THE IMPACT OF SHEAR RATE AND PROLONGED SITTING ON ENDOThelial FUNCTION IN CHILDREN

by

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Abstract

Prolonged sitting has been shown to impair endothelial function in children, yet the mechanisms underlying this remain unclear. In adults, there is a decline in endothelial function, indexed using flow-mediated dilation (FMD), after 1 hour of sitting. When localized heating was used to increase shear stress, the sitting-induced reduction in FMD can be prevented, confirming this decline was shear-stress mediated. The relationship between FMD and shear stress is weaker in children and whether limb heating increases shear stress and FMD in children is unknown. We therefore examined the time-course of changes in FMD with sitting and whether increasing shear stress during sitting would prevent reductions in FMD. Sixteen children completed measurements of superficial femoral artery (SFA) FMD in both legs before and after a 3-hour sitting period, with a subgroup of 7 children completing additional measures after 1 and 2 hours of sitting. In one leg, the calf was heated with an electronic heat pad at 42°C (i.e. heated condition), while the contralateral leg served as an internal control (i.e., non-heated condition). Heart rate and blood pressure were unchanged throughout the 3 hours confirming the heating was localized. Following 3 hours of sitting, antegrade shear rate was unchanged in the non-heated leg (pre-sit: 102.0 ±43.7 s⁻¹, 3 hr sit: 126 ±40.1 s⁻¹; \( P>0.05 \)), but increased significantly in the heated leg (pre-sit:102.2 ±33.6 s⁻¹, 3 hr sit: 216.2 ±47.3 s⁻¹; \( P<0.05 \)). FMD was not altered in either heated or unheated legs. In the subset, there was no significant main effect for time or interaction for corrected FMD; however, there was some indication of an increase in FMD in the heated leg after three hours of sitting. To conclude, we show that sitting for three hours did not reduced FMD. Passive limb heating increased blood flow and shear stress, but did not have a consistent impact on FMD.
Preface

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Chapter 2 - I wrote Chapter 2 and received extensive feedback from Dr. McManus through several editing processes.

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Chapter 4 - I wrote chapter 4 and received extensive feedback from Dr. McManus prior to finalization.

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<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>AU</td>
<td>Arbitrary Units</td>
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<td>AUC</td>
<td>Area Under the Curve</td>
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<td>IMT</td>
<td>Intima-Media</td>
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<td>Kc</td>
<td>Calcium-Activated Potassium Channel</td>
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<tr>
<td>Mhz</td>
<td>Megahertz</td>
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<td>Muscle Sympathetic Nervous Activity</td>
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<td>NO</td>
<td>Nitric Oxide</td>
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<td>PGI2</td>
<td>Prostacyclin</td>
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<td>SBP</td>
<td>Systolic Blood Pressure</td>
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<td>SFA</td>
<td>Superficial Femoral Artery</td>
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<tr>
<td>SRAUC</td>
<td>Shear Rate Area Under the Curve</td>
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Dedication

I would like to dedicate this to my family.
Chapter 1 Introduction

Sedentary behaviour is a part of everyday life in the developed world. Whether this is motorized transportation, preferential sedentary leisure-time habits, the sedentary occupations or the schooling that the majority of the population engages in, sitting is ubiquitous. The negative effects excessive sitting has on health are far reaching, including increased weight gain (Trost et al., 2001), poor psychological health (Ussher et al., 2007), poor cardiovascular health (Katzymark et al., 2009) and an increase in metabolic diseases (Ekelund et al., 2006).

Being sedentary is not unique to the 21st century. Documentation from the 1700’s by occupational physician Bernardino Ramazzini highlighted the negative health consequences of sedentary behaviour in the workplace. He noted that workers who sat for long periods of time had negative health outcomes (Franco & Fusetti, 2004), but those who could perform postural changes in the workplace prevented the hazards of prolonged sitting. One of the first comprehensive research studies was conducted in the 1950’s by Jerry Morris and colleagues, who worked with London bus drivers. This prospective work elegantly highlighted the higher prevalence of cardiovascular disease (CVD) in bus drivers compared to their mobile bus conductor colleagues (Morris et al., 1953). With advances in technology, sedentary behaviour has become a much more prominent part of modern lifestyles in westernised society and the accompanying adverse health consequences are apparent. For example, a dose-response relationship between sitting time, all-cause mortality and CVD has been shown in a representative sample of 17,013 Canadians, aged 18-90 years (Katzmarzyk et al, 2009). As little as a 1 hour increase in television time is associated with an 11% increase in risk of all-cause mortality, and an 18% increase in CVD (Dunstan, 2010). In both children and adults
decreased physical activity and increased sedentary time is a global issue (Hallal et al., 2012), and the impact this has upon physiological function remains poorly understood.

Sedentary behaviours begin early, with Canadian children spending more than 60% of their waking day sedentary (Colley et al., 2011). This is particularly concerning given CVD, metabolic disease, psychological well-being and obesity can originate in childhood (Dietz, 1998; Ekelund et al., 2006; Thijssen et al., 2010; Katzmarzyk et al, 2009). A systematic review of 170 studies in children found that more than 2 hours a day of sedentary time was associated with unfavorable body composition (Tremblay et al., 2011). Decreasing sedentary time improves body weight, BMI and weight status (Salmon et al., 2008; Goldfield et al., 2007; Ochoa et al., 2007; Epstein et al., 2005). Additionally, in children increased sedentary time is associated with greater risk for CVD. More than 2 hours of screen time has been reported to relate to higher blood pressure (BP) (Dasgupta et al, 2006; Lazarou et al., 2009) and the effects of sedentary behaviour on CVD risk appear to originate with arterial health. Most notably endothelial-dependent flow-mediated dilation (FMD), a marker of arterial health, is an antecedent of CVD, with a 1% decrease in FMD increasing cardiovascular disease risk by 13% (Inaba et al., 2010). Likewise, areas of low shear rate, that is areas where blood flow is low and does not provide the appropriate arterial wall stimulus, have more frequent atherosclerotic lesions (Caro et al., 1969). Evidence however of arterial damage in children is limited. Correlational work examining the relationship between total sedentary time and FMD in children found no association (Hopkins et al., 2012). However, it should be noted that this study assessed upper limb (brachial) FMD and it is known that the brachial artery does not present the atherosclerotic tendencies seen in lower limbs (Dalager et al., 2007). Additionally,
it is uninterrupted sitting rather than total sedentary time that appears to have the greatest health impact (Healy et al., 2008). Experimental sitting protocols provide a greater appreciation of the acute effects of uninterrupted sitting on arterial function and using such an experimental set-up, McManus and colleagues (2015) demonstrated that an acute bout of uninterrupted sitting for 3 hours causes a profound (2.2%) reduction in superficial femoral artery (SFA) FMD in healthy children. In adults, a similar decrease in FMD is seen even after 1 hour of uninterrupted sitting (Thosar et al., 2015).

Endothelial function is very responsive to changes in the hemodynamic status of the vasculature. This can be negative, such as with excessive sitting reducing blood flow and therefore the shear stress stimuli (Restaino et al., 2016). Equally this can be positive, such as increases in shear stress from exercise or localized limb heating (Thosar et al., 2015; Restaino et al., 2016). Interestingly unlike adults, the decline in FMD noted after 3 hours of sitting in children was not accompanied by changes in shear stress (McManus et al., 2015) and the relationship between shear stress as a key hemodynamic stimulus on endothelial function in childhood is less clear. Developing a better understanding of the mechanisms underlying sitting-induced endothelial dysfunction in children is therefore an essential step in the development of strategies to effectively prevent this adverse response.

