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Impact of prolonged sitting on vascular function in young girls.

Ali M McManus\textsuperscript{1}, Philip N Ainslie\textsuperscript{1}, Daniel J Green\textsuperscript{2,3}, Ryan G Simair\textsuperscript{1}, Kurt Smith\textsuperscript{1}, Nia Lewis\textsuperscript{1}

\textsuperscript{1}Centre for Heart, Lung and Vascular Health, School of Health and Exercise Sciences, University of British Columbia, Kelowna, Canada
\textsuperscript{2}School of Sport Science, Exercise and Health, The University of Western Australia, Crawley, Western Australia
\textsuperscript{3}Research Institute for Sport and Exercise Sciences, Liverpool John Moores University

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Corresponding author: Dr Ali McManus
Centre for Heart, Lung and Vascular Health, School of Health and Exercise Sciences, University of British Columbia,
1147 Research Road- ART 360,
Kelowna BC V1V V17,
Canada.
Tel: +1 250 807 8192
Email: ali.mcm anus@ubc.ca

Subject Area: Human/environmental and exercise physiology
• **What is the central question of this study?**

Children are spending more than 60% of their waking day sedentary. The consequences of excessive sedentary behavior are not well understood in the child, but there is growing evidence that with increasing sedentary time, cardiovascular risk in childhood also increases.

• **What is the main finding and its importance?**

Our findings show that a 3-hour period of uninterrupted sitting causes a profound (33%) reduction in vascular function in young girls. Importantly we also demonstrate that breaking up sitting with regular exercise breaks can prevent this.
Excessive sedentary behaviour has serious clinical and public health implications; however, the physiological changes that accompany prolonged sitting in the child are not completely understood. Herein we examined the acute effect a prolonged period of sitting has upon superficial femoral artery function in 7-10 year old girls and the impact of interrupting prolonged sitting with exercise breaks. Superficial femoral artery endothelium-dependent flow-mediated dilation, total shear rate, antegrade and retrograde shear rates and oscillatory shear index, were assessed before and after two experimental conditions: a 3-hour uninterrupted period of sitting (SIT) and a 3-hour period of sitting interrupted each hour with 10 minutes of moderate intensity exercise (EX). A mixed-model analysis of variance was used to compare between condition and within condition main effects, controlling for the within-subject nature of the experiment by including random effects for participant. Superficial femoral artery endothelium-dependent flow-mediated dilation decreased significantly from pre- to post-SIT (mean difference 2.2% flow-mediated dilation; 95% CI = 0.60 to 2.94%, P < 0.001). This relative decline of 33% was abolished in the EX intervention. Shear rates were not significantly different within conditions. Our data demonstrate the effectiveness of short but regular exercise breaks in offsetting the detrimental effects of uninterrupted sitting in young girls.
INTRODUCTION

Sedentary behaviour has reached alarming levels, with children and teens spending between five to seven hours of the waking day sedentary (Colley et al., 2011). These data are particularly concerning given the increasing body of epidemiological evidence that shows too much sitting is an independent risk factor for cardiovascular disease in both children and adults (Hamilton et al., 2008; Saunders et al., 2014) and is associated with an increase in all-cause mortality in adults (Van der Ploeg et al., 2012).

Early deterioration in vascular function, particularly alterations in endothelial integrity, are significant in the development of cardiovascular disease (Aggoun et al., 2005). In various models of enforced physical inactivity, evidence indicates that acute bouts of sedentary behavior contribute to vascular deconditioning in adults (Thijssen et al., 2010; Boyle et al., 2013). Prolonged periods of sitting alter the anatomical structure of the lower limb arteries and create unique changes to leg haemodynamics such as calf blood pooling and increased blood viscosity (Shvartz & Gaume, 1983; Hitosugi et al., 2000). Endothelial flow-mediated dilation (FMD), a marker of vascular health, is also altered with prolonged sitting in adults (Thosar et al., 2015). Here, it was demonstrated that a three-hour period of uninterrupted sitting caused a relative decline in superficial femoral artery (SFA) FMD of ~50%, which was accompanied by declines in mean shear rate (SR) and antegrade shear (Thosar et al., 2015). Prolonged sitting exerts a localised model of physical inactivity, causing disruption to vascular function specific to the lower extremities, which is associated with increased cardiovascular mortality (Thijssen et al.,...
2010); thus, it is likely that sitting induced decrements in SFA FMD contribute to increasing cardiovascular disease risk if they persist over time.

