Research Paper

Impact of prolonged sitting on vascular function in young girls

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New Findings

- **What is the central question of this study?**
  Children are spending more than 60% of their waking day sedentary. The consequences of excessive sedentary behaviour 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 h 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- to 10-year-old girls and the impact of interrupting prolonged sitting with exercise breaks. Superficial femoral artery endothelium-dependent flow-mediated dilatation, total shear rate, anterograde and retrograde shear rates and oscillatory shear index were assessed before and after two experimental conditions: a 3 h uninterrupted period of sitting (SIT) and a 3 h period of sitting interrupted each hour with 10 min 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 dilatation decreased significantly from pre- to post-SIT (mean difference 2.2% flow-mediated dilatation; 95% confidence interval = 0.60–2.94%, $P < 0.001$). 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.

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Introduction

Sedentary behaviour has reached alarming levels, with children and teenagers spending between 5 and 7 h 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 behaviour 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 dilatation (FMD), a marker of vascular health, is also altered with prolonged sitting in adults, as shown by Thosar *et al.* (2015). Here it was demonstrated that a 3 h 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 anterograde shear. Prolonged sitting provides a localized 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 haemodynamic outcomes in children has not been addressed; however, correlational data indicate that changes in endothelial health can be independent of sedentary behaviour (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.* 2015).

The aim of the present study was to examine whether 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 arterial function occur with ageing that are independent of maturational growth (Hopkins *et al.* 2015). Our hypotheses were as follows: (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 anterograde 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 (Colley *et al.* 2013; LeBlanc *et al.* 2015). 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 cross-over trial with the following two experimental conditions: (i) uninterrupted sitting (SIT), in which participants remained seated for 3 h; and (ii) breaks in sitting time (EX), in which participants completed an identical 3 h of sitting as above, except that at the beginning of each hour they cycled for 10 min 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 familiarization 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 measured manually prior to the start of both 3 h conditions. The SFA FMD was then assessed prior to the start and following each 3 h 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 h prior to each visit. In order to standardize dietary intake, parents were instructed to provide identical meals on both testing days, consisting of food normally eaten by their child, 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.

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During the SIT and EX conditions, the children sat on large beanbag seats for 3 h 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 bathroom, 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 min exercise break at the beginning of each of the 3 h, cycling on a cycle ergometer (LODE Paediatric Corival, Groningen, Netherlands) at an individualized 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 was calculated from body mass (in kilograms) divided by stature (in metres)^2. Healthy weight, overweight and thinness were classified using international references (Cole *et al.* 2000, 2007).

Blood pressure was assessed by sphygmomanometry to the nearest 2 mmHg, twice in the right arm, seated, after 10 min supine rest, and a third time if the readings were 5 mmHg apart. Diastolic pressure was defined as the point of disappearance of Korotkoff sounds (fifth phase).

Maximal oxygen uptake was determined during a ramp exercise test to volitional exhaustion on an electromagnetically braked cycle ergometer (Lode Paediatric Corival). Following a 3 min warm-up at 5 W, intensity was increased every minute by 10 W. Pedal cadence was ∼70 r.p.m. until volitional exhaustion, defined as a drop in cadence ≥10 r.p.m. for 5 s consecutively, despite strong verbal encouragement. Heart rate was measured continuously using heart rate telemetry (Polar Vantage NV; Polar Electro Oy, Kempele, Finland). Pulmonary gas exchange and ventilation were measured continuously using a breath-by-breath metabolic cart (Oxycon Pro; Carefusion, San Diego, CA, USA). 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 identified non-invasively 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 maximal oxygen uptake of the child, which severely compresses the range of work rates within a given exercise intensity (Armstrong & Barker, 2009).

Posture and movement were assessed using ActivPal accelerometers (ActivPal3^{TM} micro; PALtechnologies, Glasgow, UK) 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 t3200^{TM}; Teratech, Burlington, MA, USA) was used to image the left SFA. The FMD was assessed according to international guidelines (Thijssen *et al.* 2011*a*). A rapidly inflatable blood pressure cuff was positioned ∼5–10 cm above the knee joint, distal to the site of ultrasound capture. Baseline measurements 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 mmHg 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 used for the analysis of SFA diameter and blood flow velocity (Woodman *et al.* 2001; Thijssen *et al.* 2011*a*). This software provides continuous and simultaneous measurement of diameter and velocity, blood flow {lumen cross-sectional area and Doppler velocity (v); [4 × velocity (in centimetres per second)]/diameter (in centimetres)} and shear rate; as well as *post hoc* calculation of FMD. Velocity and flow were calculated from the Doppler envelope. Anterograde and retrograde blood flow and SRs were calculated from the anterograde 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.* 2009*b*). 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 maximal value of 0.5, an indicator of flow reversal. High values of OSI have been associated with endothelial dysfunction in adults (He & Ku, 1996). The OSI was calculated as follows:

$$\text{OSI} = |\text{retrograde shear}|/(|\text{anterograde shear}| + |\text{retrograde shear}|)$$

