The Acute and Prolonged Impact of Knee-Flexion Angle on Popliteal Hemodynamics and Flow-Mediated Dilation Responses to Sitting

by

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Abstract

Background: An acute bout of prolonged sitting reduces lower-limb artery blood flow and induces endothelium dysfunction. 'Kinking' of the popliteal artery during knee-bent sitting may exacerbate these negative effects. However, the impact of sitting at different knee-flexion angles on popliteal function is unknown. ***Objectives:*** To determine the impact of prolonged sitting (~2.5 h) at knee-flexion angles of 0° (straight leg), 45°, and 90° on popliteal blood flow (PBF) and endothelial-dependent, flow-mediated dilation (FMD) responses. ***Methods:*** Duplex ultrasonography was used to assess PBF and popliteal FMD in 8 participants (24±2 yr; 4 females). Two sitting bouts were completed on separate days with one leg positioned at a knee flexion angle of 0° or 90° and the opposite leg at 45° knee-flexion. PBF was measured at the start (pre-sitting), 0.5-h, 1.0-h, and post-sitting, while FMD was only assessed at pre- and post-sitting timepoints. ***Results:*** At the start of sitting, PBF was lower during 90° knee-flexion (36±15 $mL \cdot min^{-1}$) versus 0° (53±15 $mL \cdot min^{-1}$, P=0.021). Following prolonged sitting, PBF was lower with the knee flexed at 45° (33±10 $mL \cdot min^{-1}$) and 90° (30±12 $mL \cdot min^{-1}$) versus 0° (42±15 $mL \cdot min^{-1}$, both $P \leq 0.026$). However, sitting-induced reductions in popliteal FMD were similar between all knee flexion angles (all, $P > 0.674$). ***Conclusion:*** During an acute bout of prolonged sitting, greater knee-flexion angles negatively impacted PBF, but did not further impair endothelial-dependent vasodilatory responses.

List of Abbreviations

Ca^{2+} - calcium

cGMP - cyclic guanosine monophosphate

CO - cardiac output

DBP - diastolic blood pressure

EDHF - endothelium-derived hyperpolarization factors

eNOS - endothelial nitric oxide synthase

FMD - flow-mediated dilation

GTP - guanosine triphosphate

HR – heart rate

MAP - mean arterial pressure

METs - metabolic equivalents of task

NO - nitric oxide

PAD – peripheral artery disease

PBF – popliteal blood flow

PGI_2 – prostacyclin

RBCv - red blood cell velocity

SBP - systolic blood pressure

sGC – soluble guanylyl cyclase

SR – shear rate

SR_{AUC} – shear rate area under the curve

SV - stroke volume

TVC - total vascular conductance

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Chapter 1: Introduction

Sedentary behavior is characterized by an energy expenditure ≤1.5 metabolic equivalents of task (METs) during waking hours in a sitting, reclining, or lying posture (Tremblay et al., 2017). National 24-hour movement guidelines recommend that Canadians should limit their daily sedentary time to less than 8 hours (Ross et al., 2020). However, Canadian adults (Prince, Melvin, Roberts, Butler, & Thompson, 2020), even those who achieve national physical activity guidelines (O'Brien, Johns, Al-Hinnawi, & Kimmerly, 2020), typically spend > 9 hours per day sedentary. Independent of habitual physical activity levels, excessive sedentary time is a risk factor for cardiovascular disease (Katzmarzyk, Church, Craig, & Bouchard, 2009; Warren et al., 2010) and all-cause mortality (Katzmarzyk et al., 2009; Koster et al., 2012). Specifically, increased sedentary time is related to a greater incidence of peripheral artery disease (PAD) in the lower-limb (Unkart et al., 2020).

Peripheral artery disease is characterized by a narrowing or blockage of peripheral arteries, caused by atherosclerosis or the buildup of fatty plaques (Cohoon, Wennberg, & Rooke, 2017). This disease poses a significant public health burden that contributes to a high risk of morbidity and mortality, as well as a low quality of life (Diehm et al., 2009; Fowkes et al., 2008). Compared to the upper-limbs, the development of atherosclerosis and PAD is more likely to occur in lower-extremity arteries (Gallino et al., 2014). Of relevance to this study, the popliteal artery (located behind the knee) is highly susceptible to atherosclerosis and a common site of PAD (Jadidi et al., 2021; Watt, 1965). Endothelium dysfunction is defined as the damage and poor function of the arterial endothelium (inner cell monolayer), which commonly precedes atherosclerosis (Ross,

1999). Furthermore, endothelium dysfunction is routinely observed immediately following a bout of prolonged sitting (> 1-hour) (Padilla & Fadel, 2017). Thus, repeated bouts of prolonged sitting over the life course may contribute to the development of popliteal PAD.

The flow-mediated dilation (FMD) test is a commonly used, non-invasive assessment of endothelium-dependent vasodilation in peripheral arteries (Thijssen et al., 2019). Specifically, high-resolution ultrasonography is used to examine increases in artery diameter (i.e., vasodilation) following a distal ischemia-induced reactive hyperemia (i.e., increase in blood flow) (Thijssen et al., 2019). The FMD assessment provides an index regarding the ability of the endothelium to produce the potent vasodilator nitric oxide (NO) (Thijssen et al., 2019). Larger increase in lumen diameter (i.e., a greater FMD response) represents a healthier endothelium. In the laboratory setting, a 3-hour bout of uninterrupted sitting decreases popliteal FMD (Liu, O'Brien, Johns, & Kimmerly, 2021; O'Brien, Johns, Al-Hinnawi, et al., 2020; O'Brien, Johns, Williams, & Kimmerly, 2019; Restaino, Holwerda, Credeur, Fadel, & Padilla, 2015; Restaino et al., 2016). In fact, as little as 1-hour of sitting can decrease superficial femoral artery FMD responses (Ballard et al., 2017; Thosar, Bielko, Mather, Johnston, & Wallace, 2015).

The negative impact that sitting has on popliteal FMD responses is associated with a corresponding decrease in resting blood flow and shear rate (SR, frictional force of blood on the endothelium), which may be exaggerated when the knee is more flexed (e.g., at a 90° angle) due to 'kinking' of the artery (Poulson et al., 2018; Walsh, Restaino, Martinez-Lemus, & Padilla, 2017). Specifically, Walsh et al. (2017) observed a greater

decrease in popliteal blood flow (PBF) and FMD when people performed 3 hours of side-lying with a bent lower leg (i.e., 90° knee-flexion) versus when the lower leg was straight (i.e., 0° knee-flexion). Furthermore, they observed that popliteal FMD responses were only reduced in the bent leg following prolonged side-lying (Walsh et al., 2017). Similarly, Delis et al. (2000) found that PBF decreased between the side-lying versus seated posture with the knees flexed to 45° in both healthy and unhealthy people. However, popliteal FMD was not assessed in that study. As such, the consequence to popliteal health (i.e., blood flow and FMD responses) during sitting at different knee-flexion angles is unknown. Such information may provide evidence to support future public health recommendations (e.g., sitting with straight lower legs is better than sitting with your knees bent) that could mitigate some of the ill vascular implications associated with prolonged bouts of sitting.

Given that the impact of knee-flexion angles on popliteal health during prolonged sitting has not been previously investigated, the objectives of this study were to determine: 1) the effect of knee-flexion angles (0°, 45°, 90°) on PBF responses at the start of prolonged sitting (i.e., acute effects), and 2) the impact of these knee-flexion angles on PBF and FMD responses during and following prolonged sitting. It was hypothesized that both acute and prolonged sitting-induced impairments on PBF and FMD responses would be the greatest when sitting with a 90° knee-flexion angle, less after sitting at 45° knee-flexion angle, and the least impaired after sitting at 0° knee-flexion angle (i.e., with straight lower legs).

