Sitting cross-legged for 30 minutes alters lower limb shear stress pattern but not flow-mediated dilation or arterial stiffness
Title: Sitting cross-legged for 30 minutes alters lower limb shear stress pattern but not flow-mediated dilation or arterial stiffness

Authors: Joshua C. Tremblay\textsuperscript{1}
Taylor V. Stimpson\textsuperscript{1}
Kristen M. Murray\textsuperscript{1}
Kyra E. Pyke\textsuperscript{1}

Affiliations: \textsuperscript{1}Cardiovascular Stress Response Laboratory, School of Kinesiology and Health Studies, Queen’s University, Kingston, Canada.

Correspondence:
Dr. Kyra E. Pyke
Associate Professor
28 Division Street
Kingston, Ontario, Canada,
K7L 3N6
Phone: 613-533-6000 ext 79631
Email: pykek@queensu.ca
Abstract (max 75 words; 74 words)

Prolonged sitting decreases lower limb endothelial function via sustained reductions in mean shear rate. We tested whether 30 minutes of sitting cross-legged differentially impacts superficial femoral artery shear rate pattern, flow-mediated dilation (FMD) and leg pulse-wave velocity (PWV) compared to sitting flat-footed. Sitting cross-legged attenuated the reduction in mean and antegrade shear rate and increased arterial pressure compared to sitting flat-footed. Superficial femoral artery FMD and leg PWV were unaltered following either sitting position.

Key words: FMD, sitting position, endothelial function, retrograde shear stress, pulse wave velocity
Introduction

Prolonged sitting is associated with an increased risk of all-cause and cardiovascular disease mortality (Katzmarzyk et al. 2009) and sitting flat-footed for 1-6 hours has been shown to impair lower limb conduit artery endothelial function as assessed by flow-mediated dilation (FMD) (Padilla and Fadel 2017). Previous observations have identified that acute changes in endothelial function influence arterial tone and stiffness (Kinlay et al. 2001; Bellien et al. 2010). However, whether sitting-induced reductions in endothelial function are accompanied by increases in arterial stiffness is unclear.

The sitting-induced impairment in conduit artery endothelial function is dependent on a sustained reduction in conduit artery shear stress (Restaino et al. 2016), the frictional force that blood exerts against the endothelium. Indeed, interventions to interrupt the reduction in shear stress, including heating (Restaino et al. 2016), walking (Thosar et al. 2015), and fidgeting (Morishima et al. 2016), prevent the sitting-induced endothelial dysfunction. The decreased shear stress incurred by sitting is due to the arterial bending caused by hip and knee flexion; as illustrated by the observation that, even when laying supine, shear stress is higher with the leg straightened compared to bent (Walsh et al. 2017). However, whether sitting cross-legged versus flat-footed differentially influences shear stress, FMD or arterial stiffness is unknown.

Sitting cross-legged may elicit a distinct hemodynamic effect on the lower limb vasculature compared to sitting flat-footed. For instance, sitting cross-legged increases mean arterial pressure (MAP) compared to measurements made when sitting flat-footed (Peters et al. 1999). Furthermore, sitting cross-legged increases the angulation of the top leg compared to the flat-footed seating position (Snijders et al. 2006). Taken together, the altered perfusion pressure (Padilla et al. 2010) and increased arterial bending (Walsh et al. 2017) that manifest when sitting cross-legged have the potential to alter conduit artery shear stress patterns in the lower limb.

The purpose of this study was to compare the acute impact of sitting cross-legged versus flat-footed on lower limb conduit artery shear stress patterns, FMD and arterial stiffness. We reasoned that antegrade shear stress would be greater, due to the increased perfusion pressure, and retrograde shear stress greater, due to increased angulation and turbulent flow, in the cross-legged sitting position.
position compared to flat-footed. Furthermore, we hypothesized that 30 minutes of sitting cross-legged and flat-footed would decrease superficial femoral artery FMD and arterial stiffness (pulse-wave velocity; PWV).

Methods

Ethical approval

All experimental procedures were approved by the Queen’s University Health Sciences Research Ethics Board, which conforms to the standards set by the Declaration of Helsinki and written informed consent was obtained.