This semi-automated software provides higher reproducibility of diameter measurements and reduces both observer error and bias (Woodman *et al.* 2001). 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 (in millimetres) and relative (percentage) increases from

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the preceding baseline diameter (Thijssen *et al.* 2011*a*). In accordance with procedural recommendations (Pyke & Tschakovsky, 2005, 2007), we also measured the postdeflation area under the shear rate curve (SR_{AUC}) in order to provide the best interpretion of any changes in FMD; however, we did not normalize FMD (%) against SR because of 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) × 100] for baseline SFA diameter and SFA FMD (%). We compared between condition (SIT *versus* EX) main effects for the accelerometer data using ANOVA. We examined within and between condition (SIT *versus* EX) main effects for SFA FMD (%), SRs, velocity and flow using a mixed-model 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 (%). Carry-over effects were not formally tested, given the minimal 3 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 α-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 min per hour, with an average of <4 min taken standing or moving. During the EX trial, the girls sat for ∼45 min per hour, and in addition to the 10 min exercise break, spent an average of 5 min 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. The TEM expressed as a percentage for SFA baseline diameter and FMD (%) were 3.7 and 5.9%, respectively. The coefficients 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 that the washout period between trials was sufficient to eliminate any possible carry-over effects.

Table 1. Descriptive characteristics

	Mean (SD)	Minimum and maximum
Age (years)	9.04 ± 0.78	7.92–10.08
Stature (cm)	137.4 ± 8.5	128.4–155.5
Body mass (kg)	32.0 ± 5.6	25–44.2
BMI (kg m^{-2})	16.8 ± 1.5	14.7–18.8
Systolic BP (mmHg)	101 ± 8	84–113
Diastolic BP (mmHg)	65 ± 5	59–74
$\dot{V}_{O_2max}$ (l min^{-1})	1.24 ± 0.27	0.90–1.70
$\dot{V}_{O_2max}$ (ml min^{-1} kg^{-1})	38.8 ± 5.5	29.0–47.9
90% GET (W)	30 ± 10	17–46

Values are means ± SD (n = 9). Abbreviations: BMI, body mass index; BP, blood pressure; GET, gas exchange threshold; $\dot{V}_{O_2max}$, maximal oxygen uptake.

The SFA FMD (%)decreased significantly following the SIT condition ($P < 0.001$) but not the EX condition ($P = 0.97$; see Fig. 1). The mean decrease in corrected SFA FMD (%) was 2.2% from pre- to post-SIT (95% confidence interval = 0.60–2.94%, $P < 0.001$). Corrected SFA FMD (%) remained unchanged pre- to post-EX condition (see Table 3 and Table S1).

Mean baseline SR, anterograde and retrodgrade SRs, as well as SR under the curve and OSI 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 during reactive hyperaemia ($P > 0.05$). There was no within-condition main effect for SR_{AUC}; however, a significantly greater SR_{AUC}, as well as flow area under the curve ($flow_{AUC}$), was detected pre-EX compared with pre-SIT ($P < 0.05$).

Discussion

These are the first findings to demonstrate that 3 h of uninterrupted sitting in young girls is detrimental to vascular function, and that a 10 min 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

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Table 2. Time (in minutes) spent sitting, standing and moving each hour of the 3 h SIT and EX conditions

	SIT ($n = 9$)			EX ($n = 9$)		
Period	Sit	Stand	Move	Sit	Stand	Move
Hour 1 (min)	57.1 ± 2.3	1.9 ± 1.5	0.8 ± 0.9	45.3 ± 4.0*	3.3 ± 3.9	11.0 ± 0.9*
Hour 2 (min)	56.2 ± 3.2	2.9 ± 2.7	0.9 ± 0.7	46.0 ± 5.5*	2.2 ± 2.8	11.6 ± 2.3*
Hour 3 (min)	57.5 ± 1.6	1.7 ± 0.9	0.8 ± 0.9	44.0 ± 5.3*	4.9 ± 5.2	11.0 ± 0.6*

Values are means ± SD. *Significant difference between the SIT and EX conditions, $P < 0.001$.