The purpose of this thesis is to clarify how much sitting is harmful to endothelial health in the child and determine whether changes in shear stimuli account for changes in endothelial dependent FMD. The thesis begins with a review of the extant literature, which provides an overview of the development of the vasculature, discusses the impact of lifestyle on vascular function, with specific emphasis on sitting. The mechanism(s) underlying sitting induced
vascular dysfunction are also considered. This is followed by a methods chapter, providing details of the main outcome measures (FMD and shear stress), and outlining the study methodology. Results follow, and the thesis finishes with discussion and conclusions.

1.1. Development of the vasculature

Various vessels of different calibers are found within the arterial network, but it is the larger peripheral conduit vessels where vascular function is most regularly assessed. Figure 1.1 illustrates the anatomy of the conduit artery, with the tunica media, providing the elastic stretch in response to the pulse pressure of the cardiac cycle, the tunica intima media and the endothelium, the single-cell inner lining of the artery.

![Cross-sectional image of a conduit artery.](image-url)


The endothelium has autocrine and paracrine functions which enable the production and
release of vasoactive substances. Healthy endothelial cells respond to changes in blood flow by secreting vasodilators (e.g., nitric oxide, NO). It is the increase in blood flow through the vessel lumen that causes a frictional force or drag (i.e., shear stress) and stimulates the endothelium to produce endothelium nitric oxide synthase (eNOS) which synthesizes NO. This causes NO-mediated vasodilation to normalize the shear stress on the vessel wall, regulate vascular tone, and adjust blood flow (Di Francescomarino et al. 2009; Rauramaa & Hassinen, 2011). Therefore, vascular function in this thesis refers to changes in shear stress and the vasodilation of an artery.

Endothelium-independent vasodilatation is another measure to assess vascular health that uses an NO donor (usually nitroglycerin) to abolish the influence of the endothelium. Nitroglycerin can stimulate vessel dilation via smooth muscle, without changing shear patterns. While this method is not often used in children, it provides insight into mechanisms of dilation.

Conduit arteries progressively increase in diameter with age from childhood to young adulthood (see figure 1.2), in a sex dependent manner (Hopkins et al., 2015). The most commonly assessed vessels in children are the carotid and brachial arteries, with more limited data on the femoral and popliteal arteries. In children aged 5-9 years brachial and femoral diameter are 2.22±0.33 mm and 4.63±0.57 mm respectively (Sarkola et al., 2012). By the age of 12 years brachial diameter is 3.11±0.31 mm (Hopkins et al., 2015), while femoral diameter is 6.03±1.11 mm (Sarkola et al., 2012). By 15-18 years of age, average brachial diameter is 3.11±0.46 mm, and femoral diameter is 7.25±1.04 mm (Sarkola et al., 2012).
Figure 1.2 Baseline artery diameter (mm) of males (triangles) and females (circles) from ages 6 to 18 years. Retrieved from Hopkins et al., (2012) © permission not required.

The rate and timing of growth of the brachial arterial diameter is sex specific, with a steady increase in diameter in both boys and girls found from age 6 years, continuing to 18 years in boys, but showing a plateau in girls at 12 years of age (Hopkins et al., 2015). Brachial artery diameter is similar between girls and boys prior to puberty, but by about 12 years of age, average brachial artery diameter is 2.96 mm in girls and 3.24 mm in boys (Hopkins et al., 2015). The carotid and aortic arteries follow a similar sex specific growth pattern in diameter (Sarkola et al., 2012); however, no sex differences were noted in leg arterial lumen or wall dimensions (Sarkola et al., 2012).

From birth to adulthood there is also a thickening of the arterial wall, due to remodeling of the intima-media (IMT). Carotid IMT values during infancy (<1 year) average 0.292 ± 0.062 mm, by mid-childhood (5-9 years) have increased to 0.351 ± 0.045 mm, and by adulthood (15-18
years) are $0.414 \pm 0.072$ mm. Femoral IMT is $108 \pm 0.024$ at infancy, $0.177 \pm 0.035$ mm by age 5-9 years and $0.314 \pm 0.064$ mm by age 15-18 years. This increase is a major determinant of the increases in systolic BP over the same period. The variability of the arterial wall and IMT can largely be explained by age, sex, body surface area and the strong association between arterial wall stress and IMT, indicates developmental changes of vascular tone and BP (Sarkola et al., 2012).

Carotid artery stiffness decreases from $4.6 \pm 1.0$ to $3.0 \pm 0.8$ (in non-dimensional units) from birth to age 4 years (Sarkola et al., 2012). Increases are then apparent from 5-9 years up to 15-18 years, with evidence of an increase in ascending aorta stiffness index from $3.3 \pm 1.4$ to $4.6 \pm 12.0$. This does not appear to be influence by sex, but is influenced by systolic BP. In youngsters, there is no relationship between arterial stiffness and endothelial function (Sarkola et al., 2012). This is in contrast to adult work where brachial FMD is inversely associated with carotid IMT (Juonala et al., 2008), although it is important to note that some of the adults in the Juonala et al. study had several cardiovascular risk factors. Overall the variability in arterial endothelial function cannot be determined by wall thickness alone (Sarkola et al., 2012).

Changes also occur in endothelial function during the growing years (Celermajer et al., 1994). FMD declines with age from 6-18 years (Hopkins, 2015, see figure 1.3), and continues to decline into adult life, with declines occurring earlier in males than females. An age-related decline in FMD has been found in the brachial (Celermajer et al., 1994; Parker et al., 2006; Black et al., 2009), as well as in femoral arteries (Thijssen et al., 2006).
Sex differences in FMD are negligible once FMD is adjusted for baseline diameter (See chapter 2 for more information on scaling for diameter). Vessel diameter (size) influences flow, whereby a larger vessel will have a decreased velocity (see figure 1.4). Also, smaller vessel diameters have a larger dilator response, which may be explained by the ratio of higher smooth muscle to elastic laminae. This is noted by the positive relationship seen between wall-to-lumen ratio and vasodilator response of conduit arteries (Thijssen et al., 2011).

Figure 1.4. Illustration to show that vessels with different diameters and the same flow may represent a very different shear stress stimulus. Retrieved from Pyke & Tschakovsky 2005 with © permission.
As shown by Hopkins et al. (2015) brachial artery FMD around 12.2 years of age is 8.31% (range 7.95 to 8.66%) in boys, and 7.62% (range 7.33 to 7.91%) in girls respectively. It has been suggested that the sex difference is related to the effects of estrogen and testosterone during the pubertal period. It is known that estrogen is beneficial for vascular endothelial function, as it is a modulator of molecular pathways that allow for greater vasodilation (Arora et al., 1998), as well as greater NO production and bio-availability (Forte et al., 1998). This has been confirmed with post-menopausal women who experience a steep decline in NO (Celermajer et al., 1994). In boys, puberty is accompanied by a surge in androgens, specifically testosterone and dihydrotestosterone (Rogol et al., 2002) which promote muscle growth and increases vessel size (Hopkins et al., 2015).

Adjusting for differences in artery diameter, FMD values in girls age 6, 10 and 18 years are 8.09% (range 4.42 to 11.89%), 9.57% (range 7.89 to 11.28%) and 9.94% (range 6.99 to 12.97%) respectively. In boys of the same ages values are 12.09% (range 8.65 to 15.64%) at 6y, 9.56% (7.88 to 11.27%) at 10y and 6.61% (4.17 to 9.10%) at 18y (Hopkins et al., 2015). It is worth noting that although growth reference values are not available for the lower limb vessels, it is likely that a similar pattern of change is evident.