Far less is understood about the physiological changes that accompany prolonged sitting in the child. The influence that a prolonged bout of sitting has upon hemodynamic outcomes in children has not been addressed; however, correlational data indicate that changes in endothelial health can be independent of sedentary behavior (Hopkins et al., 2012). Thus, the physiological impact of prolonged sedentary behaviour may be smaller than in adults or even absent in the child. However, it should be noted that brachial artery FMD was assessed in the correlational study of endothelial function and sedentary behaviour in 10-year olds (Hopkins et al., 2012). Unlike the femoral artery, brachial artery FMD is not perturbed during sitting in adults (Thosar et al., 2014, Restaino et al., in press).

The aim of the present study was to examine if an acute bout of uninterrupted sitting reduces SFA FMD and shear rates in girls and whether interrupting prolonged sitting with exercise breaks prevents any adverse changes. We chose to study girls because some recent evidence suggests that changes in artery function occur with aging that are independent of maturational growth. We hypothesized that (i) prolonged sitting will result in a reduction in SFA FMD, whereas prolonged sitting interrupted with moderate intensity exercise breaks will prevent this decline; and (ii) declines in FMD following prolonged sitting will be accompanied by declines in total SR and antegrade shear.
METHODS

Nine girls participated in the study. We recruited a single sex group because of documented sex differences in vascular function (Sarkola et al., 2012; Hopkins et al., 2015) and in sedentary behaviour in children (LeBlanc et al., 2015; Colley et al., 2013). None of the girls had any physical limitations or chronic disease. Written informed consent was obtained from parents and verbal assent was obtained from the girls. The clinical ethical review committee at UBC approved the experimental procedures and the study conformed to the standards set by the latest revision of the Declaration of Helsinki.

Study Design and Procedures

We used a crossover trial with two experimental conditions: (1) uninterrupted sitting (SIT), where participants remained seated for three hours and (2) breaks in sitting time (EX), where participants completed an identical 3-hours of sitting as above, except at the beginning of each hour they cycled for 10-minutes at a moderate intensity. The two conditions were completed on two separate days, the order of which was randomized.

Prior to the experimental trial the girls attended the laboratory for familiarisation with the setting and test procedures. They completed anthropometric measurements, the vascular measures that would be completed in the subsequent two conditions and a maximal exercise test. The girls returned to the laboratory at the same time of day (either morning or afternoon) on two separate occasions to complete the two experimental conditions. Resting blood pressure was taken manually prior to the start of both 3-hour conditions. SFA FMD was then assessed prior to the start and following each 3-hour
condition. At least 3 days separated each visit. The girls were asked to maintain their regular diet and physical activity habits throughout the duration of the study, but to abstain from strenuous exercise or caffeine for 24 hours prior to each visit. Parents were instructed to provide identical meals on both testing days, consisting of food normally eaten by their child, to standardize dietary intake and the meal content was recorded. We studied the girls in the non-fasted state because this was more indicative of normal daily life than the fasted state.

During the SIT and EX conditions the children sat on large beanbag seats for three hours and watched movies, played on iPads, read or coloured books. They were allowed to move their arms to alter the volume on the video or play, but were discouraged from standing during the seated periods. If they needed the washroom they were wheeled there and back. Leg movement was monitored on the dominant side of the body (right side for all) throughout both trials using accelerometers. During the EX condition participants completed a 10-minute exercise break at the beginning of each of the three hours, cycling on a cycle ergometer (LODE Paediatric Corival) at an individualised moderate intensity.