Chapter 2: Review of Literature

2.1 Sedentary Behavior and Cardiovascular Health

Sedentary behavior is defined as any waking behavior with a low energy expenditure (≤1.5 metabolic equivalents of task, METs) in a sitting, reclining or lying posture (Tremblay et al., 2017). National guidelines suggest that Canadian adults limit their daily sedentary time to less than 8 hours (Ross et al., 2020), whereas most Canadians spend at least 9 hours per day engaged in sedentary behaviours (Carson et al., 2014; O'Brien, Johns, Al-Hinnawi, et al., 2020; Prince et al., 2020). If spending time sedentary is inevitable, perhaps sitting with a straighter leg and reduced knee-flexion angles can minimize sitting-induced reductions in popliteal endothelial health. This potential recommendation could improve future public health guidelines related to sedentary behavior.

It is well known that excessive sedentary behavior is a significant risk factor for multiple adverse health outcomes (Thorp, Owen, Neuhaus, & Dunstan, 2011) and is positively associated with all-cause mortality, independent of habitual physical activity levels (Ekelund et al., 2019; Katzmarzyk et al., 2009; Koster et al., 2012; Warren et al., 2010). Specifically, increased daily sitting time is related to a higher cardiovascular disease incidence and mortality (Katzmarzyk et al., 2009; Park, Moon, Kim, Kong, & Oh, 2020; Warren et al., 2010). Moreover, a prolonged bout (≥1 hour) of uninterrupted sitting has negative impacts on peripheral (e.g., popliteal artery) and central (e.g., aorta) vascular health (Credeur et al., 2019; Padilla & Fadel, 2017; Unkart et al., 2020). A contributing mechanism linked with prolonged sitting-induced impairments in cardiovascular health is lower-limb artery endothelium dysfunction (Kruse, Hughes, Benzo, Carr, & Casey, 2018;

Morishima, Restaino, Walsh, Kanaley, & Padilla, 2017; Morishima et al., 2016; Padilla & Fadel, 2017; Paterson et al., 2020; Restaino et al., 2015, 2016). Consequently, endothelium dysfunction initiates and leads to the development of atherosclerosis (i.e., the buildup of fatty plaque in the artery) (McLenachan, Williams, Fish, Ganz, & Selwyn, 1991; Ross, 1999; Widlansky, Gokce, Keaney, & Vita, 2003). Therefore, it is important to first understand the structure and function of peripheral arteries before fully appreciating the mechanisms associated with prolonged sitting-induced lower-limb endothelium dysfunction. The artery under investigation for this project will be the popliteal artery.

2.2 Arterial Structure and Function

The popliteal artery is considered a muscular or conduit artery, which typically has an internal lumen diameter between 0.4 to 0.6 cm and contains a large amount of vascular smooth muscle (~65% of vessel wall) (Smith & Fernhall, 2011). One of the main functions of arteries and downstream arterioles (~30 μm internal diameter, ~60% vascular smooth muscle in the vessel wall) is to distribute blood to tissues and organs. The amount of blood flowing through the conduit artery is primarily controlled by adjusting the lumen diameter of the distal arterioles via contraction or relaxation of vascular smooth muscle cells. Specifically, relaxation of the vascular smooth muscle results in vasodilation and increased blood flow. In contrast, contraction of the vascular smooth muscle results in vasoconstriction and decreased blood flow. The relative amount of vascular smooth muscle contraction/relaxation plays an important role in the regulation of arterial blood pressure via changes in peripheral vascular resistance, which impacts the distribution of cardiac output (CO) to the systematic circulation (Smith & Fernhall, 2011).

2.2.1 Arterial Anatomy

In general, there are three distinct layers of muscular arteries and downstream arterioles: the *tunica adventitia (*or *externa)*, the *tunica media*, and the *tunica intima* (Figure 2-1; Smith & Fernhall, 2011). The *tunica adventitia* is the outermost layer and contains post-ganglionic sympathetic nerve axons, connective tissue (elastin/collagen) and the *vaso vasorum* that provides blood supply to the vessel. The *tunica media* is the middle layer and mostly contains vascular smooth muscle cells embedded in a matrix of collagen, elastin, and glycoproteins. Importantly, this layer is responsible for changes in lumen diameter. The *tunica intima* is the innermost layer and primarily contains a single layer of endothelial cells and a basal lamina (basement membrane). The endothelium is the barrier between the blood and the underlying tissue, is essential for optimal cardiovascular health by responding to mechanical forces (e.g., shear force of red blood cells) and chemical signals (e.g., vasoactive hormones) from the blood (Smith & Fernhall, 2011).

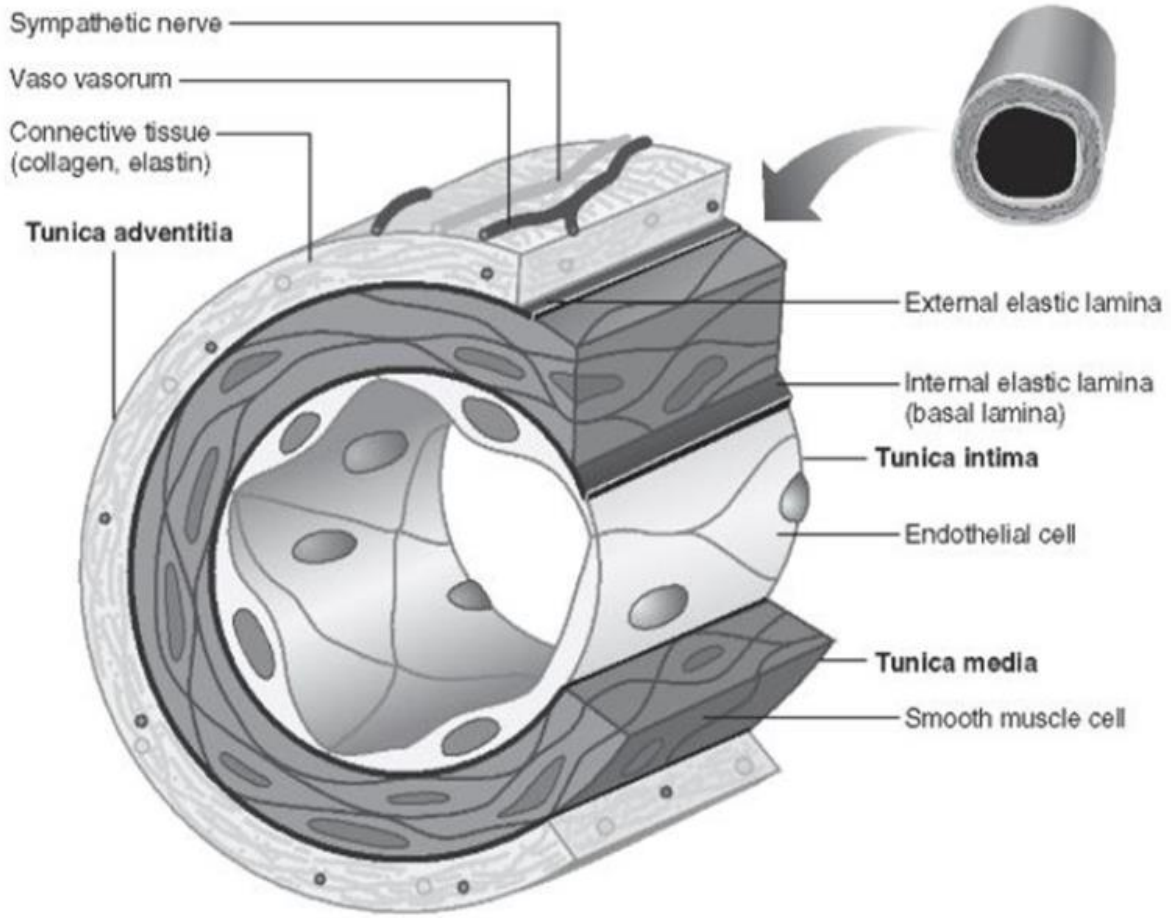


Figure 2-1. A transversal view of the 3 layers of a muscular artery or arteriole (Smith & Fernhall, 2011, p106).