1.2 Vascular function and lifestyle

In addition to developmental changes, arterial health also changes as a function of other challenges such as lifestyle or environment. Chronic disease emanates from poor lifestyle, including sedentary behaviour, low levels of physical activity and poor diet. All of these impact upon the child’s vascular health and increase the risk of future cardiovascular events. With poor lifestyle, the endothelium is damaged (Giannotti et al., 2007) and is an early event marker of atherosclerosis, even before the formation of plaque (Celermajer et al., 1992).
Comparing healthy weight children with obese, FMD has been shown to be reduced in the obese whether at the brachial artery (Obese: 6.00 ± 0.69%; healthy weight: 12.32 ± 3.14%; Watts et al., 2004) or radial artery (Obese: 5.81 ± 3.42%; healthy weight: 9.29 ± 1.87%; Meyer et al., 2006). Obesity also affects arterial structure. In 14 year olds with obesity, Meyer et al. (2006) found an increased common carotid artery and carotid bifurcation intima media thickness (0.47 ± 0.06 mm) compared to lean adolescents (0.37 ± 0.05 mm), lowering arterial compliance and elasticity/distensibility. Hypertension is also negatively correlated with FMD (Juonala et al., 2006). Interestingly, the question remains if elevated BP has a cause or effect relationship with endothelial dysfunction in the obese. As higher arterial stiffness affects the ability of the large arteries to cushion cardiac output and the higher blood volume of these children influences BP regulation (Meyer et al., 2006).

1.3 The impact of sedentary behaviour on the endothelium

Different experimental modes of inactivity have been used to investigate the impact of excessive sedentary time on the vasculature. Bed rest, immobilization via casting, and subjects with spinal cord injury have all been studied in inactivity research. These all face confounding factors beyond typical sedentary behaviours such as a continuous ‘lack of movement’ in bed rest, the impact of healing and inflammatory processes from trauma (Green et al., 1997), and vascular adaptation that may occur from loss of sympathetic vascular tone in spinal cord injury (Thijssen et al., 2007). Experimental models such as bed rest have shown reduction in limb volume of 26-48% during 4-120 days of head down tilt bed rest (Christ et al. 2001; Convertino et al. 1989; Louisy et al. 1997; Pawelczyk et al. 2001), and a 24% reduction following 28 days of limb immobilization using venous occlusion plethsmography (Bleeker et al., 2005a).
Interestingly despite acute and chronic deconditioning of the peripheral conduit arteries, endothelium dependent vasodilation is preserved in spinal cord injury (de Groot et al., 2005) following bed rest (Bleeker et al., 2005b), and with unilateral lower limb suspension (Bleeker et al., 2005a). This may be related to the upregulation of smooth muscle cell sensitivity to NO due to deconditioning induced changes in shear rate, in order to preserve or normalize vascular function (Green et al., 2010).

Long term, deconditioning results in conduit artery diameter decreases and decreased blood flow. For example, leg casting resulted in a 6% reduction in artery diameter after 7 days (Sugawara et al. 2004), a 13% decrease in diameter after 28 days of bed rest (Bleeker et al. 2005a) and up to 17% reduction after 25 to 52 days of bed rest (Bleeker et al. 2005b).

Experimental manipulations of sitting provide an appreciation of the acute effects of sitting on endothelial function, and overcome some of the deficits of the previously discussed inactivity paradigms. Although it is important to note that short-term sitting experiments cannot provide any understanding into the chronic impact sitting may have on the vasculature.

In adults, the acute of effect of sitting causes a decline in endothelial function, reflected through changes in FMD and shear stress. After three hours of sitting in healthy individuals, SFA FMD decreases from baseline values of 4.72 ± 3.78% to 0.52% ± 0.85% at one hour, 1.66 ± 1.11% at two hours and 2.2 ± 2.15% at three hours (Thosar et al., 2015). These results were replicated by Restaino et al., (2016) who showed that three hours of sitting causes a decline in FMD of the popliteal artery from 7.1± 1.4% at baseline to 2.8 ± 0.9% at three hours. There may be a limb specific response to sitting, as three hours of sitting causes a decline in lower limb FMD, but no change in upper limb FMD (Thosar et al., 2014).
Sitting as a laboratory model of physical inactivity is highly relevant for children given the elevated levels of sitting noted in the younger population, however, the use of experimental models of sitting in children and adolescents are scarce. One study of adolescents, used an 8-hour period of sitting, but did not find any deficits in insulin, glucose or lipids (Saunders et al., 2012). More recent findings in healthy weight children have found individualized moderate intensity exercise breaks are effective in offsetting metabolic dysfunction created by an acute period of sitting for three hours (Belcher et al., 2015). Breaking up the three hours of sitting with three minutes of exercise at an individualized moderate intensity every 30 minutes resulted in a 32% reduction in insulin (area under the curve) compared to continuous sitting in 7-11 year-olds. The only study to focus on the vasculature, has shown a relative decline of 33% in SFA FMD following three hours of sitting in 7-10 year-old girls, which corresponds to a decline from 7.04% at baseline to 4.71% (see figure 1.5).

Figure 1.5 Superficial femoral artery flow-mediated dilatation before (Pre) and after (Post) sitting. Retrieved from McManus et al., 2015 with © permission.
1.3.1 Sex differences in sitting responses

New evidence shows that a sex difference in the response to sitting may exist (Vranish et al., 2017). After three hours of uninterrupted sitting, a decline in popliteal artery FMD has been shown in men, but not in women (Men: PreSit: 5.5 ± 0.9% and PostSit: 1.6 ± 0.4%; Women: (PreSit: 4.4 ± 0.6% and PostSit: 3.6 ± 0.6%). Conversely, looking at the reactive hyperemia response (hyperemic blood flow area under the curve) a decline was noted in both men and women (women: 28,860 ± 5,742 arbitrary units and men: 28,691 ± 9,685 arbitrary units). Interestingly, both sexes presented similar responses in shear rate, so this may indicate the sitting induced impairment in women is at the microvascular level. It has been suggested that due to the greater NO bioavailibility in women, even with decreases in shear from sitting, conduit artery vasodilatation is preserved (Vranish et al., 2017). The microvascular response (noted from the reactive hyperemic response) is less dependent on NO, and perhaps more influenced by changes in myogenic or local metabolites that alter with prolonged sitting (Doshi et al., 2001; Joannides et al., 1995). Sex differences have not been explored in children.

1.3.2 Time course of sitting induced endothelial function.

The decline in endothelial health from acute sitting can be seen after as little as 10 minutes of sitting in adults (Vranish et al., 2017), with the most pronounced decline noted after one hour (see figure 1.6, Thosar et al., 2014). The compensatory mechanisms that result in the rise in FMD with 2 and 3 h of sitting are not understood and whether the same time-course exists for children has yet to be determined.
Figure 1.6 The decline in FMD after 1, 2 and 3 hours of sitting noted in the black circles. Retrieved from Thosar et al., 2014 with © permission.

1.4 Mechanisms underlying sitting-induced endothelial dysfunction

1.4.1 Shear stress and FMD

Shear stress is considered a strain stress on vessel walls, acting parallel to the length of the vessel. It is the external force caused by friction between fluid particles. Blood flow creates a drag on the vessel’s wall causing shear stress (Luo et al., 1992). Normal stressors such as BP act on all layers of the vessel walls, whereas shear stress acts on the endothelial wall of the blood vessel. Blood flow therefore has a role in varying the amount of shear stress, and shear patterns, with both antegrade and retrograde flow components. Antegrade flow is known to promote endothelial health (Wang et al., 2013) and retrograde flow is associated with negative effects (Widlansky et al., 2003; Thijssen et al., 2009). Blood flow therefore determines vessel dilation via this shear stress mechanism, stimulating the endothelium to release vasoactive chemicals such as NO (Joannides et al., 1995; Pohl et al., 1986).