**Measures**

Body mass was measured with electronic scales with subjects barefoot and dressed in light clothing. Stature was measured barefoot with a Harpenden stadiometer. Body mass index (BMI) was calculated from body mass (kg) divided by stature (m)². Healthy weight, overweight and thinness were classified using international references (Cole et al., 2000; Cole et al., 2007).
Blood pressure (BP) was assessed by sphygmomanometry to the nearest 2mm Hg, twice in the right arm, seated, after 10 minutes supine rest, and a third time if the two readings were 44mm Hg apart. Diastolic pressure was defined as the point of disappearance of Korotkoff sound (5th phase).

Maximum oxygen uptake (VO2max) was determined during a ramp exercise test to volitional exhaustion on an electro-magnetically braked cycle ergometer (Lode Paediatric Corival). Following a 3 minute warm up at 5 watts (W), intensity was increased every minute by 10 W. Pedal cadence was ~70 revolutions per minute (RPM) until volitional exhaustion, defined as a drop in cadence ≥ 10 rpm for 5 consecutive seconds, despite strong verbal encouragement. Heart rate (HR) was measured continuously using HR telemetry (Polar Vantage NV, Polar Electro Oy). Pulmonary gas exchange and ventilation were measured continuously using a breath-by-breath metabolic cart (Oxycon Pro, Carefusion). The moderate intensity workload for each child was determined from the maximal exercise data and defined as a work-rate that falls below the gas exchange threshold (GET - a non-invasive equivalent of the blood lactate threshold). The GET was noninvasively identified using the V slope method (Beaver et al., 1986) and the corresponding wattage at 90% of GET was noted for the exercise breaks. Anchoring intensity to the GET provides greater methodological rigor because of the smaller absolute maximum VO2 of the child which severely compresses the range of work-rates within a given exercise intensity (Armstrong & Barker, 2009).
Posture and movement was assessed using ActivPal accelerometers (ActivPal3™ micro, PALTechnologies) attached to the thigh. Time spent sitting, standing and moving was recorded.

**Principal Outcome Measure**

A 10-MHz multifrequency linear array probe attached to a high-resolution ultrasound machine (Terason t3000™, Teratech) was used to image the left SFA. FMD was assessed according to international guidelines (Thijssen et al., 2011a). A rapid inflatable blood pressure cuff was positioned ~5-10cm above the knee joint, distal to the site of ultrasound capture. Baseline measures of SFA diameter and Doppler measurements of SFA blood flow velocity were continuously recorded for 1 min prior to cuff inflation, after which the cuff was inflated to 50 mm Hg above resting systolic blood pressure (mean cuff inflation level 151±8 mmHg) for 5 min. Continuous diameter and blood velocity recordings resumed 30 s prior to cuff deflation and continued for 3 min thereafter.

Custom-designed edge-detection and wall-tracking software, which is independent of investigator bias, was utilised for the analysis of SFA diameter blood flow velocity (Woodman et al., 2009; Thijssen et al., 2011a). This software provides continuous and simultaneous measurement of diameter and velocity, blood flow (lumen cross-sectional area and Doppler velocity \([v]\); \([4*velocity\ cm.s^{-1}]/diameter\ cm\)) and shear rate; as well as post hoc calculation of FMD. Velocity and flow were calculated from the Doppler envelope. Antegrade and retrograde blood flow and SRs were calculated from antegrade and retrograde area under the curve data that were subsequently averaged from
positive or negative data points respectively. In animal and in-vitro models, increased oscillatory shear stress characterized by increased retrograde SR is associated with acutely decreased endothelial cell function (Thijssen et al., 2009b). Oscillatory shear index (OSI) was used as an indicator of the magnitude of shear oscillation or shear reversal. For purely oscillatory flow, the OSI attains a maximum value of 0.5, an indicator of flow reversal. High values of OSI have been associated with endothelial dysfunction in adults (He & Ku, 1996). OSI was calculated as:

\[
\text{OSI} = \frac{|\text{retrograde shear}|}{(|\text{antegrade shear}| + |\text{retrograde shear}|)}
\]