2.2.2 The Endothelium

The endothelium is composed of endothelial cells and the basal lamina, which separates the endothelial cells from the underlying vascular smooth muscle cells (Figure 2-2; Smith & Fernhall, 2011). Endothelial cells have an organized cytoskeleton to support cellular structure, attaching to the basal lamina through integrin proteins, and attaching adjacent endothelial cells via junctional proteins. The gap between adjacent endothelial cells is called the intracellular junction and helps to determine the degree of permeability of substances from the lumen. The luminal surface of endothelium contains the glycocalyx, which has a negative charge to allow water and small solutes to pass through

the intracellular junction, but not large negatively charged proteins (e.g., lipoproteins, albumin). Such large molecules can pass from the circulation to the underlying tissue across endothelial cells via a caveola-vesicle system (Smith & Fernhall, 2011). Importantly, the integrins and glycocalyx serve as mechanoreceptors that detect laminar shear stress (i.e., frictional force of red blood cells), resulting in production of the potent vasodilator, NO (Chatterjee, 2018).

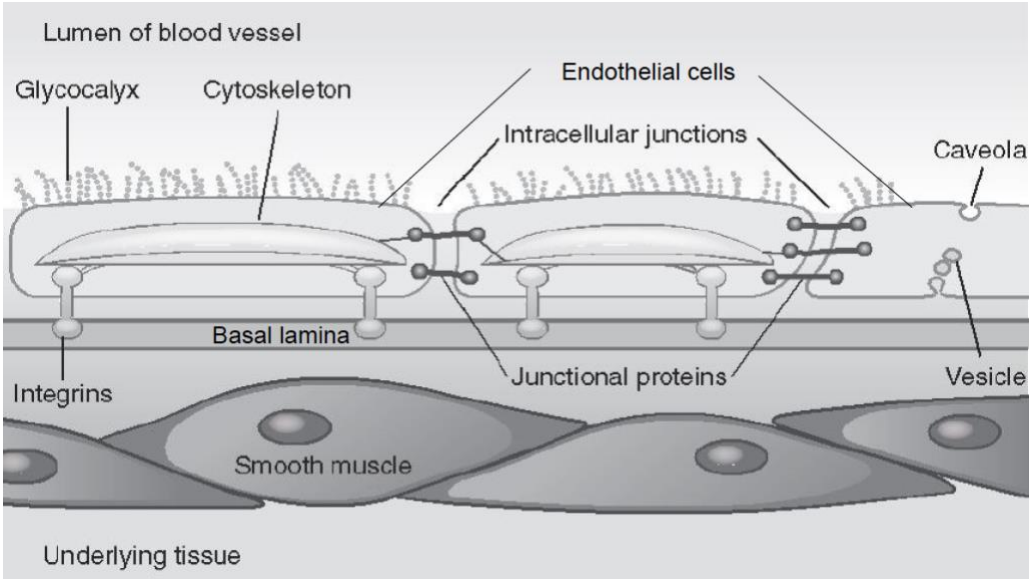


Figure 2-2. Anatomy of the vascular endothelium (Smith & Fernhall, 2011, p109).

2.2.2.1 Endothelium Nitric Oxide Production

One of the primary functions of the endothelium is to regulate vascular tone (i.e., the degree of vasoconstriction relative to the maximally dilated state), which controls blood flow distribution to organs and regulates blood pressure (Smith & Fernhall, 2011). Endothelial cells regulate vascular smooth muscle contraction through the production and release of chemical mediators that cause either vasodilation (e.g., NO; prostacyclin, PGI_2; endothelium-derived hyperpolarization factors, EDHFs) or vasoconstriction (e.g., endothelin-1). Importantly, NO is the most potent vasodilator produced by the endothelium, which also inhibits platelet aggregation and the accumulation of adhering

molecules (i.e., has an anti-clotting function) (Smith & Fernhall, 2011). The focus of this project will be on endothelium-derived NO production. As such, it is important to better understand how the endothelium produces NO and the mechanisms by which NO causes vascular smooth muscle relaxation (i.e., vasodilation).

As indicated above, endothelium NO production can be regulated by shear stress and/or by receptor-bound agonists (e.g., acetylcholine, thrombin, bradykinin, adenosine triphosphate, adenosine diphosphate, and substance P) (Smith & Fernhall, 2011). For the methodological procedures used in this study (i.e., the FMD test), shear stress detected by mechanoreceptors (i.e., glycocalyx and integrins) represents the predominant stimulus for endothelial NO production (Chatterjee, 2018; Smith & Fernhall, 2011). The transduction of shear stress and receptor-bound agonists increase intracellular calcium (Ca^{2+}) concentration, leading to phosphorylation of the enzyme endothelial nitric oxide synthase (eNOS), which produces NO by converting the amino acid L-arginine to NO and citrulline (Chatterjee, 2018; Sandoo, Veldhuijzen van Zanten, Metsios, Carroll, & Kitas, 2010; Zhao, Vanhoutte, & Leung, 2015). In addition, the increased intracellular Ca^{2+} binds with the protein Calmodulin (calcium-modulated protein) with the resulting calcium-calmodulin complex activating eNOS to promote greater NO production (Sandoo et al., 2010; Zhao et al., 2015). The activation of eNOS to produce endothelium-derived NO is illustrated in Figure 2-3.