Shear stress leads to vessel dilation by triggering a signal which is transduced by endothelial cells to stimulate the release of vasoactive substances as shown in figure 1.7. This acts on the

Figure 1.7 A schematic of the interplay between shear mediated stimulation of the endothelium and the short-term (*), medium-term (minutes, **) and longer-term (hours, ***) changes in vasodilators that occur at the cellular level that impact FMD. Prostacycline (PGI₂); endothelium-derived hyperpolarizing factor (EDHF); calcium-activated potassium channel (Kc). Retrieved from Moens et al., 2005 with © permission.

As blood flow slows from sitting, shear rate appears to be an important underlying mechanism of endothelial dysfunction resulting from prolonged sitting. The relationship between shear and FMD has been confirmed in animals. In experimental mice (pro-atherogenic apoE null mice) it has been demonstrated that reductions of carotid artery shear stress impairs endothelial function (Nam et al., 2009). Low shear stress, as occurs in periods of sitting, decreases the production of NO (Malek et al., 1999). In humans, short term periods of low shear stress or oscillatory periods from distal cuff occlusion also decrease FMD in the upper and lower
extremities. Jenkins et al., (2013) showed that the disturbance of blood flow induces change in mean blood flow, mean shear, antegrade and retrograde shear, and causes acute endothelial activation and apoptosis in humans. Indeed, FMD is ~67% shear stress mediated in adults (Green et al., 2013). In the brachial artery, with increased oscillatory and retrograde SR by using cuff compression, a mean decrease in shear and FMD are noted (Johnson et al., 2012). The lower limb (femoral artery) reacts similarly to acutely increased retrograde shear, again causing a decline in FMD (Schreuder et al., 2014).

1.4.2 Age dependent shear-FMD relationships

Worthy of note is the relationship between shear rates and FMD varies with age. There was no correlation found between any of the shear stimuli variables and the magnitude of the brachial artery FMD response in children (Thijssen et al., 2009). Further, Hopkins et al. (2012) found no relationship between hyperaemic shear rate and FMD in 6 to 18 year olds (r=0.04, P=0.348). Age differences in the relationship between FMD and shear rates may be attributed to vascular wall characteristics such as elasticity (Monahan et al., 2001), wall thickness (Dinenno et al., 2000), and baseline diameter (Dinenno et al., 1999). Other factors could be the age differences in relation to vasoactive substances such as vasodilators (NO) (Seals et al., 2008) and vasoconstrictors (angiotensin-II, endothelin-I) (Thijssen, 2007). It has been suggested that prostacyclin (PGI₂) is the primary mediator in youth, NO in adulthood and changes again in older adulthood. Higher levels of cyclooxygenase metabolites have been found in children may contribute to the increased production of PGI₂ (Beyer et al., 2017).

1.4.3 Low shear stress from sitting

Accompanying sitting-induced reductions in FMD in adults, are significant declines in shear
rate and shear patterns (Reistano et al., 2013; Thosar et al., 2015). For example, across three hours of sitting Thosar et al. (2015) noted declines in SFA FMD and these were accompanied by declines in mean shear rate and antegrade shear rate, again indicating the role shear stimuli plays in mediated the FMD response.

Evidence from experimentally increasing the shear stimuli during sitting confirms shear stress as a mediator of FMD in adults. Restaino et al. (2016) manipulated shear by submerging one foot in 42°C water to sustain blood flow during a three hour sit, which kept shear stress elevated. This allowed for the contralateral limb to be an internal control, and in this experimental set-up they found that after three hours of sitting, popliteal artery mean shear rate had significantly declined in the non-heated leg (pre-sit: 49.9 ± 4.5 s⁻¹, post sit 23.6 ± 3.3 s⁻¹; \(P<0.05\)). In contrast, there was no decline in the heated leg (pre-sit: 38.9 ± 3.4 s⁻¹, post sit: 63.9±16.9 s⁻¹; \(P>0.05\)). Popliteal artery FMD also differed after three hours of sitting between the heated and non-heated legs. Declines from pre-sit values of 7.1 ± 1.4% to post-sit values of 2.8 ± 0.9% were noted in the non-heated leg, but in the heated leg FMD was preserved (pre-sit: 7.3 ± 1.5% vs. post-sit, 10.9 ± 1.8%). Morishima et al. (2016) used single leg intermittent muscle contractions or “fidgeting” (cycles of 1 min of fidgeting with 4 min rest) to maintain blood flow and thus shear rate and showed that the increased shear rate increased FMD (pre-sit: 3.7 ± 0.6%; post-sit: 6.6 ± 1.2%), which declined in the control non-fidgeting leg (pre-sit: 4.5 ± 0.3% to post-sit: 1.6 ± 1.1%). These data indicate that shear stress mediates sitting induced endothelial dysfunction in the leg, at least in adults.

More recently, McManus and colleagues (2015) found no relationship between post-sitting declines in FMD and shear rate or pattern in children. The lack of experimental verification of the shear-FMD relationship in children and reliance on correlational data may have masked
the relationship between shear and FMD in the child. Alternatively, there may be developmental divergent mechanisms underlying sitting induced endothelial dysfunction. A first step would be to experimentally confirm the relationship between shear stimuli and FMD during prolonged sitting in children.

1.5 Summary, objectives and hypotheses

To summarize, the rising levels of sedentary behaviour in children necessitates a better understanding of the negative health consequences. Limited evidence has shown that prolonged sitting causes a decline in vascular health indexed from endothelial dependent FMD. In adults, this decline in FMD occurs with as little as 10 min of sitting, and is most acute after one hour of sitting, but the time-course of sitting induced vascular dysfunction is not known in children. This reduction in FMD is shear mediated in adults, but the relationship between FMD and shear stimuli is less clear in children.

The objective of this thesis therefore is to clarify how much sitting is harmful to endothelial function in the child and determine whether changes in shear stimuli account for changes in endothelial dependent FMD. To achieve this, children were asked to sit for three hours with a shear rate manipulation. This entailed localized heating of one leg, with the contralateral leg left unheated. SFA FMD was simultaneously recorded in both legs at baseline and following the three hours in all children, and in a sub-set of children following each hour of sitting. We also intended to see if these responses were sex specific in younger children, however, we had a lot more difficulty recruiting boys to the study and therefore were underpowered to explore sex differences.
1.5.1 Hypotheses

We hypothesized that in 7 to 10 year-old girls and boys

(i) shear rate and antegrade flow would not differ from pre-sitting values in the unheated leg, whereas these would be increased in the heated leg,

(ii) despite increases in shear stimuli in the heated leg, sitting for 3 hours would result in a reduced SFA FMD in both heated and unheated legs after 3 hours,

(iii) in comparison to pre-sitting values, SFA FMD would decline the most after 1 hour compared to 2 and 3 hours.
Chapter 2 Methods

2.1 Participants

Twenty-two healthy 7-11 year olds (15 girls, 7 boys) participated in the study. All participants were classified as pre-pubertal on the basis of parental assessment of Tanner stage. Girls were rated Tanner 1 for pubic hair and breast development. Boys were rated Tanner 1 for pubic hair and genitalia development (Rasmussen et al., 2015). Written consent was received prior to testing by parents and the children provided written assent. The experimental procedures were approved by the Clinical Review Board of UBC (see Appendix A).