This semi-automated software provides higher reproducibility of diameter measurements and reduces both observer error and bias (Woodman et al., 2009). A single individual who was blinded to the study codes conducted all the measurements and data processing for this study. Diameter data are presented as absolute (millimetres) and relative (percentage) rises from the preceding baseline diameter (Thijssen et al., 2011a). In accordance with procedural recommendations (Pyke & Tschakovsky, 2005; Pyke & Tschakovsky, 2007), we also measured the post-deflation area under the shear rate curve (SR_{AUC}) in order to best interpret any changes in FMD; however, we did not normalize FMD (%) against SR due to the established limitations of this approach (Atkinson et al., 2009). As a complementary measure, allometric scaling was also used to adjust for variability in baseline diameter and to improve the specificity and interpretation of the FMD protocol (Atkinson et al., 2013). These results are presented as “corrected” FMD (%).
STATISTICS

Using pre-SIT and pre-EX values we calculated the technical error of measurement (TEM) and the percentage coefficient of variation ([SD of the paired differences/ overall mean/ square root of 3] x 100) for baseline SFA diameter and SFA FMD (%). We examined within and between condition (SIT vs. EX) main effects for SFA FMD (%), SRs, velocity and flow using a mixed-model analysis of variance (ANOVA). This model enabled control for the within-subject nature of the experiment by including random effects for participant. The time varying covariate baseline SFA diameter was used to scale the change in logarithmically transformed SFA FMD (%). The resulting mean differences between conditions and pre to post were back-transformed to the original units of FMD (%) providing corrected SFA FMD (%). Carryover effects were not formally tested, given the minimum three-day washout period between conditions and the fact that trials were performed in a randomized order; however, we did compare baseline diameter using ANOVA between those who began the trial with the SIT condition and those who began the trial with the EX condition. The alpha level was set a priori at 0.05. Statistical analyses were performed using SPSS for Windows (version 21).

RESULTS

All nine girls recruited completed the study (see Table 1). Two of the girls were classified as overweight; the remaining girls were healthy weight.
The ActivPAL data for each hour of the two conditions are presented in Table 2. These data show that during the SIT trial the girls remained seated for at least 56 minutes per hour, with an average of less than four minutes taken standing or moving. During the EX trial, the girls sat for about 45 minutes per hour, and in addition to the 10 minute exercise break, spent an average of 5 minutes per hour standing or moving.

The SFA diameters at baseline and with reactive hyperaemia prior to and following the SIT and EX trials are presented in Table 3. Percentage TEM for SFA baseline diameter and FMD (%) were 3.7% and 5.9% respectively. The coefficient of variation for SFA baseline diameter and FMD (%) were 6.3% and 8.8% respectively. There were no significant differences in baseline diameter prior to the SIT or EX conditions between the girls starting with the SIT condition or those starting with the EX condition, verifying the washout period between trials was sufficient to eliminate any possible carry-over effects.

SFA FMD (%) significantly decreased following the SIT condition (P < 0.001) but not the EX condition (P = 0.97, see Figure 1). The mean decrease in corrected SFA FMD (%) was 2.2% from pre to post SIT (95% CI = 0.60 to 2.94%, P < 0.001). Corrected SFA FMD (%) remained unchanged pre to post the EX condition (see Table 3).
Mean baseline SR, antegrade and retrograde SRs, as well as SR under the curve and OSI index during reactive hyperaemia, pre and post the two conditions are presented in Table 3. There were no significant main effects, within or between conditions, for baseline shear rates or OSI index during reactive hyperaemia ($P > 0.05$). There was no within condition main effect for $\text{SR}_{\text{AUC}}$; however, a significantly greater $\text{SR}_{\text{AUC}}$, as well as $\text{flow}_{\text{AUC}}$ was detected pre EX compared to pre SIT ($P < 0.05$).