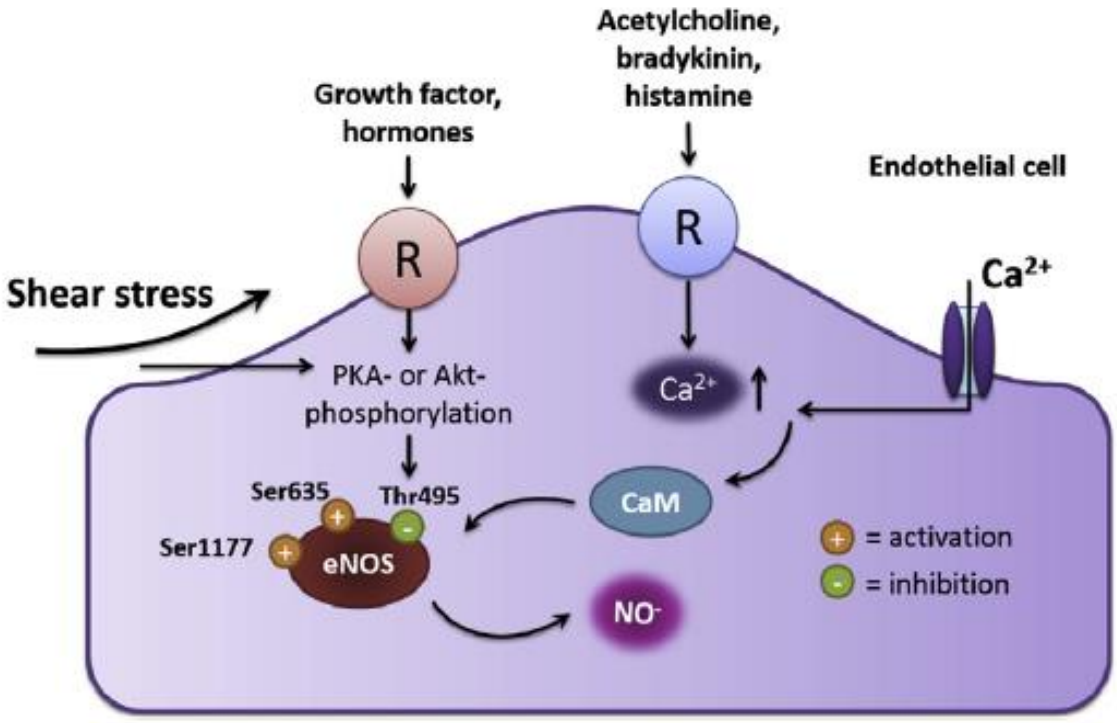


Figure 2-3. The activation of endothelial nitric oxide synthase (eNOS). The enzyme, eNOS, can be activated by calcium (Ca^{2+})-dependent and -independent pathways in the endothelial cell. The Ca^{2+}-dependent mechanism to activate eNOS is due to increased intracellular concentration of Ca^{2+} from shear stress activating K^{+}/ Ca^{2+} channels and from agonists (e.g., acetylcholine, bradykinin, histamine) acting on specific receptors (R) on the cell membrane. Ca^{2+} binds to calmodulin (CaM) and results in the activation of eNOS. The Ca^{2+}-independent pathway to activate eNOS is due to phosphorylation of eNOS by protein kinase A (PKA) or protein kinase B (Akt) on the serine (non-essential amino acid) activation sites (Ser1177 or Ser 635) (Zhao et al., 2015).

In contrast, the bioavailability of NO can be reduced in response to high levels of oxidative stress, which produces excessive amounts of superoxide radicals (O_2^-) (Smith & Fernhall, 2011). As depicted in Figure 2-4, NO reacts with O_2^- to produce the inactive form peroxynitrate ($ONOO^-$), which does not result in vascular smooth muscle relaxation. Thosar et al. (2015) conducted randomized, 3-hour uninterrupted sitting trials with or without antioxidant supplementation (vitamin C) in 11 healthy males. They found that sitting-induced lower-limb endothelium dysfunction was prevented following the administration of vitamin C. This indirect evidence suggests that sitting-induced

endothelium dysfunction (i.e., reduced NO bioavailability) might be related to an oxidative stress-dependent mechanism. Endothelium dysfunction is commonly observed in people with atherosclerosis, and often precedes the development of fatty plaques (Davignon & Ganz, 2004; McLenachan et al., 1991; Ross, 1999). Moreover, lower-limb endothelium dysfunction often occurs following bouts of prolonged sitting (Paterson et al., 2020).

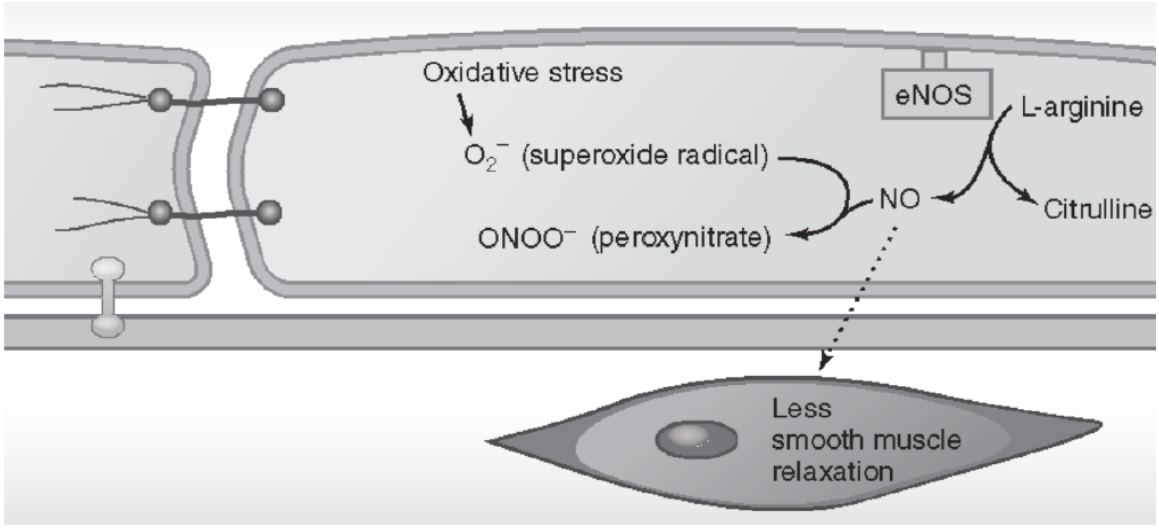


Figure 2-4. Diminished nitric oxide (NO) bioavailability with oxidative stress. NO is synthesized by the enzyme endothelial nitric oxide synthase (eNOS), which initiates vascular smooth muscle relaxation. High oxidative stress results in corresponding elevated levels of superoxide radicals (O_2^-) within the endothelial cell. O_2^- interacts with NO to produce peroxynitrate ($ONOO^-$), which prevents NO-mediated vasodilation (Smith & Fernhall, 2011, p113).

2.2.3 Vascular Smooth Muscle Structure and Function

The vascular smooth muscles are composed of spindle-shaped cells containing the contractile actin and myosin filaments. However, vascular smooth muscle lacks the striated appearance of skeletal or cardiac muscle cells because these filaments are not organized in parallel, repeated sections (i.e., no sarcomeres) (Figure 2-5; Smith & Fernhall, 2011). The actin filaments are attached to the cell surface via dense bands and

anchored by dense bodies. The myosin filaments are interwoven between the actin filaments. Myosin-actin crossbridge formation can help vascular smooth muscle contraction, and the detailed mechanism is indicated in Figure 2-6 (Webb, 2003). Adjacent vascular smooth muscle cells are interconnected by gap junctions, which are primarily composed of the protein connexin. These gap junctions allow ions to flow between adjacent cells, which promotes cell-to-cell depolarization. As such, vascular smooth muscle cells are an example of an excitable cell.