2.2 Study design and procedures

We used an experimental trial with two conditions: heated leg and non-heated leg. Heating was achieved using a heat-pad heated to 42°C and wrapped around either the right or left calf (randomly assigned). The contralateral leg was unheated and served as the control limb. Heating at 42°C has been shown to be an effective stimulus to increase localized blood flow and shear stress without causing any systemic changes (Padilla et al., 2011).

The children attended a single laboratory visit. They were asked to avoid vigorous exercise 24h before testing and not to eat at least 4 hours prior to attending the laboratory. Parents were asked to avoid giving the child foods containing fat and vitamin C, which may acutely effect endothelial function (Nicholls et al., 2006; Thosar et al. 2015b). We tested children in a non-fasted state as this is more indicative of normal daily life.

During the laboratory visit the children completed anthropometric measurements. They then sat upright on a couch in a temperature controlled room (22 °C), which allowed them to put
their legs up horizontally so the vascular ultrasound scans could be conducted *in situ*. Baseline measures of SFA function were completed, as were resting BP and heart rate (HR). The children continued to sit for 3 hours, watching movies or playing on iPads during the seated period. They were allowed to make small arm movements to perform these light activities, but were discouraged from standing. To allow for bathroom breaks children were wheeled in a chair to the washroom. Their activity was monitored throughout the trials with ActivPAL monitors (ActivPal3™ micro; PALtechnologies, Glasgow, UK) on their dominant leg. BP and HR were measured manually at baseline and at the end of each hour of sitting. SFA FMD was measured simultaneously in both legs before sitting (pre-sit) and post sitting (post 3h) in all 22 children (hypotheses 1 and 2). In a subset of 10 children SFA FMD was also assessed at the end of first (1h) and second hours (2h) of sitting (hypotheses 3). Files were coded to ensure analysis was blinded. Sonographers scanned the same leg throughout study, with the condition randomized.

### 2.3 Measures

Body mass (kg) was measured to the nearest 0.1 kg with electronic scales (Tanita TBF-410). Stature (cm) was measured to the nearest 0.1 cm with a stadiometer (Seca 217). Body mass index was calculated from mass (kg) divided by height (m) squared. All children were classified as healthy weight (mean BMI 16.44 ± 1.53 m.kg²).

Systolic (SBP) and diastolic blood pressure (DBP) were measured using a manual sphygmomanometer (Prestige Medical 79-BLK standard aneroid, Northridge CA) to the nearest 2 mmHg twice in the right arm after 5 min seated pre-sit and at the end of each hour of sitting. A third reading was taken if the values differed by more than 5mmHg. Diastolic
pressure was defined as the point of disappearance of Korotoff sounds (fifth phase). HR was measured pre-sit and at the end of each hour of sitting using HR telemetry (Polar Vantage NV, Polar Electro Oy).

Posture and movements were continuously monitored using an ActivPAL accelerometer (ActivPal3 micro; PALtechnologies, Glasgow, UK) placed on the dominant leg. Time spent sitting, standing and moving was recorded.

2.3.1 Principle outcome measures

FMD is calculated as the change from baseline diameter to peak diameter after the ischemic period, and expressed as a percent change. The recommended guidelines by Thijssen et al. (2011) call for subjects to rest in a dimly lit room for ≥ 20 minutes. Participants must avoid vigorous exercise, caffeine and ideally come in fasted state as FMD is affected by fat (Nicholls et al., 2006) and vitamin c (Thosar et al., 2015b). FMD is also lower in the morning, and affected by some medication and the menstrual cycle (Otto et al., 2004; Magen et al., 2005; Celermajer et al., 1994).

Measurement with duplex ultrasound should be continuous providing simultaneous acquisition of b-mode diameter and pulse wave velocity Doppler velocity signals. An ultrasound linear probe of >7.5 MHz, with the angle of insonation set to ≤ to 60 should be used. Cuff position influences the magnitude of shear and the contribution of vasoactive substance like NO and the cuff should be distal to the vessel of interest. Measurement of baseline of at least 1 minute is necessary, resuming 30 seconds before cuff deflation and continuing for 5 minutes post deflation. The timing of occlusion/ischemia can also impact the FMD and the recommended time is 5 minutes.
For this study, SFA blood flow and diameter were assessed using high-resolution duplex ultrasound (Terason USMART3300™; Teratech, Burlington, MA, USA). Following 10 minutes of seated rest BP cuffs controlled with fast deflating aneroids (Hokanson SC5, Bellevue, WA) were placed on each leg distal (~5cm) to the ultrasound probe (15-MHz Broadband Multi Frequency Linear Array Transducer). Once optimal imaging of the lumen-arterial wall interface was obtained, baseline SFA diameter, blood flow, and shear rates (mean, antegrade, retrograde) were continuously measured for 1 minute prior to cuff inflation. The cuffs were simultaneously inflated to 50mmHg above SBP for 5 minutes to induce ischemia. Recordings were resumed 30 seconds prior to cuff deflation and continuing for 5 minutes following deflation.

Simultaneous diameter and velocity signals were corrected at an insonation angle of 60 degrees. Recording software Camtasia (TechSmith, Okemos, MI) recorded at five frames per second and files were stored as .avi files. Edge-detection and wall-tracking custom designed software was used to provide continuous and simultaneous measurement of diameter and velocity, blood flow (lumen cross-sectional area and Doppler velocity \(v\); \([4\times\text{velocity cm.s}^{-1}]\)/diameter cm) and shear rate; as well as post hoc calculation of FMD. The Doppler envelope was used to calculate velocity and flow. Antegrade and retrograde blood flow and shear rates were calculated from antegrade and retrograde area under the curve data that were subsequently averaged from positive or negative data points respectively.

Edge-detection and wall-tracking software reduces investigator bias and adheres to international measurement recommendations (Woodman et al., 2001). One investigator conducted the analysis and was blinded to the study codes and participant files. Two researchers conducted the FMD measurements, each measuring one leg on every child,
counter-balanced for heat and no-heat. Inter-tester variation was minimized by comparing the pre-sit baseline diameter and FMD between the two legs of the same child, and only accepting scans that were within 0.5% for diameter (=0.33%) and 1% for FMD (=0.61%). The intra-tester coefficients of variation for FMD and baseline diameter were 10% and 5.0% for the two testers.

2.3.2 Shear stress

Post-scan analysis provides shear rate, shear patterns and hyperaemic shear rate area under the curve (SRAUC). Using SRAUC following cuff deflation has been used as an appropriate determinant of the FMD response rather than peak shear (Thiijsen et al., 2009).

2.4 Scaling for diameter

Arterial diameter impacts FMD because the calculation of FMD uses baseline diameter ($D_{\text{base}}$) expressed as function of the hyperemic peak diameter ($D_{\text{peak}}$), and therefore 64% of the variability in FMD can be explained by $D_{\text{base}}$ (Celermajer et al., 1992).

$$\text{FMD} = \left( D_{\text{peak}} - D_{\text{base}} \right) \times 100$$

$$D_{\text{base}}$$ (Thijssen et al., 2011)

The ratio nature of the FMD calculation has an inherent statistical bias (Packard & Boardman, 1999), which means FMD is overestimated in small diameter vessels, and underestimated in larger diameter vessels (Atkinson et al., 2013b). Therefore, to appropriately scale FMD for variations in diameter a, $D_{\text{base}}$ should be used as a covariate in a logarithmic model, providing an allometric correction.
2.5 Statistics