**DISCUSSION**

These are the first findings to demonstrate that three hours of uninterrupted sitting in young girls is detrimental to vascular function, and that a 10-minute exercise break each hour prevents this adverse decline.

*Impact and implications of uninterrupted sitting:* In support of our hypothesis, prolonged sitting caused significant vascular dysfunction, shown by a mean decrease in corrected SFA FMD (%) from 7.04% at baseline to 4.71%. This change in FMD represents a relative decline of 33% and parallels the adult response to uninterrupted sitting (Thosar et al., 2015). It has been reported that resting SR in the SFA are lower than those in the brachial artery (Wu et al., 2004), and that the normal pattern of SR in the SFA includes a larger retrograde component in comparison to the brachial artery (Newcomer et al., 2008). This chronically lower wall shear stress, along with higher turbulence through the SFA due to anatomical structure, has been suggested to contribute
to higher incidence of atherosclerosis in comparison to the upper extremity blood vessels (Wu et al., 2004; Wood et al., 2006). Further, vascular disease risk is often detectable in the blood vessels of the legs rather than the arms [i.e. intermittent claudication, peripheral arterial disease] (Ouriel, 2001). The implications of our findings is that reductions in vascular function are predictive of poorer cardiovascular outcomes and worse vascular health, with a 1% decline in FMD (%) estimated to increase risk of a future cardiac event by up to 13% (Inaba et al., 2010).

The longer-term consequences in terms of vascular health of uninterrupted sitting are currently unknown, especially in children. Prior studies using alternative models of inactivity such as bed-rest and spinal cord injury in adults have considered chronic vascular responses to inactivity, although it should be noted that these models of inactivity do not present the same stimuli to the vasculature as prolonged sitting (Thijssen et al., 2010). Nonetheless, there seems to be consistent findings of an initial decline in FMD up following a period of approximately 3 weeks of inactivity (Thijssen et al., 2010). Thereafter there is detectable arterial remodeling, sometimes accompanied by thickening of the arterial walls (Thijssen et al., 2011b). As a consequence of the now smaller vessel and altered wall thickness, FMD is has been reported to paradoxically increase with continued inactivity, an finding that occurs in the presence of enhanced vasoconstrictor activity (de Groot et al., 2006). In therefore seems that, in adults, long-term inactivity and activity have distinct mechanistic pathways and are not simply the reverse of each other. The time course of recovery from uninterrupted sitting and the chronic vascular response to inactivity remains to be confirmed in the child.
Mechanism(s) of action: Although we found a substantial decrease in SFA FMD following prolonged sitting, these were not accompanied by significant declines in mean shear rate or shear patterns at baseline. The decrease in SFA FMD following sitting was also not accompanied by changes in the eliciting SR\textsubscript{AUC} stimulus in this group of young girls, suggesting that diminished shear stress mediated stimulation of endothelial NO bioavailability was not responsible for the reduced vasodilation. Thosar et al. (2015) also found a decrease in SFA FMD (%) was not accompanied by a decline in SR\textsubscript{AUC} following three hours of sitting, although mean shear rate and antrograde shear rate did diminish. In contrast, Restaino and colleagues (2015) showed that six hours of sitting resulted in a decline in the FMD SR\textsubscript{AUC}. It is possible that differences in the sitting protocols (i.e., six hours of sitting versus three hours of sitting) between these studies resulted in differing shear stimuli. Additionally, previous work has shown that the association between FMD (%) and post-deflation SR is weaker in children than in young adults (Thijssen et al., 2009a). The sensitivity to vasodilators such as nitric oxide appears to be age-dependent, as are vasoconstrictor pathways and this may account for the differences in our findings (Seals et al., 2008; Thijssen et al., 2007). Other reasons may account for flow-mediated dilatory responses to sitting in the young, such as differences in vascular wall properties between the young and old (Dinenno et al., 2000), or sympathetic nerve activity (Thijssen et al., 2006). Moreover, although alterations in both blood viscosity and fibrinogen have been noted in adults following prolonged sitting (Shvartz & Gaume, 1983; Hitosugi et al., 2000), it is unclear if such changes may also occur in children.
Although SR\textsubscript{AUC} and flow\textsubscript{AUC} were higher during the pre EX hyperaemic condition compared to the pre SIT hyperaemic condition, the order of the trials was counter-balanced, thereby eliminating trial order as a reason for this difference. It is probable that this difference represents day-to-day measurement variation. Regardless of these subtle differences, the pre interventional FMDs were similar between trials and the principal finding, a decrease in FMD post SIT, could not be explained by decreased SR\textsubscript{AUC}.

