083, 0.2	-

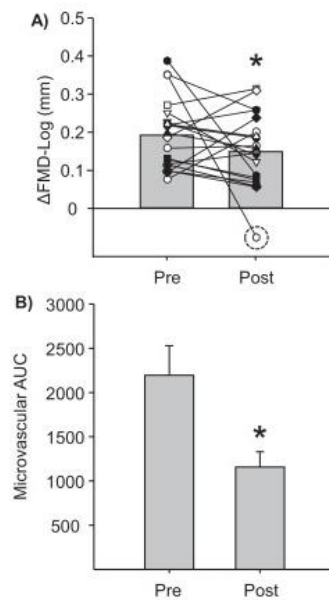


Figure 4. Mean summary data for vasodilatory [i.e., log-transformed FMD (mm)] (**Panel A**) and microvascular function (microvascular AUC) (**Panel B**) responses to prolonged sitting. *Denotes $p < 0.05$ versus Pre Sit. A subanalysis was performed by removing the observed outlier (dashed circle, bottom right of Panel A) and revealed a decrease in the log-transformed FMD ($p = 0.02$).

Discussion

The purpose of this study was to examine the peripheral and central vascular health response to prolonged sitting. Uniquely, our data demonstrate that central hemodynamics, namely aPWV and Alx are significantly affected by sitting; characterized by an increase in aPWV, but a reduction in Alx. Prolonged sitting also led to a reduction in overall vasoreactivity of arteries in the leg, which was primarily mediated by a reduction in vasodilatory function. Collectively, our data demonstrate that in relatively healthy adults, a single bout of prolonged, uninterrupted sitting negatively impacts markers of peripheral and central vascular health.

Prolonged sitting resulted in a large reduction in Alx. Limited evidence exists regarding the impact of prolonged sitting on central hemodynamics; however, previous reports using orthostatic stressors (i.e., head-up tilt)²¹ provide insight for comparison. Albeit a variable response, a reduction in Alx has been observed during upright tilting in healthy adults.^{21, 22} The upright posture promotes venous pooling,²³ which in turn may dampen pulse wave reflection²¹ and Alx. To account for this, Pb and RM% can be examined and are believed to reflect peripheral vascular resistance to pressure waves descending from the aorta.^{13, 14} Although a moderate effect for a decrease was noted, these variables did not significantly differ from baseline after sitting, thus, alterations in peripheral resistance are not likely contributing to our observed Alx reduction. Alternatively, a reduction in Alx after sitting could be related to alterations in stroke volume such that venous pooling, in theory, leads to a decrease in venous return, reduction in stroke volume, and subsequently, a decrease in Alx. The Pf is largely dependent on preload, and a reduction in Pf should correspond to a reduction in Alx.²⁴ At present, this theory is not confirmed. One final consideration is that Alx may not necessarily reflect the pressure wave components in isolation but is associated with aortic reservoir function which

could minimize both forward and backward wave contributions to overall aortic pressure.²² If a reduction in stroke volume occurred with sitting then one could speculate that aortic reservoir function also decreased, thus, decreasing AIx. This theory may also partially explain the increase in aPWV after sitting. While intriguing, this hypothesis will need to be confirmed with future work.

Our study is the first to report that prolonged sitting results in a moderate, statistically significant increase in aPWV in relatively healthy adults, although, this variable did not reach the clinically significant threshold for change (i.e., 1 m/s).¹⁸ Despite being below this threshold, an acute increase in aPWV in 3 hours could become more meaningful over time. This finding is supported by one report which demonstrated that systemic arterial stiffness (carotid-ankle PWV) was higher during sitting as compared with interrupting with frequent standing bouts during a simulated workday in overweight/obese adults.²⁵ The investigators postulate a greater carotid-ankle PWV during sitting might be the result of either a reduction in renal perfusion pressure, with subsequent renin-angiotensin-aldosterone activation, or decreased vasodilatory function, with concomitant stiffening. To account for this, we examined the relation between changes in vasodilation (i.e., FMD-mm) and aPWV but found no apparent link ($r = 0.008$, $p = 0.975$). We cannot definitively state if a reduction in vasodilatory function of a proximal vessel such as the femoral artery better correlates to changes in aortic stiffening. Although not the primary purpose, a secondary analysis of our results indicates a potential gender difference in the vascular stiffness response to sitting, such that males exhibit greater aortic stiffening compared with females. This finding is supported by one report examining vasodilatory function and demonstrated females do not exhibit a reduction in vasodilatory function after sitting (3 hours), perhaps due to hormonal differences.²⁶ Indeed, future work in a larger cohort of men and women is needed to confirm this.