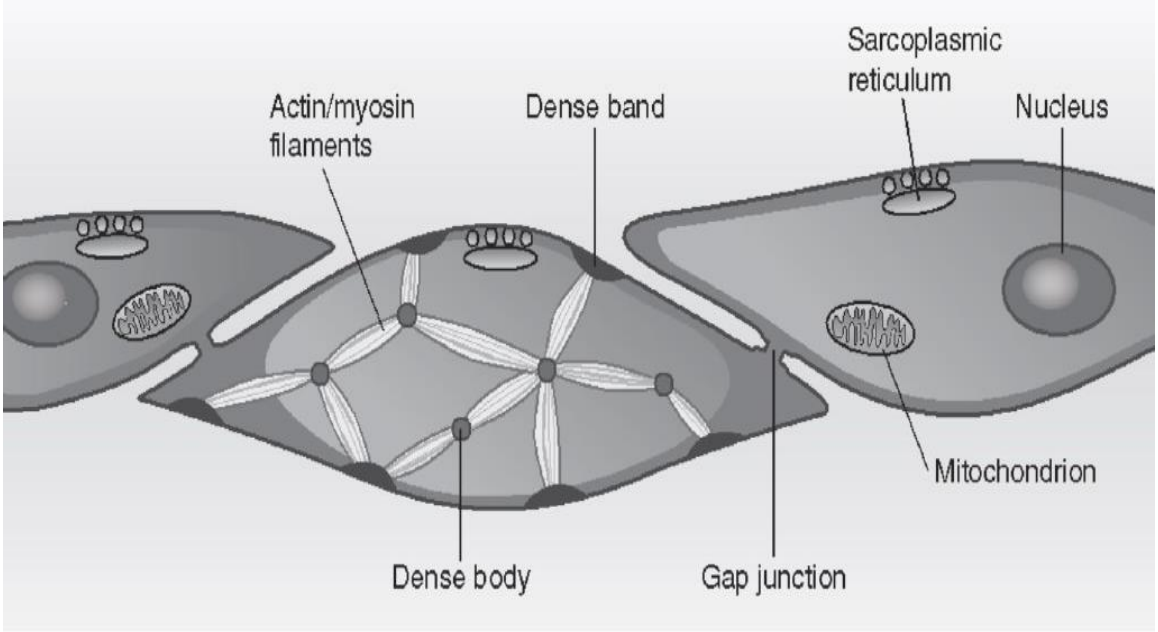


Figure 2-5. Vascular smooth muscle anatomical structure (Smith & Fernhall, 2011, p116).

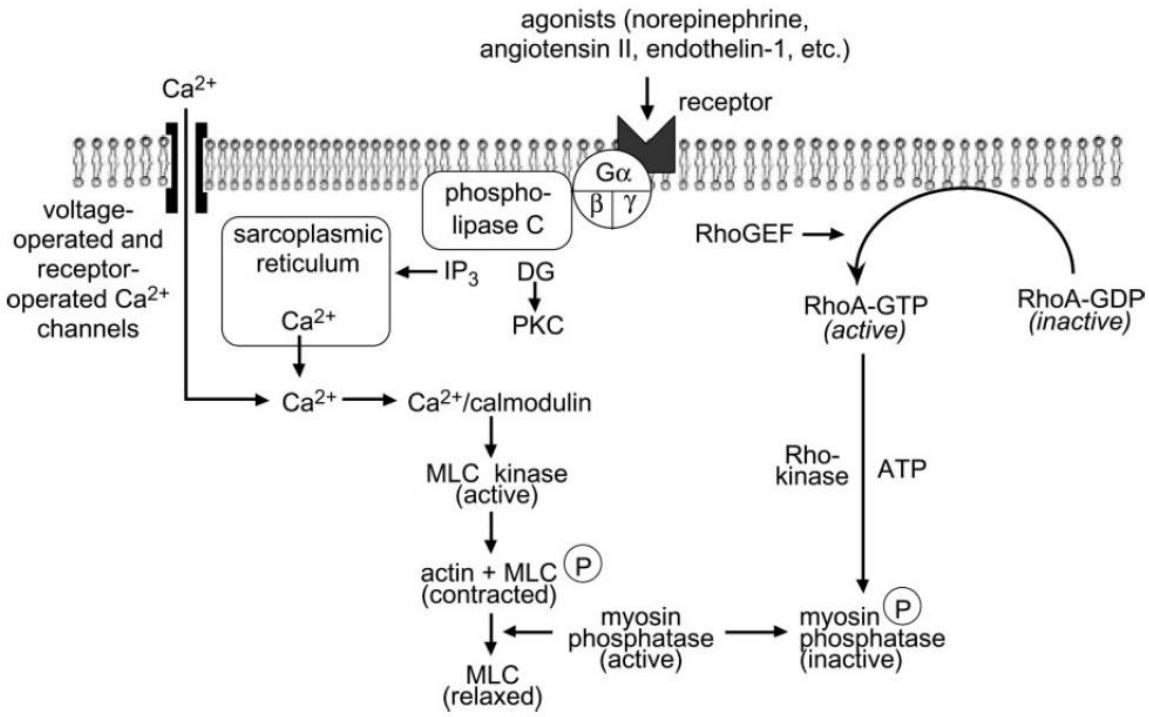


Figure 2-6. Vascular smooth muscle contraction via myosin-actin crossbridge formation. Cytosolic Ca^{2+} is increased in vascular smooth muscle cells through extracellular Ca^{2+} (entry through voltage-dependent Ca^{2+} channels via electromechanical innervation) and intracellular stores released from sarcoplasmic reticulum. Agonists (norepinephrine, angiotensin II, endothelin-1, etc.) binding to G-protein coupled receptors activate phospholipase C, which produces two second messengers: diacylglycerol (DG) and inositol triphosphate (IP_3). DG activates protein kinase C (PKC), which phosphorylates Ca^{2+} channels or other specific proteins, and IP_3 binds to specific receptors on the sarcoplasmic reticulum to release Ca^{2+}. The formation of the Ca^{2+}-Calmodulin complex stimulates the activation of myosin light chain kinase (MLC kinase), which phosphorylates myosin filaments and permits connection to actin filaments (i.e., the formation of cross-bridges) and causes vascular smooth muscle contraction. However, increased cytosolic Ca^{2+} is transient. A guanine nucleotide exchange factor (RhoGEF) contributes to the activation of RhoA-guanosine triphosphate (GTP) conversion from RhoA-guanosine diphosphate (GDP). The RhoA-GTP increases Rho-kinase activity leading to the inhibition of MLC phosphatase activity (which would dephosphorylate the MLCs). This inhibition of MLC phosphatase is initiated at the same time that phospholipase C is activated and allows the MLCs to remain phosphorylated and maintain vasoconstriction. (Webb, 2003).

Furthermore, vascular smooth muscle contains cellular organelles, particularly the sarcoplasmic reticulum that stores and releases calcium. However, compared to skeletal or cardiac cells, vascular smooth muscle cells have a relatively underdeveloped sarcoplasmic reticulum. Consequently, intracellular calcium stores are limited, and

vascular smooth muscle is dependent on extracellular calcium to initiate contraction. The degree of vascular smooth muscle contraction versus relaxation determines the size of the lumen diameter and magnitude of blood flow to an organ (Figure 2-7). Furthermore, the extent of vascular smooth muscle contraction and relaxation of the arterioles has a large effect on total vascular conductance (TVC) and mean arterial pressure (MAP) [MAP = CO/TVC] (Smith & Fernhall, 2011).