*Amelioration of impairment in vascular function by exercise breaks:* Our second major finding was that the deleterious effect of sitting on SFA FMD (%) was prevented by interrupting sitting with short exercise breaks, with FMD (%) remaining unchanged from baseline when the three-hour sit was interrupted with a 10-minute moderate intensity exercise break once an hour. We choose this exercise break protocol because in adults this has been established as effective in ameliorating sitting induced vascular dysfunction in the SFA (Thosar et al., 2015). Prior experiments using sitting as a model for physical inactivity have varied the exercise break protocol (Saunders et al., 2012; Altenburg et al., 2013; Thosar et al., 2014). In adults, lower intensity, more frequent breaks have been effective in preventing sitting induced metabolic dysfunction (Dunstan et al., 2010), however our previous work and that of others has shown low intensity exercise does not alter metabolic health in children (McManus et al., 2011b; Saunders et al., 2012). Since shear patterns did not clearly explain the prophylactic benefits of exercise in offsetting the sitting-induced impairment in vascular function, the favourable mechanism(s) of action warrant further research. Moreover, since higher intensity
exercise has been found to be more effective than moderate intensity exercise for improving aerobic fitness and arterial function in children (McManus et al., 2005; Mills et al., 2013), future work could investigate the protective effect of shorter, more frequent and intense exercise breaks, which may provide a better match to the child’s normal physical activity patterns than a continuous 10 minute exercise break (McManus et al., 2011a).

Methodological considerations: A major strength of the current study is the objective monitoring of the sitting conditions, achieved by assessing posture and movement using ActivPAL accelerometers. During the three-hour SIT condition children sat on average for 171 minutes. The remaining time was spent standing (~6 minutes) or moving (~2 minutes) and this movement was largely due to postural adjustments in sitting position. During the EX condition 135 minutes was spent seated and 30 minutes exercising on the cycle ergometer. The remaining time was spent standing (~10 minutes) or moving (~3 minutes). The increased time standing in the EX condition was because of the necessity to stand up to get on and off the cycle ergometer. Clearly 171 minutes of sitting was still sufficient to cause reductions in vascular function, but this does illustrate the importance of carefully monitoring how much time is spent in various postures when using experimental manipulations of sitting. This study is also strengthened by the FMD analysis approach we use, which is largely operator independent, hence limited operator bias. Previous reports of SFA FMD coefficient of variation or the technical error of measurement have not been documented. We report 5.9% technical error and 8.8%
coefficient of variation, the latter of which is lower that those reported in a recent large-scale study of FMD across childhood and adolescent (Hopkins et al., 2015). The analysis approach is internationally accepted as best practice and minimises the technical and experimental error in the primary outcome measured here (Thijssen et al., 2011a).