Table 3. Superficial femoral artery parameters before and after the SIT and EX conditions

		SIT		EX	
		Pre	Post	Pre	Post
Baseline	Mean diameter (mm)	4.00 ± 0.07	4.09 ± 0.09	3.96 ± 0.11	4.07 ± 0.10
	Mean blood flow velocity (cm s^{-1})	16.62 ± 0.96	19.04 ± 2.89	15.86 ± 0.95	19.37 ± 1.97
	Mean blood flow (ml min^{-1})	128.3 ± 9.9	149.0 ± 21.6	119.3 ± 9.9	150.2 ± 12.4
	Mean SR (s^{-1})	161 ± 8	181 ± 27	167 ± 14	196 ± 27
	Mean anterograde SR (s^{-1})	181 ± 7	197 ± 23	184 ± 13	210 ± 25
	Mean retrograde SR (s^{-1})	−20 ± 3	−17 ± 5	−17 ± 2	−14 ± 2
Reactive hyperaemia	Peak diameter (mm)	4.27 ± 0.07	4.28 ± 0.09	4.23 ± 0.11	4.34 ± 0.11
	Absoulte FMD (mm)	0.27 ± 0.02	0.19 ± 0.01*†	0.27 ± 0.01	0.27 ± 0.02
	Corrected FMD (%)	7.04 ± 0.30	4.71 ± 0.20*†	7.25 ± 0.30	7.04 ± 0.50
	SR_{AUC} (A.U.)	16,651 ± 1995	19,266 ± 2747	30,161 ± 5495*	26,865 ± 6694
	OSI	0.10 ± 0.02	0.10 ± 0.03	0.12 ± 0.03	0.07 ± 0.01
	$Flow_{AUC}$ (ml s^{-1})	233 ± 37	258 ± 35	371 ± 157*	353 ± 76

Values are means ± SEM. *Significant difference between the SIT and EX conditions ($P < 0.01$). †Significant difference ($P < 0.01$) from baseline within the SIT or EX conditions. Abbreviations: AUC, area under the curve; FMD, flow-mediated dilatation; OSI, oscillatory shear index; SR, shear rates.

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 SRs 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 because of the anatomical structure, has been suggested to contribute to a higher incidence of atherosclerosis in comparison to the upper extremity blood vessels (Wu *et al.* 2004; Wood *et al.* 2006). Furthermore, 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 implication 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 the risk of a future cardiac event by up to 13% (Inaba *et al.* 2010).

The longer-term consequences of uninterrupted sitting in terms of vascular health 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 seem to be consistent findings of an initial decline in FMD following a period of ∼3 weeks of inactivity (Thijssen *et al.* 2010). Thereafter, there is detectable arterial remodelling, sometimes accompanied by thickening of the arterial walls (Thijssen *et al.* 2011*b*). As a consequence of the now smaller vessel and altered wall thickness, FMD has been reported to increase paradoxically with continued inactivity, a finding that occurs in the presence of enhanced vasoconstrictor activity (de Groot *et al.* 2006). It 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 remain to be confirmed in the child.

Mechanism(s) of action

Although we found a substantial decrease in SFA FMD following prolonged sitting, this was not accompanied by significant declines in mean shear rate or shear patterns

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at baseline. The decrease in SFA FMD following sitting was also not accompanied by changes in the eliciting SR_{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 vasodilatation. Thosar *et al.* (2015) also found that a decrease in SFA FMD (%) was not accompanied by a decline in SR_{AUC} following 3 h of sitting, although mean shear rate and anterograde shear rate did diminish. In contrast, Restaino *et al.* (2015) showed that 6 h of sitting resulted in a decline in popliteal artery FMD SR_{AUC}. It is possible that differences in the sitting protocols (i.e. 6 h of sitting *versus* 3 h of sitting) between these studies resulted in differing shear stimuli. Additionally, previous work has shown that the association between FMD (%) and postdeflation SR is weaker in children than in young adults (Thijssen *et al.* 2009*a*). 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 (Thijssen *et al.* 2007; Seals *et al.* 2008). 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 whether such changes may also occur in children.