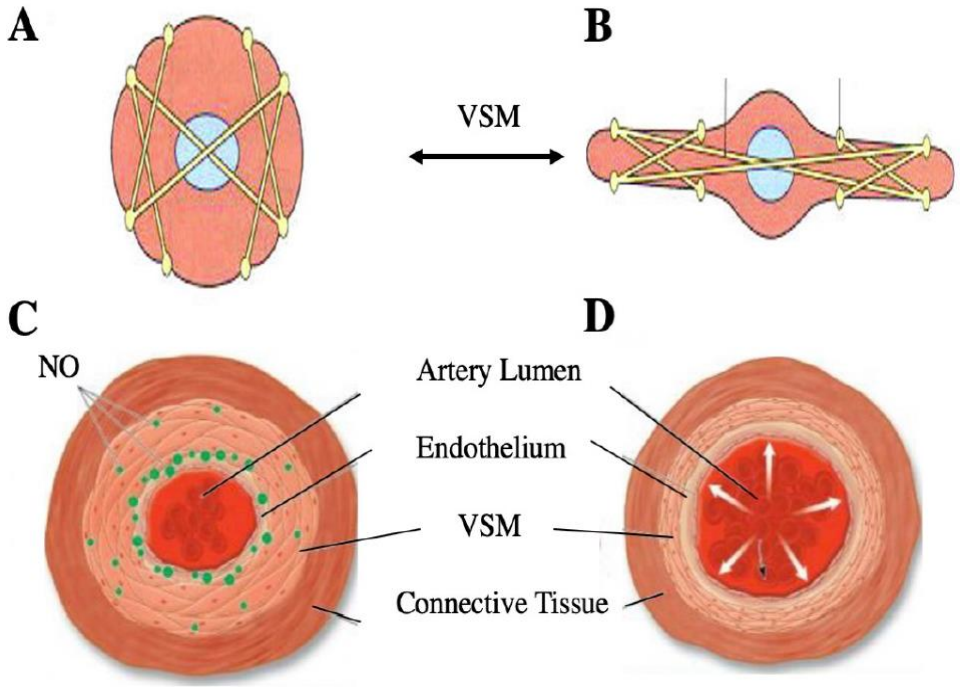


Figure 2-7. The degree of shortening of the actin and myosin contractile filaments in vascular smooth muscle (VSM). Panel A, the contracted state of the VSM due to the shortening of the actin and myosin contractile filaments. Panel B, the relaxed state of the VSM due to the lengthening of the actin and myosin contractile filaments. Panel C, a cross-sectional view of an artery in a more contracted state of the VSM and a smaller lumen diameter. Panel D, a cross-sectional view of an artery in a more relaxed state of the VSM and a larger lumen diameter due to the potent vasodilator, nitric oxide (NO, green dots).

2.2.3.1 Nitric Oxide-Mediated Relaxation

Nitric oxide synthesized in the endothelium rapidly diffuses into the vascular smooth cells causing them to relax. This occurs because NO stimulates soluble guanylyl

cyclase (sGC), which converts guanosine triphosphate (GTP) to the second messenger cyclic guanosine monophosphate (cGMP) (Sandoo, Veldhuijzen van Zanten, Metsios, Carroll, & Kitas, 2010). This second messenger activates protein kinase G (PKG), which closes voltage-dependent Ca^{2+} channels and reduces Ca^{2+} entry into the cells, as well as prevents Ca^{2+} release from the sarcoplasmic reticulum by IP_3 (Webb, 2003; Zhao et al., 2015). In addition, PKG also activates the enzyme calcium-ATPase which promotes Ca^{2+} uptake into sarcoplasmic reticulum. Intracellular Ca^{2+} is also removed by Ca^{2+}/Mg-ATPase and Na^{+}/Ca^{2+} exchangers on the plasma membrane. As a result of these mechanisms, intracellular Ca^{2+} concentration is reduced in the vascular smooth muscle. Consequently, there is less Ca^{2+}-Calmodulin activation of myosin light chain (MLC) kinase. Furthermore, intracellular Ca^{2+} depletion increases MLC phosphatase activity. This removes phosphates from the MLCs and breaks the myosin-actin cross-bridge, causing vascular smooth muscle relaxation (vasodilation). Thus, the relaxation of vascular smooth muscle is due to decreased intracellular Ca^{2+} concentration and increased MLC phosphatase activity (Sandoo et al., 2010; Webb, 2003; Zhao et al., 2015). A summary of how the endothelial cell produces NO to cause vascular smooth muscle relaxation is indicated in Figure 2-8 (Sandoo et al., 2010). A detailed description and illustration of the mechanisms associated with vascular smooth muscle relaxation is provided in Figure 2-9 (Webb, 2003).

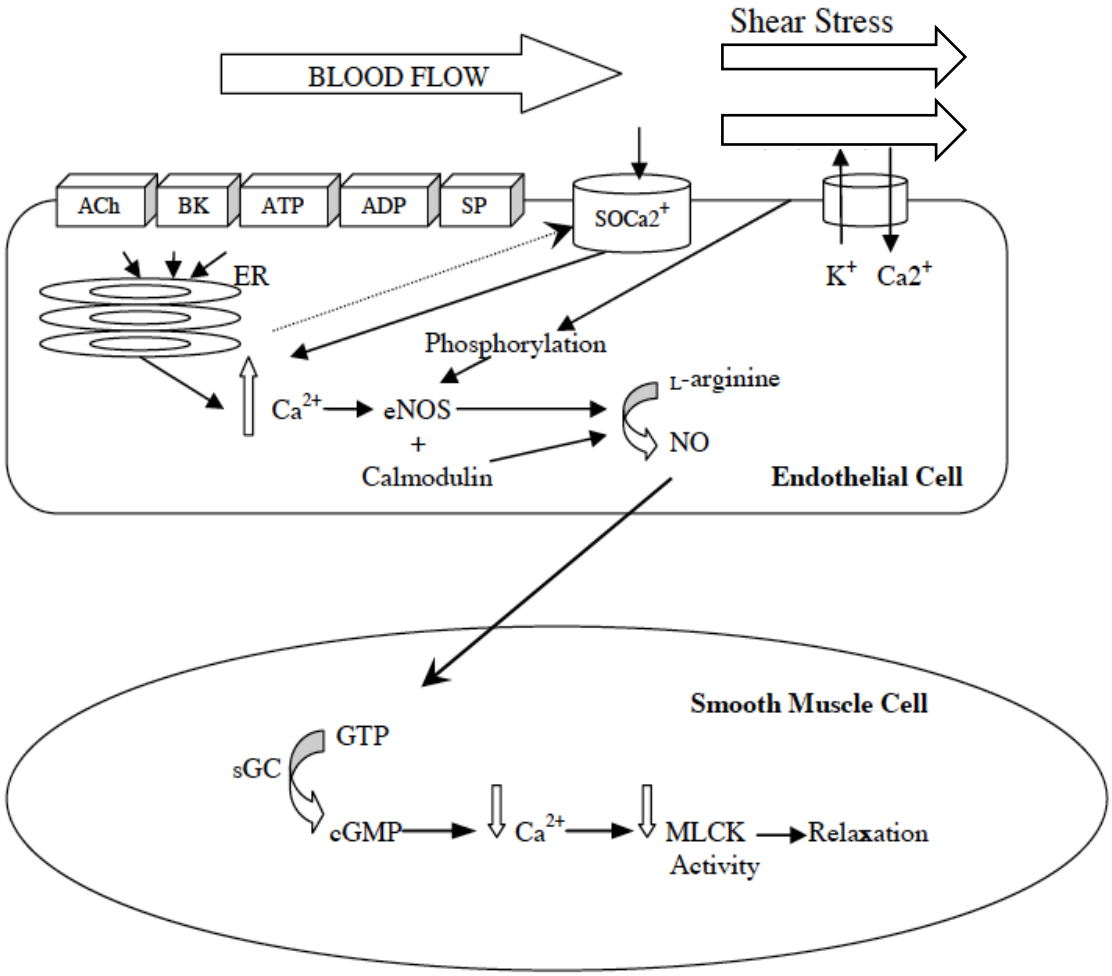


Figure 2-8. The pathway of endothelial nitric oxide (NO) production acting on a vascular smooth muscle (VSM) cell. In the endothelial cell, increased blood flow (i.e., shear stress) results in increased intracellular calcium (Ca^{2+}) concentrations through activating K^{+}/Ca^{2+} channels and binding NO agonists [e.g., acetylcholine (ACh), thrombin, bradykinin (BK), adenosine tri- and di-phosphate (ATP; ADP), substance P (SP)] with receptors on the endothelial cell surface. Ca^{2+} is released from the endoplasmic reticulum (ER) due to agonists and extracellular stores via storage-operated calcium ($SOCa^{2+}$) channel when the Ca^{2+} stores of ER are depleted. Ca^{2+} binds with Calmodulin to activate endothelial nitric oxide synthase (eNOS) phosphorylation and subsequently convert the amino acid L-arginine to NO. NO binds with soluble guanylyl cyclase (sGC) after rapidly diffusing into VSM. In the VSM cell, guanosine triphosphate (GTP) is converted to cyclic guanosine-3'5-monophosphate (cGMP), which reduces Ca^{2+} concentrations. The activity of myosin light chain kinase (MLCK) is also reduced, while myosin light chain phosphatase is activated (not shown). Consequently, the actin-myosin complex (cross-bridge) is detached to cause VSM relaxation (Sandoo et al., 2010).