Descriptive analysis of participant characteristics are presented as means and standard deviations. HR and BP during the sitting trial were compared to pre-sit values using a one-way analysis of variance (ANOVA). To address hypotheses 1 and 2, repeated measures ANOVAs were used to examine within (time: pre-sit, post-3h sit) and between (heat vs no heat) condition main effects and interaction for blood flow, shear rates and SFA FMD. To address hypothesis 3, time (pre-sit, 1h, 2h and post-3h sit) by heat (heat vs no heat) repeated measures ANOVAs were performed. Where necessary, within subject main effects were deconstructed using t-tests with Bonferroni correction. To determine the relationship between shear rates and SFA FMD, Pearson’s correlations were performed. To account for changes in baseline diameter, a log-linear mixed model, with baseline diameter as the time-varying covariate was used to examine the effect of limb heating (heat, no heat) on FMD pre- and post-3h sit and the time-course of these responses (pre-sit, 1h, 2h and post-3h sit). Mean differences were back-transformed to the original units of FMD (%), providing corrected SFA FMD (%) mean ± standard error. Statistical significance was set at $P \leq 0.05$. Statistical analyses were performed using SPSS 23.0 (SPSS, Chicago, IL, USA).
Chapter 3  Results

3.1 Descriptive data

Of the 22 children tested 16 (12 girls, 4 boys) had pre- and post-3h sitting data. Data was lost on 6 children because of movement during the scanning resulting in large inter-leg variation in baseline diameter and FMD. The mean age of the remaining 16 was 9.61 ± 1.39 y, stature was 138.0 ± 10.6 cm and body mass 31.87 ± 7.55 kg.

The ActivPal data confirmed the children sat for 99% of trial. An average of 59.53 ± 0.29 min was spent sitting each hour with less than 0.16 ± 0.22 min standing and 0.06 ± 0.13 min moving, which was attributed to postural changes, or when getting up to be wheeled to the washroom.

HR (pre-sit 78 ± 6 beats.min⁻¹; 1h 76 ± 5 beats.min⁻¹; 2h 75 ± 6 beats.min⁻¹; post-3h 74 ± 6 beats.min⁻¹), SBP (pre-sit 108 ± 6 mmHg; 1h 105 ± 5 mmHg; 2h 106 ± 4 mmHg; post-3h 106 ± 5 mmHg) and DBP (pre-sit 74 ± 6 mmHg; 1h 72 ± 7 mmHg; 2h 74 ± 6 mmHg; post-3h 74 ± 6 mmHg) remained constant throughout trial, confirming that the localised heated did not have any systemic impact.

3.2 Blood flow and shear stimulus pre- and post-3h of sitting, with and without limb heating

Baseline blood flow changed with time (F(1,30)=27.431, P<0.001; η²= 0.478), with a time by heat interaction (F(1,30)=31.002, P<0.001; η²=0.508). Table 3.1 shows that baseline blood flow was increased post-3h sit in the heated (P<0.001), but not the unheated leg (see Table 3.1).
Hyperaemic blood flow AUC is presented in figure 3.1. There was a significant main effect for time (F(1,30)=27.254, \( P<0.001; \eta^2=0.476 \)), and an interaction (F(1,30)=37.325, \( P<0.001; \eta^2=0.556 \)). Simple effects showed in comparison to pre-sit values, there was an increase in blood flow in the heated (\( P<0.001 \)), but not unheated leg.

![Figure 3.1. Hyperemic Blood Flow AUC from pre-sit to post-3h in the unheated (white bars) and heated (grey bars) conditions. Data are means and SD. Dots represent individual data points.](image)

Shear patterns pre- and post 3h sitting are presented in Table 3.1. Antegrade shear rate changed with time (F(1,30)=12.930, \( P<0.001; \eta^2=0.301 \)), with a time by heat interaction (F(1,30)=20.104, \( P<0.001; \eta^2=0.401 \)). Values were higher in the heated leg only compared to pre-sit (\( P<0.001 \)). Retrograde shear rate altered with time (F(1,30)=7.121, \( P<0.05; \eta^2=0.192 \)), with a significant interaction (F(1,30)=7.709, \( P<0.01; \eta^2=0.204 \); see Table 3.1). At post-3h, retrograde shear rate in the unheated leg was not different from pre-sit values, but values had declined in the heated leg (\( P<0.01 \)).
Table 3.1. Superficial femoral artery hemodynamics pre- and post-sitting for 3 hours, with and without passive limb heating.

<table>
<thead>
<tr>
<th></th>
<th>Unheated leg (n=16)</th>
<th>Heated leg (n=16)</th>
<th>RM ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-sit</td>
<td>Post-3h</td>
<td>Pre-sit</td>
</tr>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diameter (mm)</td>
<td>4.08±0.32</td>
<td>4.12±0.31#</td>
<td>4.05±0.32</td>
</tr>
<tr>
<td>Blood flow (ml·min⁻¹)</td>
<td>163.1±59.2</td>
<td>155.2±56.1</td>
<td>168.8±65.1</td>
</tr>
<tr>
<td>Antegrade Shear (s⁻¹)</td>
<td>128.6±43.2</td>
<td>118.0±36.9</td>
<td>137.0±52.6</td>
</tr>
<tr>
<td>Retrograde Shear (s⁻¹)</td>
<td>-23.90±16.5</td>
<td>-24.25±16.7</td>
<td>-25.81±17.5</td>
</tr>
<tr>
<td>Reactive Hyperemia</td>
<td>4.34 ± 0.29</td>
<td>4.39 ± 0.24#</td>
<td>4.33 ± 0.28</td>
</tr>
</tbody>
</table>

#Significant difference from baseline, P<0.01
Hyperaemic SRAUC altered with time ($F(1,30)=11.174, P<0.01; \eta^2=0.271$), with a significant interaction ($F(1,30)=14.763, P<0.001; \eta^2=0.330$). Figure 3.2 illustrates the increase post-3h sit in the heated leg only ($P<0.01$).

![Figure 3.2](image)

Figure 3.2 SRauc pre-sit and post-3h in the unheated (white bars) and unheated (grey bars) conditions. Data are means and SD. Dots represent individual data points.

### 3.3 Superficial femoral artery flow-mediated dilation pre- and post- 3 h of sitting, with and without limb heating

SFA baseline and peak diameters pre-sit and post-3h are presented in Table 3.1. Baseline diameter changed with time ($F(1,30)=7.538, P<0.05; \eta^2=.201$) with no significant interaction, increasing after 3h of sitting ($P< 0.01$) in both heated and unheated legs. Peak diameter also changed with time ($F(1,30)=13.658, P < 0.001; \eta^2=.313$), but with no significant interaction, again increasing from pre-sit to post-3h in both legs.

SFA FMD is presented in figure 3.3. There was no main effect for time ($P=0.063$) or an interaction ($P>0.05$), with values remaining constant from pre- to post-3h sit in both conditions. Heat (pre $7.0 \pm 3.072\%$) post $7.12 \pm 3.34\%$). No heat (pre $6.38 \pm 2.56\%$; post
6.51 ± 1.70%

Figure 3.3. Delta change in FMD (%) from pre-sit to post-3h in the unheated (white bars) and heated (grey bars) conditions. Data are means and SD. Dots represent individual data points.

FMD (%) was corrected for changes in baseline diameter using a log-linear model. Pre- and post-3h sit corrected values were 6.18 ± .06% and 6.61 ± .06% in the unheated limb respectively. In the heated limb corrected FMD values were 6.82 ± .06% and 7.47 ± .06%, pre- and post-3h sit respectively. There were no significant main effects or interaction.