In agreement with previous work,² our results demonstrate a reduction in mean shear rate and a decrease in microvascular function in leg arteries after prolonged sitting. A reduction in FMD% was not observed; however, we would like to emphasize our data were collected in a distal vessel which has a relatively high vasodilatory capacity, likely due to its small resting diameter.²⁷ The rationale for choosing this vessel stems not only from its location, but because it does not experience the same level of contortion during sitting as compared with other proximal arteries (e.g., popliteal).²⁸ After sitting, there was a significant reduction in resting diameter despite no significant change in the shear stimulus. Considering this, it has been purported that for a given shear stimulus, smaller vessels dilate more than larger ones.²⁷ Thus, the FMD% not changing pre–post sitting may actually reflect macrovascular dysfunction. In this case, we found it appropriate to substantiate our FMD analysis with the allometric scaling procedure,¹⁹ the results of which supported the interpretation that vasodilatory function is attenuated after sitting. The underlying mechanism for this is unclear, however, we postulate this was likely mediated by the reduction in mean shear rate during sitting,²⁹ as the OSI, an index of oscillatory shear and a proatherogenic signal,¹⁶ decreased. Together, these findings highlight that sitting-induced vascular impairment occurs not only in large proximal arteries but advances across multiple distal segments, including smaller arteries of the leg and foot.

Recently, it has been reported in overweight/obese adults that prolonged sitting augments circulating levels of endothelial-derived vasoconstrictors (i.e., Endothelin-1),³⁰ which in theory, could attenuate vasodilatory function and perhaps even enhance vasoconstrictor responses to reductions in blood flow. However, our data are not supportive of

this, as L-FMC did not change after sitting. Thus, reductions in overall vasoreactivity (FMD+L-FMC) after prolonged sitting appear to stem primarily from alterations in vasodilatory function. Although a tendency for a decrease was noted ($p = 0.083$), sitting did not significantly impact Tone%, a measure believed to reflect the vasoactive balance between dilator and constrictor pathways.³ We would like to note, after sitting HRV responses did favor greater parasympathetic activity (i.e., greater SDNN, and lower LF), which should correspond to a decrease in Tone%. Together, these data emphasize that in relatively healthy adults, local factors affecting vasodilation (reduced shear rate, and decreased nitric oxide production) are perhaps more greatly impacted by prolonged sitting compared with other vasoconstrictor pathways (increased sympathetic tone, endothelin-1).

A discussion of potential limitations is warranted. We recruited a heterogeneous group of participants ranging in age, gender, and body mass index. Our rationale for this selection was to be inclusive and to provide a better representation of the cardiovascular health response to prolonged sitting in the general population. Indeed, future work will be needed to confirm the extent to which individual differences (e.g., age, gender, and disease status) impact the cardiovascular health response to prolonged sitting. This study tested the acute cardiovascular response; however, it was not designed to evaluate the time-course of change throughout a typical sitting day. Future work will be needed to determine the time-course of change and long-term impact of prolonged sitting on cardiovascular health, as well as strategies for preventing sitting-induced vascular impairments.

In conclusion, these findings indicate that prolonged sitting negatively affects markers of peripheral and central vascular health, signified primarily by a reduction leg vasodilatory function and an increase in aortic vascular stiffening. Our data support the view that a single bout of prolonged, uninterrupted sitting may serve as a precursor for initiating the deleterious cardiovascular health response associated with long-term sedentarism.