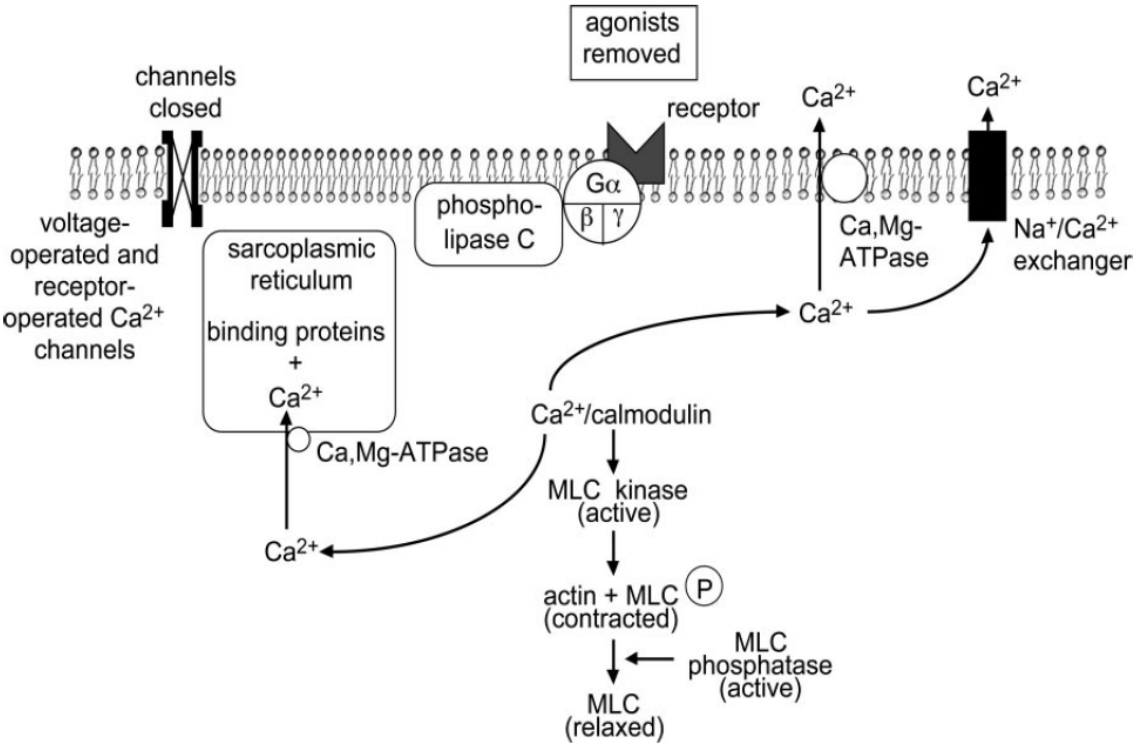


Figure 2-9. Vascular smooth muscle relaxation. When the contractile stimulus is removed or substances (e.g., nitric oxide) directly stimulate the inhibition of vascular smooth muscle contraction, vascular smooth muscle cells are relaxed due to decreased intracellular Ca^{2+}. Intracellular Ca^{2+} is reduced by increasing calcium-ATPase (i.e., the calcium pump) activity on the sarcoplasmic reticulum and plasma membrane, as well as by activation of the Na^+/Ca^{2+} exchanger on the plasma membrane. The voltage- and receptor-operated Ca^{2+} channels in the plasma membrane close to prevent calcium influx. Reduced intracellular Ca^{2+} concentration inactivates calmodulin and reduces myosin light chain (MLC) kinase activity, while increasing MLC phosphatase activity. MLC phosphatase removes phosphate groups from the MLC, which decreases myosin-actin crossbridge formation, resulting in vascular smooth muscle relaxation (Webb, 2003)

2.3 Endothelium-Dependent Vasodilation

Endothelium-dependent vasodilation can be achieved by both invasive and non-invasive endothelial function testing (a biomarker of atherosclerosis) in coronary and peripheral circulations (Verma, Buchanan, & Anderson, 2003). In the coronary circulation, quantitative coronary angiography is an invasive test to examine the changes in vascular diameter through infusion of an endothelium-dependent vasodilator, such as acetylcholine, bradykinin, substance P, or serotine (Verma et al., 2003). In the peripheral

circulation, the ultrasound-based FMD assessment is a non-invasive and commonly used technique to assess endothelial-dependent vasodilation (Verma et al., 2003).

2.3.1 Flow-Mediated Dilation Assessment

Impaired peripheral arterial endothelium-dependent vasodilation (i.e., endothelium dysfunction) is associated with an increased cardiovascular disease risk (Adachi et al., 2015; Calver, Collier, & Vallance, 1992; Panza, Quyyumi, Brush, & Epstein, 1990; Vita et al., 1990; Widmer & Lerman, 2014). Specifically, endothelium dysfunction precedes the development of atherosclerosis (Ross, 1999). There is a continued interest by researchers and clinicians to quantify muscular artery endothelial function via the non-invasive and ultrasound based FMD assessment. The FMD test was originally developed in 1992 (Celermajer et al., 1992), and has multiple published and similar methodological guidelines (Corretti et al., 2002; Thijssen et al., 2011, 2019).

The FMD test takes advantage of the phenomenon that increased blood flow (shear stress) in the arterial lumen leads to greater NO production and vasodilation (Smith & Fernhall, 2011). Based on published guidelines (Thijssen et al., 2019), the FMD test requires the placement of a pressure cuff distal to the conduit artery of interest, which is rapidly inflated to pressures greater than systolic blood pressure (e.g., 250 mmHg) and maintained for 5-minutes. This induces a period of distal ischemia (i.e., no blood flow). Following the ischemic period, the cuff is instantly deflated, resulting in an increased blood flow and shear stress (i.e., hyperemia), which provides the stimulus for endothelial cells to produce and release NO to cause vasodilation. The popliteal FMD test used in the present study is 12-minutes in duration, separated into the following periods: 2-minute baseline, 5-minute cuff inflation and 5-minute post-cuff deflation. Figure 2-10 represents

the time course of the shear stress and FMD responses from a 2-minute example of the post-cuff deflation period (time 0-s) (Thijssen et al., 2011).