3.4 The time course of blood flow and shear during three hours of sitting, with and without limb heating.

Of the 10 children who completed time course measurements, 7 were included in the final analyses (5 girls and 2 boys). The 3 children who were not included each had one scan out of the 8 (4 time points x 2 legs) that could not be analysed satisfactorily because of movement artefact. The mean age of the 7 children was 9.91 ± 1.52 y, stature was 140.13 ± 11.09 cm and body mass was 32.70 ± 7.18 kg.
Baseline and hyperemic blood flow, shear patterns, shear rate are presented in Table 3.2. Baseline blood flow altered with time (F(2,082,24.982)=19.828, $P<0.001$, $\eta^2=.623$), with a significant interaction (F(2,082,24.982)=16.391, $P<0.001$; $\eta^2=.577$). Values increased from pre-sit each hour in the heated leg only ($P$’s < 0.01).

There was a significant main effect for time for hyperemic blood flow AUC (F(3,36)=6.387, $P<0.01$; $\eta^2=.347$), with a significant interaction (F(3,36)=7.871, $P<0.001$; $\eta^2=.396$). Hyperemic blood flow AUC increased each hour from pre-sit in the heated leg only ($P$’s < 0.01).

Antegrade shear rate changed with over time (F(2.001, 24.018)=4.656, $P<0.05$; $\eta^2=.280$), with a significant interaction (F(2.001, 24.018)=2.468, $P>0.05$; $\eta^2=.171$). Increases were apparent in the heated, but not the unheated leg after 1, 2 and 3 hours of sitting ($P$’s < 0.01). Retrograde shear rate altered with time (F(1.510,18.116)=3.390, $P>0.05$; $\eta^2=.220$), with a significant interaction (F(1.510,18.116)=4.110, $P<0.05$; $\eta^2=.255$). A reduction in retrograde shear rate was apparent after 1, 2 and 3 hours of sitting in the heated leg only ($P$’s < 0.01). Hyperemic SRAUC increased with time (F(3,36)=4.563, $P<0.05$; $\eta^2=.275$), with a significant interaction (F(3,36)=3.246, $P<0.05$; $\eta^2=.213$), with values in the heated leg only greater than baseline after each hour ($P$’s < 0.01).
Table 3.2. The time course of superficial femoral artery hemodynamics over 3-hours of sitting, with and without passive limb heating

<table>
<thead>
<tr>
<th></th>
<th>Unheated leg (n=7)</th>
<th>Heated leg (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-sit 1h 2h</td>
<td>3h Pre-sit 1h 2h 3h</td>
</tr>
<tr>
<td>Baseline (mm)</td>
<td>4.23±0.32 4.20±0.27 4.17±0.25</td>
<td>4.19±0.28 4.25±0.23 4.29±0.21 4.23±0.30 4.31±0.28</td>
</tr>
<tr>
<td>Blood flow (ml·min⁻¹)</td>
<td>84.6±38.2 92.4±31.4 77.3±30.8</td>
<td>104.1±41.7 89.6±27.6 154.6±41.0# 193.4±50.1# 237.8±45.9#</td>
</tr>
<tr>
<td>Antegrade shear rate (s⁻¹)</td>
<td>102.0±43.7 138.9±115.7 100.6±55.9</td>
<td>126±40.1 102.2±33.6 156.6±33.45# 170.3±42.4# 216.2±47.3#</td>
</tr>
<tr>
<td>Reactive Hyperemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak diameter (mm)</td>
<td>4.48±0.30 4.49±0.28 4.47±0.26</td>
<td>4.48±0.28 4.53±0.22 4.53±0.20 4.56±0.25 4.66±0.29</td>
</tr>
<tr>
<td>SRₐUC (AU)</td>
<td>25693±15830 31376±17515 21316±13243</td>
<td>29317±7846 22565±10068 33707±1408# 36444±1335# 47811±2033#</td>
</tr>
<tr>
<td>Blood flow (ml·min⁻¹)</td>
<td>424.6±84.0 476.4±168.5 387.7±93.8</td>
<td>423.7±100.0 332.0±139.8 520.7±143.9# 631.6±132.2# 728.7±148.1#</td>
</tr>
<tr>
<td>FMD (%)</td>
<td>6.14±2.71 6.60±2.06 6.94±1.95</td>
<td>6.97±1.42 6.44±3.14 5.26±3.33 6.60±2.06 8.20±4.30</td>
</tr>
</tbody>
</table>

# Significant difference from baseline. AU, arbitrary units; FMD, flow-mediated dilatation.
3.5 The time course of corrected superficial femoral artery flow-mediated dilation during 3 h of sitting, with and without limb heating.

When corrected for baseline diameter there was no main effect or interaction. Figure 3.4 shows no change in corrected FMD in the non-heated leg. Although there was no main effect for time there is an indication of a rise in corrected FMD in the heated leg from 2 hours onward. Simple effects do show that corrected FMD in the heated leg rises significantly above baseline after 3 hours ($P<0.01$; 95% confidence intervals pre-sit - 3.98% to 9.31%; post-3h -6.08% to 11.52%), but not 2 hours ($P=0.60$) of sitting.

Figure 3.4 Corrected FMD (%) pre-sit and at 1h, 2h and post-3h of sitting in the unheated (open squares) and heated (black circles) conditions (values are means and standard error).
3.6 Relationship between superficial femoral artery flow-mediated dilation during 3 h of sitting and shear rate.

A positive correlation exists between SR$_{AUC}$ (AU) and FMD (%) in the heated condition ($r^2=0.419$, $P=0.01$), but not in the unheated condition.

Figure 3.5 Relationship between FMD (%) and SR$_{AUC}$ (AU) from pre-sit to post-3h of sitting in the heated condition.
Chapter 4 Discussion

We hypothesized that heating would increase shear rates and patterns, but these would remain constant in the unheated leg. Herein we show that the heat stimulus did cause substantial increases in blood flow and shear rates in children, improving microvascular function. In contrast to our hypotheses however, we do not see a decline in FMD in the unheated or heated legs following 3 hours of sitting.

Prolonged sitting has been shown to result in declines in FMD, that are accompanied by reductions in shear rates and antegrade shear in adults (Morishima et al., 2016; Padilla et al., 2011; Restaino et al., 2016; Thosar et al., 2015; Vranish et al., 2017), but not children (McManus et al., 2015). In contrast to this work, we do not show any decline in FMD, shear rates or patterns with sitting. It is possible that the sitting posture used in our study compared to McManus and colleagues has impacted the findings. In the prior study (McManus et al., 2015) children sat upright with the knee at a right angle and the lower leg vertical, with the FMD scans conducted semi-supine on a bed. To improve upon this design, we sat children on deep couches so that the legs could be stretched out horizontally, and we could scan in situ. During the sitting period the children generally sat with their legs up in this horizontal position or folded to the side, but not with the lower leg vertical. It is possible the sitting posture in the current study aids venous return so blood pooling is not as acute, attenuating the impact of sitting. This small postural difference has the potential to impact blood pooling and sympathetic nerve activity (Ray et al., 1993) and recent work in adults has shown posture does impact antegrade shear in younger adults (Trinity et al., 2014). These authors showed supine antegrade shear rate was reduced when the posture was changed to sitting in younger adults.
(n=8, 24±1 years). It would be important to confirm if the sitting posture makes sitting more or less harmful in children. Alternatively, we, like McManus and colleagues (2015) show large variation in the FMD response to sitting. There is not a homogenous response to an acute period of sitting, partly illustrated by the apparent rise in the heated leg corrected FMD in the subset of children who completed the time course FMD assessment, a rise that was not apparent in the larger group. Given the lack of consistency in this finding, the meaningfulness of the finding is drawn into question.