Disclosures

The authors have no conflicts of interest to disclose.

Acknowledgment

The investigators would like to thank all of the volunteer subjects for their time participating.

References

- 1) SS Thosar, SL Bielko, CC Wiggins, JP Wallace **Differences in brachial and femoral artery responses to prolonged sitting** Cardiovasc Ultrasound, 12 (2014), p. 50
- 2) RM Restaino, SW Holwerda, DP Credeur, PJ Fadel, J Padilla **Impact of prolonged sitting on lower and upper limb micro- and macrovascular dilator function** Exp Physiol, 100 (2015), pp. 829-838
- 3) PL Bell, ET Kelley, SM McCoy, DP Credeur **Influence of aerobic fitness on vasoreactivity in young men** Eur J Appl Physiol, 117 (2017), pp. 2075-2083

- 4) T Gori, U von Henning, S Muxel, S Schaefer, F Fasola, M Vosseler, B Schnorbus, H Binder, JD Parker, T Munzel **Both flow-mediated dilation and constriction are associated with changes in blood flow and shear stress: two complementary perspectives on endothelial function** Clin Hemorheol Microcirc, 64 (2016), pp. 255-266
- 5) RN Larsen, BA Kingwell, P Sethi, E Cerin, N Owen, DW Dunstan **Breaking up prolonged sitting reduces resting blood pressure in overweight/obese adults** Nutr Metab Cardiovasc Dis, 24 (2014), pp. 976-982
- 6) PC Dempsey, JW Sacre, RN Larsen, NE Straznicki, P Sethi, ND Cohen, E Cerin, GW Lambert, N Owen, BA Kingwell, DW Dunstan **Interrupting prolonged sitting with brief bouts of light walking or simple resistance activities reduces resting blood pressure and plasma noradrenaline in type 2 diabetes** J Hypertens, 34 (2016), pp. 2376-2382
- 7) S Ahmadi-Abhari, S Sabia, MJ Shipley, M Kivimaki, A Singh-Manoux, A Tabak, C McEniery, IB Wilkinson, EJ Brunner **Physical activity, sedentary behavior, and long-term changes in aortic stiffness: the Whitehall II study** J Am Heart Assoc, 6 (2017), p. 6
- 8) T Morishima, RM Restaino, LK Walsh, JA Kanaley, PJ Fadel, J Padilla **Prolonged sitting-induced leg endothelial dysfunction is prevented by fidgeting** Am J Physiol Heart Circ Physiol, 311 (2016), pp. H177-182
- 9) DH Thijssen, MA Black, KE Pyke, J Padilla, G Atkinson, RA Harris, B Parker, ME Widlansky, ME Tschakovsky, DJ Green **Assessment of flow-mediated dilation in humans: a methodological and physiological guideline** Am J Physiol Heart Circ Physiol, 300 (2011), pp. H2-12
- 10) DP Credeur, LM Vana, ET Kelley, L Stoner, DR Dolbow **Effects of intermittent pneumatic compression on leg vascular function in people with spinal cord injury: a pilot study** J Spinal Cord Med (2017), pp. 1-9
- 11) **Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology** Circulation, 93 (1996), pp. 1043-1065
- 12) RP Troiano, D Berrigan, KW Dodd, LC Masse, T Tilert, M McDowell **Physical activity in the United States measured by accelerometer** Med Sci Sports Exer, 40 (2008), pp. 181-188
- 13) M Butlin, A Qasem, AP Avolio **Estimation of central aortic pressure waveform features derived from the brachial cuff volume displacement waveform** Conf Proc IEEE Eng Med Biol Soc, 2012 (2012), pp. 2591-2594
- 14) A Qasem, A Avolio **Determination of aortic pulse wave velocity from waveform decomposition of the central aortic pressure pulse** Hypertension, 51 (2008), pp. 188-195
- 15) L Stoner, KK McCully **Peak and time-integrated shear rates independently predict flow-mediated dilation** J Clin Ultrasound, 40 (2012), pp. 341-351
- 16) MH Laughlin, SC Newcomer, SB Bender **Importance of hemodynamic forces as signals for exercise-induced changes in endothelial cell phenotype** J Appl Physiol, 104 (2008), pp. 588-600
- 17) Reference Values for Arterial Stiffness' Collaboration **Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'establishing normal and reference values'** Eur Heart J, 31 (2010), pp. 2338-2350
- 18) C Vlachopoulos, K Aznaouridis, C Stefanadis **Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis** J Am Coll Cardiol, 55 (2010), pp. 1318-1327
- 19) G Atkinson, AM Batterham **Allometric scaling of diameter change in the original flow-mediated dilation protocol** Atherosclerosis, 226 (2013), pp. 425-427
- 20) KB Watson, SA Carlson, DD Carroll, JE Fulton **Comparison of accelerometer cut points to estimate physical activity in US adults** J Sports Sci, 32 (2014), pp. 660-669
- 21) L Stoner, K Stone, ED Hanson, J Faulkner, S Fryer, D Credeur **Reliability of pulse waveform separation analysis responses to an orthostatic challenge** Hypertens Res, 41 (2018), pp. 176-182
- 22) WE Hughes, DP Casey **Aortic wave reflection during orthostatic challenges: influence of body position and venous pooling** Am J Hypertens, 30 (2017), pp. 166-172