Participants

Healthy adults (n=11 [2 female], 23±2 years, body mass index=24±3 kg m\(^{-2}\) [mean±SD]) participated in two counterbalanced experimental visits. Participants were normotensive, nonsmokers without cardiovascular disease and not taking any medications besides oral hormonal contraceptives (n=1). Women had regular menstrual cycles (>10 cycles per year) and were tested in days 1-5 of the menstrual cycle.

Experimental design

Each participant performed two laboratory visits at the same time of day within one week with standard pre-FMD testing preparation (Thijssen et al. 2011). Upon arrival at the laboratory, participants laid supine in a temperature-controlled room. After laying supine for 10 minutes, arterial blood pressure was acquired four times (first measure discarded; BD215, BIOS Diagnostics, Canada). The pre-intervention PWV and FMD (right leg) were performed after laying supine for 30 minutes. Following the pre-intervention PWV and FMD, the participant assumed a flat-footed or cross-legged seated position for 30 minutes (Figure 1, panels A and B). Shear rate (right leg, top leg when crossed) and blood pressure were acquired at 10, 20, and 25-minutes during the sitting intervention. Immediately post-intervention, the participant was assisted to a supine position with care taken to avoid muscle activation in the experimental (right) leg. Upon returning to the supine position, blood pressure was acquired four times (first measure discarded). Subsequently, the post-intervention PWV and FMD were performed with the FMD test initiated 5 minutes following entry to the supine position.
Experimental Measurements

Superficial femoral artery diameter
Superficial femoral artery diameter was obtained using two-dimensional ultrasound in B-mode (12 MHz; Vivid i2; GE Medical Systems, Canada). Ultrasound images were recorded as previously described (Jazuli and Pyke 2011). Standardized software approaches were used to analyze the ultrasound recordings (FMD/BloodFlow Software Version 5.1, Reed C, Australia) (Woodman et al. 2001).

Superficial femoral artery blood velocity
Superficial femoral artery blood velocity was obtained at an insonation angle of 68° using Doppler ultrasound operating at 4 MHz (Vivid i2; GE Medical Systems) as described previously (Pyke et al. 2008). Three second average time bins of antegrade (positive), retrograde (negative), and mean (sum of antegrade and retrograde) blood velocity were analyzed offline using data acquisition software (LabChart; ADInstruments). Shear rate was calculated as 4*blood velocity / diameter.

Superficial femoral artery FMD
FMD was performed in adherence with recent guidelines (Thijssen et al. 2011). One-minute of baseline superficial femoral artery diameter and blood velocity were acquired via Duplex ultrasound. Subsequently, a pneumatic cuff placed proximal to the knee, and distal to the site of ultrasound imaging, was inflated to suprasystolic pressure (250-mmHg) for 5 minutes. The cuff was then deflated, and ultrasound imaging persisted for 4 minutes post-deflation. Screen capture of the ultrasound was saved as an .avi file (Camtasia Studio, Techsmith Co, Ltd, USA) for future analysis using edge-detection software (Woodman et al. 2001). FMD was calculated as the absolute (mm) and relative (%) change from baseline to peak diameter (3-second average). The FMD stimulus was calculated as the shear rate area under the curve (SR_AUC) from cuff deflation to peak diameter (Pyke and Tschakovsky 2007).

Arterial Stiffness
Lower limb arterial stiffness was assessed using PWV via applanation tomometry (Millar Instruments, USA) (Tanaka et al. 1998). A minimum of 10 consecutive pulse waves were acquired.
simultaneously in the femoral and posterior tibial arteries; the pulse transit distance was calculated as the distance between the two measurement sites. PWV was calculated for each beat as the distance (m) / pulse transit time (s).