Notably, there is a strong positive relationship between $\text{SR}_{\text{AUC}}(\text{AU})$ and FMD(%) in the heated condition after 3 hours of sitting. This suggests that with sufficient increases in shear, FMD is positively impacted in children. Heating appears to increase blood flow and antegrade shear rates to higher levels than exercise breaks throughout sitting seen in previous work in children (McManus et al., 2015). Heating increased SFA blood flow from $168.8 \pm 65.1 \text{ ml} \cdot \text{min}^{-1}$ to $359 \pm 114.0 \text{ ml} \cdot \text{min}^{-1}$ with passive leg heating, while exercise breaks increased SFA blood flow from $119.3 \pm 9.9 \text{ ml} \cdot \text{min}^{-1}$ to $150 \pm 22.34 \text{ ml} \cdot \text{min}^{-1}$, but this difference was not significant. Additionally, antegrade shear increased with passive leg heating from $137.0 \pm 52.6 \text{ ml} \cdot \text{min}^{-1}$ to $233.2 \pm 66.9 \text{ ml} \cdot \text{min}^{-1}$, while exercise did not significantly change antegrade shear with mean values at $184 \pm 14 \text{ ml} \cdot \text{min}^{-1}$ increasing to $210 \pm 25 \text{ ml} \cdot \text{min}^{-1}$. The substantial increased from heat improves both shear patterns and blood flow. Of note is the improvement in microvascular function indexed from the hyperemic shear response ($\text{SR}_{\text{AUC}}(\text{AU})$), which showed large increases with passive leg heating. A recent report from the Framingham study suggested that hyperemic shear stress was more strongly correlated with cardiovascular risk factors than FMD (Mitchell et al., 2004).
Neither the current or previous work in children has explored the impact of sex on findings. In adults, despite similar declines in shear patterns and reactive hyperemia in men and women, women do not show a decline in FMD with sitting (Vranish et al., 2017). This was found irrespective of hormonal contraceptive use and assessment during the early follicular phase of the menstrual cycle when estrogen is lowest, given the protective effect estrogen has on arterial function (Celemajer et al., 1994). The children in our study were pre-pubertal and our initial intention was to compare sex differences in the responses, however, recruitment of boys was harder than girls, and although we have a larger sample than other studies (n=16), there are only 4 boys. It is possible that sex differences may have influenced our findings, but it is worth noting that the prior work used only girls and unlike our findings, still report reductions in FMD from sitting (McManus et al., 2015). Future studies would benefit from including sufficient numbers of boys and girls so comparisons between the sexes can be explored.

4.1 Mechanism(s) of action

Our findings would suggest that sufficient shear stimuli may improve microvascular function, but the impact of sitting on shear rate and patterns does not appear to be sufficient to influence FMD in children. It is likely alternative mechanisms such as blood pooling, blood viscosity and sympathetic nerve activity are possible alternative mechanisms which may underlie the change endothelial reactivity in children in response to sitting.

4.1.1 Blood Pooling

First, blood pooling can occur with a lack of muscle contractions, and the increase in hydrostatic pressure from sitting (Kitano et al., 2005). It has been shown in an acute prolonged sit in adults that ankle circumference increases (3-hr sit: 2.58 ± 0.7 %, relative to pre-sit, \( P<0.05 \)), indicating blood pooling (Morishima et al., 2016). Calf circumference has also been
used to reflect venous pooling (Vranish et al., 2017) and increases after 3 hours of sitting in both men and women (men: $37.9 \pm 0.4 \text{ cm } 10\text{-min sit vs. } 38.9 \pm 0.5 \text{ cm } 3\text{-h sit, } P < 0.001$; women: $37.0 \pm 0.8 \text{ cm } 10\text{-min sit vs. } 38.1 \pm 0.8 \text{ cm } 3\text{-h sit}$). Blood pooling while sitting may also result in an increase in blood viscosity. As little as two hours of sitting has been shown to increase blood viscosity (Hitosugi et al., 2000). This in turn can lead to impaired endothelial function due to potential increased coagulation and inflammatory markers (Kwaan, 2010) and lower shear rates, which has been associated with the clumping of red blood cells and increased viscosity (Ku, 1997).

Blood pooling can lead to venous distension, causing arterial constriction and myogenic constriction (Kitano et al., 2005). Venous distension may cause sympathetic activation through the stimulation of limb afferents (Cui et al., 2012). This could further support the notion that muscle sympathetic activity is greater in the upright seated position than supine (Ray et al., 1993). It has been suggested that this may have a greater impact on the microvasculature than the macrovasculature (Vranish et al., 2017). Unfortunately, we did not measure calf circumference as a surrogate of blood pooling to confirm whether or not this might have influenced FMD.

4.1.2 Muscle sympathetic nerve activity (MSNA)

MSNA has been shown to be greater in the upright sitting posture compared to supine (Ng et al., 1995). Increased BP from sitting may also occur to compensate for blood pooling (Shvartz et al., 1983), although we do not see any changes in BP throughout the trial. An increase in MSNA is linked to proatherogenic shear patterns of the peripheral vasculature, with increases in retrograde and oscillatory shear patterns noted (Padilla et al., 2010). Children demonstrate lower sympathetic nerve activity (Quigley & Stifter, 2006), and our findings of no change in
retrograde shear in the unheated leg, may imply the impact of sitting on MSNA in children is nominal.

4.2 Limitations

This study is not without its limitations. Based on BMI, it can be assumed that the children recruited in this study were generally healthy and active. With this in mind, the presumably healthy vasculature of these children may already be functioning close to fully dilated, and not have much room for further dilation with heating. Additionally, the vasculature of the young may be more protective and maintain flow better than in adults under challenging conditions (as we saw no decline in shear or hyperemic flow with sitting). While we recruited a total of 22 children, our sample size was reduced to 16 after unusable scans were removed. This meant the sample size in the time-course sub-sample was small and likely underpowered to detect differences, as there is large individual variation in the FMD response. Additionally, due to our relatively small sample size, we were not able to compare sex differences. With only 4 boys and 12 girls, a comparison would not have value, but this may worth investigating in the future.

In addition, we did not measure endothelium independent dilation, therefore our FMD reflects global vascular response and we cannot rule out smooth muscle contribution. Using nitroglycerin as an NO donor would be a viable option in the future. Last, as mentioned, the posture the child sat in may also be an important piece, and we did not anticipate this change in the seating arrangement causing a change in the response which potentially it could have.
Chapter 5 Conclusion

To conclude, passive leg heating improves shear rates and patterns, and blood flow while sitting. Passive leg heating also significantly improved microvascular function, as indexed by SRAUC. The change in SRAUC with passive leg heating was positively correlated to FMD during 3 hours of sitting. Although sitting did not cause reductions in FMD in either the heated or unheated conditions, the benefits in blood flow, shear rates and patterns from passive leg heating are likely important.

Future studies should determine the role sitting posture plays in endothelial function. A follow-up study with the addition of calf circumference to obtain a blood pooling index, would also be helpful to determine the impact of blood pooling on hemodynamics during sitting. We examined whether an acute period of prolonged sitting was detrimental to vascular health, but did not determine the long-term impact of sitting in children. It is possible that prolonged and repeated periods of sitting create a bigger impact on shear rates, regardless of the impact on FMD, although this has yet to be examined. Confirming the benefit of increases in shear rates on endothelial health beyond FMD, is also important. Increasing shear rates via heating, exercise or postural changes is possible, at least in adults. Postural changes that require small muscle contractions may be a low effort action, but are possibly sufficient for eliciting blood flow changes and an increase in shear. If improved shear rates are confirmed as beneficial, this could be a useful tool in workplaces and classrooms alike, as standing desks are becoming more attainable in these settings. Testing these interventions in children would be viable and applicable to many settings.
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