Limitations to our study include only examining an acute exposure to uninterrupted sitting in a single day and limiting the exercise breaks to a fixed frequency and duration. It is however difficult to experimentally manipulate sitting over longer periods of time in children without disrupting normal school schedules. We also felt it was important to establish the short-term effect prolonged sitting has, as well as the return of values to baseline, which were normalized in all children within the three-day period. To better understand the implications for longer-term cardiovascular health, it will be important to establish if the changes induced in vascular function after an acute period of sitting persist with repeated exposure to sitting. It will also be important to establish the dose–response relationships associated with different frequencies, durations, and intensities of exercise breaks and how these can be applied to real-life environments such as the school or home. The study is also limited by the small single-sex sample, although our sample was adequate to detect decreases in FMD and the findings are unlikely to change with an increased sample. Our results are limited to girls and whether the SFA response to prolonged sitting is the same in boys remains to be established. Finally, we were not able to administer a NO-donor such as nitroglycerine to assess endothelium-independent vasodilation in children this age, and our FMD results therefore reflect global vascular change and cannot be definitively ascribed to the endothelium or smooth muscle cell lines.
To conclude, our study suggests that prolonged sitting is detrimental for vascular health in children. This effect is, however, preventable if exercise breaks are instituted. Given the increasing periods of time that children are spending seated, these data highlight the importance of not just sitting there, but taking regular exercise breaks.
ACKNOWLEDGMENTS

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REFERENCES


ADDITIONAL INFORMATION

Competing Interests. There are no competing interests.

Author Contributions: The study took place in the Pediatric Inactivity Physiology Laboratory at the University of British Columbia. All authors were actively involved in the study including: the conception and design of the study (AM, PA); the collection, analysis and interpretation of data (AM, PA, DG, KS, RS and NL), drafting the article (AM, PA, DG, KS, RS and NL) and revising the article for intellectual content (AM, PA, DG and NL). All authors have approved the manuscript and AM had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy
of the data analysis.

*Funding:* We would like to thank the School of Health & Exercise Sciences and the National Health and Medical Research Council Australia (grant number APP1062338) for supporting this work.
# TABLES

Table 1. Descriptive characteristics. Values are means ± standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>n=9</th>
<th>Mean (SD)</th>
<th>Minimum &amp; maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>9.04 ± 0.78</td>
<td>7.92 - 10.08</td>
<td></td>
</tr>
<tr>
<td>Stature (cm)</td>
<td>137.4 ± 8.5</td>
<td>128.4-155.5</td>
<td></td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>32.0 ± 5.6</td>
<td>25-44.2</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>16.8 ± 1.5</td>
<td>14.7-18.8</td>
<td></td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>101 ± 8</td>
<td>84-113</td>
<td></td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>65 ± 5</td>
<td>59-74</td>
<td></td>
</tr>
<tr>
<td>VO₂max (L.min⁻¹)</td>
<td>1.24 ±0.27</td>
<td>0.90-1.70</td>
<td></td>
</tr>
<tr>
<td>VO₂max (ml.min.kg⁻¹)</td>
<td>38.8 ±5.5</td>
<td>29.0-47.9</td>
<td></td>
</tr>
<tr>
<td>90% GET (W)</td>
<td>30 ± 10</td>
<td>17-46</td>
<td></td>
</tr>
</tbody>
</table>

BMI, body mass index; BP, blood pressure; VO₂max, maximal oxygen uptake; GET, gas exchange threshold.
Table 2. Time in minutes spent sitting, standing and moving each hour of the 3-hour SIT and EX conditions. Values are means ±standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>SIT (n=9)</th>
<th></th>
<th>EX (n=9)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sit</td>
<td>Stand</td>
<td>Move</td>
<td>Sit</td>
</tr>
<tr>
<td>Hour 1 (min)</td>
<td>57.1 ± 2.3</td>
<td>1.9 ± 1.5</td>
<td>0.8 ± 0.9</td>
<td>45.3 ± 4.0*</td>
</tr>
<tr>
<td>Hour 2 (min)</td>
<td>56.2 ± 3.2</td>
<td>2.9 ± 2.7</td>
<td>0.9 ± 0.7</td>
<td>46.0 ± 5.5*</td>
</tr>
<tr>
<td>Hour 3 (min)</td>
<td>57.5 ± 1.6</td>
<td>1.7 ± 0.9</td>
<td>0.8 ± 0.9</td>
<td>44.0 ± 5.3*</td>
</tr>
</tbody>
</table>

* Significant difference between the SIT and EX condition, P < 0.001
Table 3. Superficial femoral artery parameters before and after the SIT and EX conditions. Values are means ± standard error.