- 23) KJ Stone, SM Fryer, T Ryan, L Stoner **The validity and reliability of continuous-wave near-infrared spectroscopy for the assessment of leg blood volume during an orthostatic challenge** *Atherosclerosis*, 251 (2016), pp. 234-239
- 24) A Tahvanainen, J Koskela, A Tikkakoski, J Lahtela, M Leskinen, M Kahonen, T Nieminen, T Koobi, J Mustonen, I Porst **Analysis of cardiovascular responses to passive head-up tilt using continuous pulse wave analysis and impedance cardiography** *Scand J Clin Lab Invest*, 69 (2009), pp. 128-137
- 25) B Barone Gibbs, RJ Kowalsky, SJ Perdomo, JM Taormina, JR Balzer, JM Jakicic **Effect of alternating standing and sitting on blood pressure and pulse wave velocity during a simulated workday in adults with overweight/obesity** *J Hypertens*, 35 (2017), pp. 2411-2418
- 26) JR Vranish, BE Young, J Kaur, JC Patik, J Padilla, PJ Fadel **Influence of sex on microvascular and macrovascular responses to prolonged sitting** *Am J Physiol Heart Circ Physiol*, 312 (2017), pp. H800-805
- 27) F Jazuli, KE Pyke **The impact of baseline artery diameter on flow-mediated vasodilation: a comparison of brachial and radial artery responses to matched levels of shear stress** *Am J Physiol Heart Circ Physiol*, 301 (2011), pp. H1667-1677
- 28) LK Walsh, RM Restaino, LA Martinez-Lemus, J Padilla **Prolonged leg bending impairs endothelial function in the popliteal artery** *Physiol Rep*, 5 (2017), p. 5
- 29) RM Restaino, LK Walsh, T Morishima, JR Vranish, LA Martinez-Lemus, PJ Fadel, J Padilla **Endothelial dysfunction following prolonged sitting is mediated by a reduction in shear stress** *Am J Physiol Heart Circ Physiol*, 310 (2016), pp. H648-653
- 30) RE Climie, MJ Wheeler, M Grace, E Lambert, N Cohen, N Owen, B Kingwell, DW Dunstan, DJ Green **Simple intermittent resistance activity mitigates the detrimental effect of prolonged unbroken sitting on arterial function in overweight and obese adults** *J Appl Physiol* (1985) (2018 Sep 6), 10.1152/jappphysiol.00544.2018 [Epub ahead of print].

Funding

This work was supported by a grant through the American Kinesiotherapy Association grant# GR05488.