Although SR_{AUC} and $flow_{AUC}$ were higher during the pre-EX hyperaemic condition compared with the pre-SIT hyperaemic condition, the order of the trials was counterbalanced, 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 (%) from pre- to post-SIT, could not be explained by decreased SR_{AUC}.

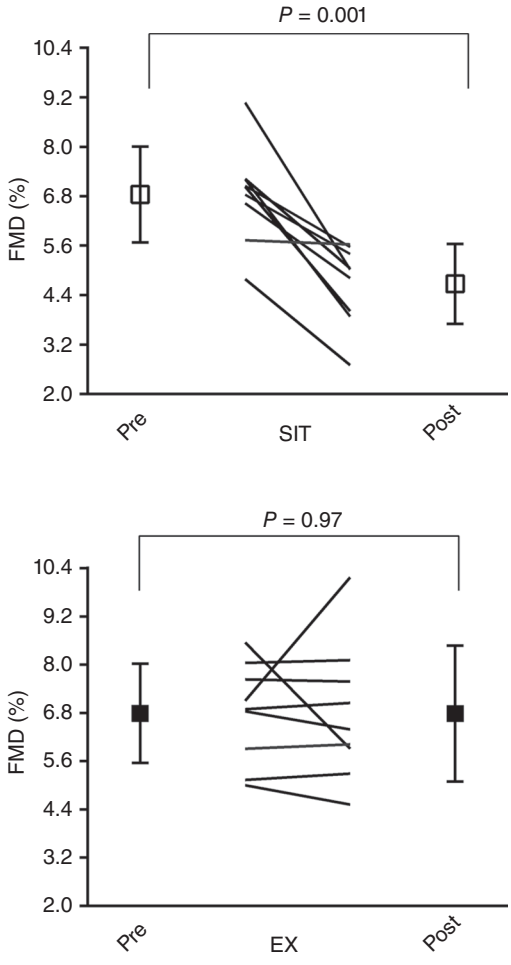

Figure 1. Superficial femoral artery flow-mediated dilatation (FMD, %) before (Pre) and after (Post) the SIT and EX conditions
Values are means ± SD. The *P* values denote significance for the *post hoc* pairwise comparison, Pre *versus* Post EX and SIT.

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 3 h sit was interrupted with a 10 min moderate-intensity exercise break once an hour. We chose 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.* 2012); however, our previous work and that of others has shown that low-intensity exercise does not alter metabolic health in children (McManus *et al.* 2011*b*; Saunders *et al.* 2012). Given that 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, as 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

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activity patterns than a continuous 10 min exercise break (McManus *et al.* 2011*a*).

Methodological considerations

A major strength of the present study is the objective monitoring of the sitting conditions, achieved by assessing posture and movement using ActivPAL accelerometers. During the 3 h SIT condition, children sat on average for 171 min. The remaining time was spent standing (∼6 min) or moving (∼2 min), and this movement was largely due to postural adjustments in sitting position. During the EX condition, 135 min was spent seated and 30 min exercising on the cycle ergometer. The remaining time was spent standing (∼10 min) or moving (∼3 min). 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 min 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 limiting 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 than those reported in a recent large-scale study of FMD (%) across childhood and adolescence (Hopkins *et al.* 2015). The analysis approach is internationally accepted as best practice and minimizes the technical and experimental error in the primary outcome measured here (Thijssen *et al.* 2011*a*).

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 manipulate sitting experimentally over longer periods of time in children without disrupting normal school schedules. We also felt that it was important to establish the short-term effect of prolonged sitting, as well as the return of values to baseline, which were normalized in all children within the 3 day period. In order to gain a better understanding of the implications for longer-term cardiovascular health, it will be important to establish whether 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 an NO donor, such as nitroglycerine, to assess endothelium-independent vasodilatation in children of this age, and our FMD results therefore reflect global vascular change and cannot be ascribed definitively to the endothelium or smooth muscle cell lines.

Conclusion

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 merely sitting there, but taking regular exercise breaks.

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Additional information

Competing interests

None declared.

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 following aspects: the conception and design of the study (A.M.M. and P.N.A.); the collection, analysis and interpretation of data (A.M.M., P.N.A., D.J.G., K.S., R.G.S. and N.L.); drafting the article (A.M.M., P.N.A., D.J.G., K.S., R.G.S. and N.L.); and revising the article for intellectual content (A.M.M., P.N.A., D.J.G. and N.L.). All authors have approved the manuscript, and A.M.M. 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.

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Supporting information

Table S1. Supplementary data for flow mediated dilation, shear rates and flow velocity.

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