Statistics
All statistical analyses were performed using IBM SPSS 24 (International Business Machines Corp, USA). Data were compared within-participants with significance set at P<0.05 and are presented as mean±SD. Data were analyzed using a linear mixed model with a compound symmetry co-variance structure. Two-factor linear mixed models were performed with time (repeated variable; pre-intervention and post-intervention) and condition (repeated variable; cross-legged versus flat-footed) for FMD, SR_{AUC}, baseline diameter, peak diameter, and PWV. The change in shear rate from pre-intervention supine to during the intervention was also assessed (repeated variable; cross-legged versus flat-footed; repeated variable; 10, 20, and 25 minutes during the intervention). Shear rates, were assessed using a two-factor linear mixed model with time (repeated variable; pre-intervention, 10, 20, and 25 minutes during the intervention, and post-intervention) and condition (repeated variable; cross-legged versus flat-footed) as factors. The change in MAP from pre-intervention to 10, 20, and 25 minutes during the intervention, and post-intervention was assessed using a two-factor linear mixed model with time (repeated variable; 10, 20, and 25 minutes during the intervention, and post-intervention) and condition (repeated variable; cross-legged versus flat-footed) as factors. Pre-intervention baseline values were compared between conditions via paired t-tests. When significant main effects were detected, Bonferroni-corrected post-hoc tests were used to make pairwise comparisons. To account for stimulus differences, FMD was also assessed with SR_{AUC} included as a covariate (Harris and Padilla 2007).

Results
Arterial Pressure
Pre-intervention supine MAP was not different between conditions (flat-footed, 85±7 mmHg; cross-legged, 87±7 mmHg; P=0.210). A main effect of condition was observed for delta MAP such that MAP increased by 3.6±4.2 mmHg during the cross-legged trial compared to 1.7±4.1
mmHg during the flat-footed trial (P=0.039) (values collapsed across time points); there was no main effect of time (P=0.913) or interaction effect (P=0.987).

Shear Rates
Shear rates are presented in Figure 1 (panels C, D, E). Mean (panel C), antegrade (panel D), and retrograde (panel E) shear rate were greater during the cross-legged condition compared to the flat-footed condition. Pre-intervention shear rate did not differ significantly between conditions for any shear rate parameter, therefore these main effects of condition were driven by the time points following the onset of the intervention. Analysis of the change in shear rate from pre-intervention to during the intervention revealed significantly greater decreases in mean (P=0.005) and antegrade (P<0.001) shear rate in the flat-footed condition (retrograde shear rate: effect of condition P=0.111). In both conditions (main effect of time p<0.001) mean and antegrade shear rate were decreased at 10, 20, 25, and post-intervention compared to pre-intervention, and retrograde shear rate was decreased during the intervention compared to pre- and post-intervention.

FMD and PWV
Superficial femoral FMD and leg PWV were not different following either sitting intervention (Table 1). The SR_{AUC} was decreased following the interventions. Covariate-adjustment of FMD for SR_{AUC} did not alter interpretation.

Discussion
For the first time, lower limb conduit artery shear rate pattern was assessed during a commonly-observed cross-legged sitting posture. Sitting cross-legged significantly mitigated the reduction in antegrade and mean shear rates, and increased MAP, compared to sitting flat-footed. Sitting in these postures for 30 minutes did not impact measures of FMD and PWV acquired in the supine position. These findings highlight the important influence of sitting position on lower limb hemodynamics. Whether a greater time spent in these distinct positions or the accumulation of habitual cross-legged sitting elicits functional consequences is currently unclear.

Sitting cross-legged created a distinct hemodynamic environment in the lower limb compared to sitting flat-footed. Previous investigations have focused on the systemic effects of leg crossing,
indicating that sitting cross-legged increases arterial pressure (Peters et al. 1999). Indeed, leg-crossing has been suggested as a useful strategy to maintain arterial pressure in patients with orthostatic hypotension (Takishita et al. 1991). Here, we extend these findings by demonstrating that in the top leg, sitting cross-legged mitigates the flat-footed sitting-induced reduction in mean and antegrade shear rates. The higher shear rates observed during the cross-legged intervention are likely the result of the increased perfusion pressure present in that posture.