<table>
<thead>
<tr>
<th></th>
<th>SIT</th>
<th></th>
<th>EX</th>
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<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Diameter (mm)</td>
<td>4.00 ± 0.07</td>
<td>4.09 ± 0.09</td>
<td>3.96 ± 0.11</td>
<td>4.07 ± 0.10</td>
</tr>
<tr>
<td>Mean blood flow</td>
<td>16.62 ± 0.96</td>
<td>19.04 ± 2.89</td>
<td>15.86 ± 0.95</td>
<td>19.37 ± 1.97</td>
</tr>
<tr>
<td>velocity (cm·s⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean blood flow</td>
<td>128.3 ± 9.9</td>
<td>149.0 ± 21.6</td>
<td>119.3 ± 9.9</td>
<td>150.2 ± 12.4</td>
</tr>
<tr>
<td>(ml/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean SR (1/s)</td>
<td>161 ± 8</td>
<td>181 ± 27</td>
<td>167 ± 14</td>
<td>196 ± 27</td>
</tr>
<tr>
<td>Mean Antrograde SR</td>
<td>181 ± 7</td>
<td>197 ± 23</td>
<td>184 ± 13</td>
<td>210 ± 25</td>
</tr>
<tr>
<td>(1/s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Retrograde SR</td>
<td>-20 ± 3</td>
<td>-17 ± 5</td>
<td>-17 ± 2</td>
<td>-14 ± 2</td>
</tr>
<tr>
<td>(1/s)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reactive hyperemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak Diameter (mm)</td>
<td>4.27 ± 0.07</td>
<td>4.28 ± 0.09</td>
<td>4.23 ± 0.11</td>
<td>4.34 ± 0.11</td>
</tr>
<tr>
<td>Absolute FMD (mm)</td>
<td>0.27 ± 0.02</td>
<td>0.19 ± 0.01#*</td>
<td>0.27 ± 0.01</td>
<td>0.27 ± 0.02</td>
</tr>
<tr>
<td>Corrected FMD (%)</td>
<td>7.04 ± 0.30</td>
<td>4.71 ± 0.20#*</td>
<td>7.25 ± 0.30</td>
<td>7.04 ± 0.50</td>
</tr>
<tr>
<td>SR_{AUC}</td>
<td>16651 ± 1995</td>
<td>19266 ± 2747</td>
<td>30161 ± 5495*</td>
<td>26865 ± 6694</td>
</tr>
<tr>
<td>OSI_{AUC}</td>
<td>0.10 ± 0.02</td>
<td>0.10 ± 0.03</td>
<td>0.12 ± 0.03</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>Flow_{AUC} (ml/s)</td>
<td>233 ± 37</td>
<td>258 ± 35</td>
<td>371 ± 157*</td>
<td>353 ± 76</td>
</tr>
</tbody>
</table>

#Indicates significant difference (P<0.01) from baseline within the SIT or EX conditions.

*Indicates significant difference between the SIT and EX conditions (P<0.01). SR. shear rates, FMD, flow mediated dilation, AUC, area under the curve; OSI, oscillatory shear index.
FIGURE LEGEND

Figure 1. Superficial femoral artery flow mediated dilation (FMD, %) before (Pre) and after (Post) the SIT and EX condition. Values are mean and standard deviations. The P values denote significance for the post-hoc pairwise comparison, Pre vs. Post EX and SIT.