Previous studies have observed decrements in leg FMD after sitting still (flatfooted) for 1-6 h (Padilla and Fadel 2017), or following just 30 minutes of cuff inflation-induced decreased mean shear stress, accompanied by increased retrograde shear stress (Schreuder et al. 2014; Totosy de Zepetnek et al. 2014). We did not observe impairments in endothelial function or arterial stiffness following the 30-minute sitting intervention, suggesting a longer duration, or greater disruption in shear stress may be necessary to elicit impairments in these parameters. Indeed, although shear rate remains lower than baseline measures (supine), intermittent fidgeting (Morishima et al. 2016) and standing (Morishima et al. 2017) attenuate the reduction in shear rate elicited with prolonged flat-footed sitting and prevent the consequent reductions in FMD. This suggests that a minor attenuation of the reduction in mean shear rate (as seen in the cross-legged condition) might prevent sitting-induced vascular impairment. Whether sitting cross-legged preserves FMD over a longer duration (1-6h) compared to sitting flat-footed merits future investigation, as do the hemodynamics in the bottom leg.

**Conflict of Interests**

The authors have no conflicts of interest to report.


Table 1. Parameters for flow-mediated dilation (FMD) and pulse-wave velocity (PWV) in the supine position pre- and post- sitting interventions.

<table>
<thead>
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<th>Flat-footed</th>
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<th>Cross-legged</th>
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<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
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<tr>
<td>Baseline Diameter (mm)</td>
<td>5.78±0.58</td>
<td>5.76±0.59</td>
<td>5.79±0.58</td>
<td>5.72±0.56</td>
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<td></td>
<td><em>Time, P=0.245; Condition, P=0.660; Interaction, P=0.492</em></td>
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<td><em>Time, P=0.245; Condition, P=0.660; Interaction, P=0.492</em></td>
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<tr>
<td>Peak Diameter (mm)</td>
<td>5.98±0.59</td>
<td>5.94±0.57</td>
<td>5.98±0.61</td>
<td>5.93±0.61</td>
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<td><em>Time, P=0.206; Condition, P=0.865; Interaction, P=0.857</em></td>
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<td><em>Time, P=0.206; Condition, P=0.865; Interaction, P=0.857</em></td>
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<td>Absolute FMD (mm)</td>
<td>0.21±0.05</td>
<td>0.18±0.08</td>
<td>0.20±0.07</td>
<td>0.21±0.08</td>
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<td><em>Time, P=0.613; Condition, P=0.517; Interaction, P=0.220</em></td>
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<td><em>Time, P=0.613; Condition, P=0.517; Interaction, P=0.220</em></td>
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<td>Relative FMD (%)</td>
<td>3.6±0.8</td>
<td>3.2±1.4</td>
<td>3.4±1.1</td>
<td>3.6±1.3</td>
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<td><em>Time, P=0.690; Condition, P=0.678; Interaction, P=0.250</em></td>
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<td>SR_AUC (10⁴au)</td>
<td>14.9±4.5</td>
<td>11.4±3.9</td>
<td>13.6±7.0</td>
<td>10.5±3.9</td>
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<td><em>Time, P=0.013; Condition, P=0.393; Interaction, P=0.845</em></td>
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<td><em>Time, P=0.013; Condition, P=0.393; Interaction, P=0.845</em></td>
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<td>SR_AUC-covariate corrected FMD (%)</td>
<td>3.6±1.2</td>
<td>3.2±1.2</td>
<td>3.4±1.2</td>
<td>3.6±1.2</td>
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<td><em>Time, P=0.706; Condition, P=0.690; Interaction, P=0.257</em></td>
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<td><em>Time, P=0.706; Condition, P=0.690; Interaction, P=0.257</em></td>
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<td>PWV (m s⁻¹)</td>
<td>7.2±1.9</td>
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<td><em>Time, P=0.765; Condition, P=0.372; Interaction, P=0.982</em></td>
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<td><em>Time, P=0.765; Condition, P=0.372; Interaction, P=0.982</em></td>
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SR_AUC, shear rate area under the curve. Data are presented as mean±SD.
Figure 1. Panel A: flat-footed position. panel B: cross-legged position. The site of ultrasound measurement is identified by the ultrasound probe. Mean (panel C), antegrade (panel D), and retrograde (panel E) shear rate for the flat-footed (solid circles) and cross-legged (open circles) visits. †, P<0.05 cross-legged versus flat-footed for mean, antegrade, and retrograde shear rate; *, P<0.05 versus pre-intervention; **, P<0.05 versus pre- and post-intervention. Data are mean±SD.