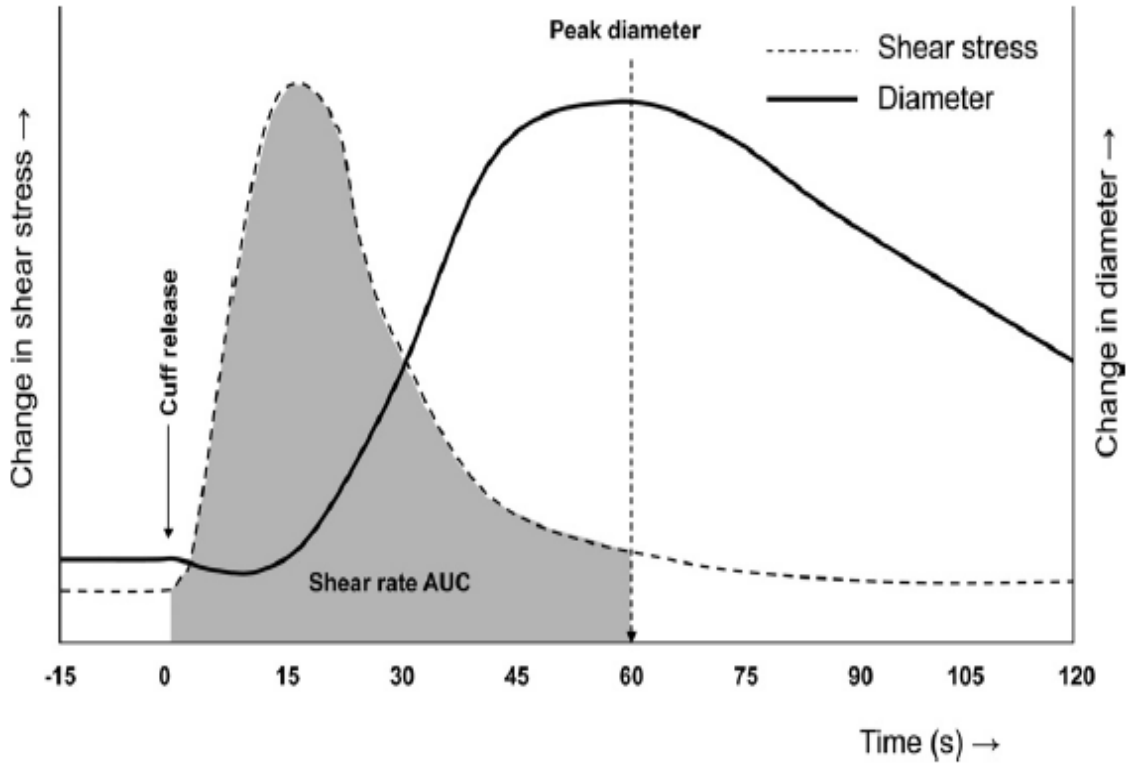


Figure 2-10. The changes in artery lumen diameter (right axis) and shear stress (left axis) during the first 2-minutes of the post-cuff deflation period. The grey area represents the shear rate area under the curve (SR_{AUC}) from the time of cuff deflation to when peak diameter occurred. The SR_{AUC} quantifies the stimulus for the resultant FMD response.

Typically, absolute (mm) and relative (%) increases between the post-cuff deflation peak lumen diameter (versus baseline diameter) are used to quantify the FMD response. Under certain conditions, it is recommended that the shear rate area under the curve (SR_{AUC}) be used to normalize relative FMD responses to minimize inter-individual variations related to the increases in shear stress after cuff deflation (Padilla et al., 2008; Pyke & Tschakovsky, 2007). Specifically, there are analytical tests that need to be performed before determining whether relative FMD should be normalized to the SR_{AUC}. These include: 1) that the SR_{AUC} correlate positively with relative FMD, and 2) that the

y-intercept of the regression is not significantly different than ‘0’ (Pyke & Tschakovsky, 2007). In addition, an independent normalization procedure, called allometric scaling, may also need to be performed if the slope of the relationship between the logarithmically transformed baseline and peak FMD diameters is not equivalent to ‘1’, and has an upper level 95% confidence interval (CI) < 1 (Atkinson & Batterham, 2013).

During the FMD test, duplex ultrasonography is used to simultaneously image the artery to record changes in lumen diameter and red blood cell velocity (RBCv) (Thijssen et al., 2019). The locations of the pressure cuff and ultrasound probe when administering the popliteal FMD test is demonstrated in Figure 2-11.

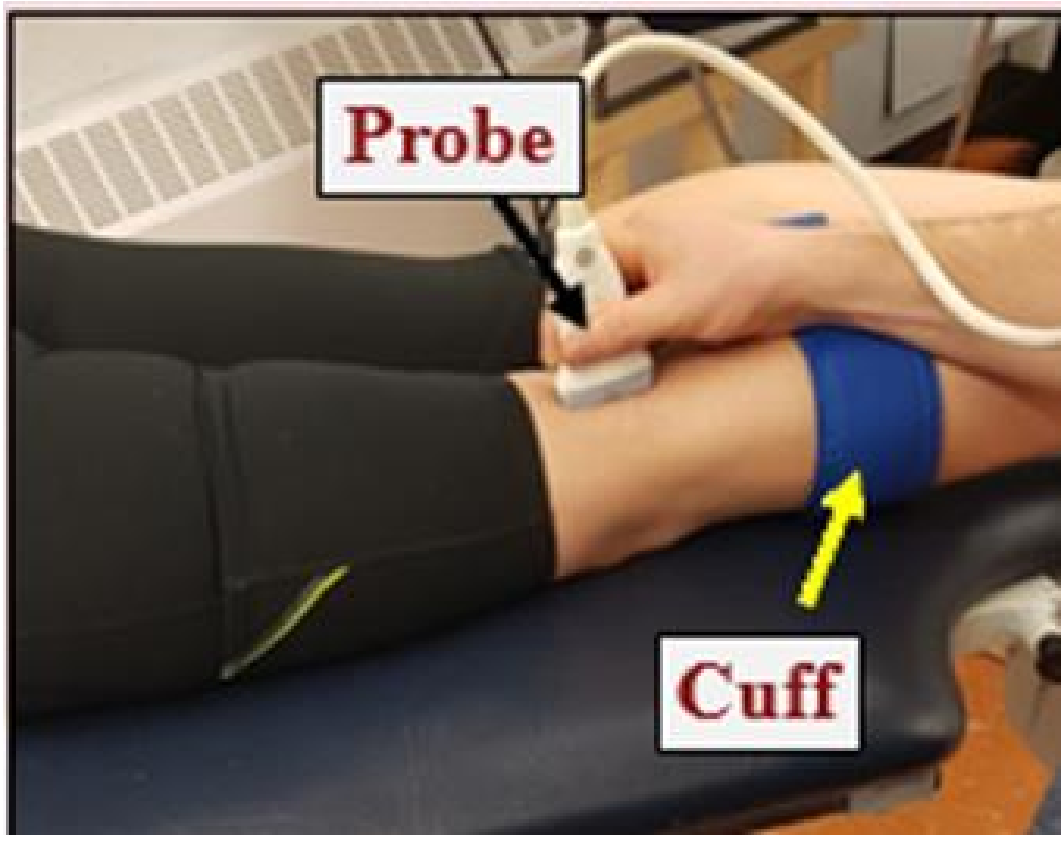


Figure 2-11. A display of the distal pressure cuff and ultrasound probe locations during a popliteal artery flow-mediated dilation (FMD) test.

2.3.2 Principles of Ultrasound

Vascular ultrasonography involves the use of ultra-high frequency sound waves transmitted into the human body to measure the size and amount of blood flowing through vessels (Revzin et al., 2019). The term 'duplex' means two modes used by the ultrasound machine. One is B-mode (brightness mode), which is used to obtain a 2-dimensional image of the vessel being studied. The second is pulsed-wave Doppler mode, which is used to record sound waves reflecting off moving objects towards or away from the probe (e.g., red blood cells). Pulsed-wave Doppler is used to evaluate the velocity and direction of blood flow in the vessel (Revzin et al., 2019).

Ultrasound in B-mode typically uses emitting frequencies between 2- and 12-MHz (Deane, 2002). This transmission frequency can be adjusted to control the level of penetration and to obtain an optimal image of the artery. Decreasing the frequency can increase the depth of penetration for deeper arteries. Increasing the frequency can decrease the depth of penetration for more superficial arteries (Deane, 2002). For example, we use B-Mode emitting frequencies between 8-10 MHz for the popliteal artery.

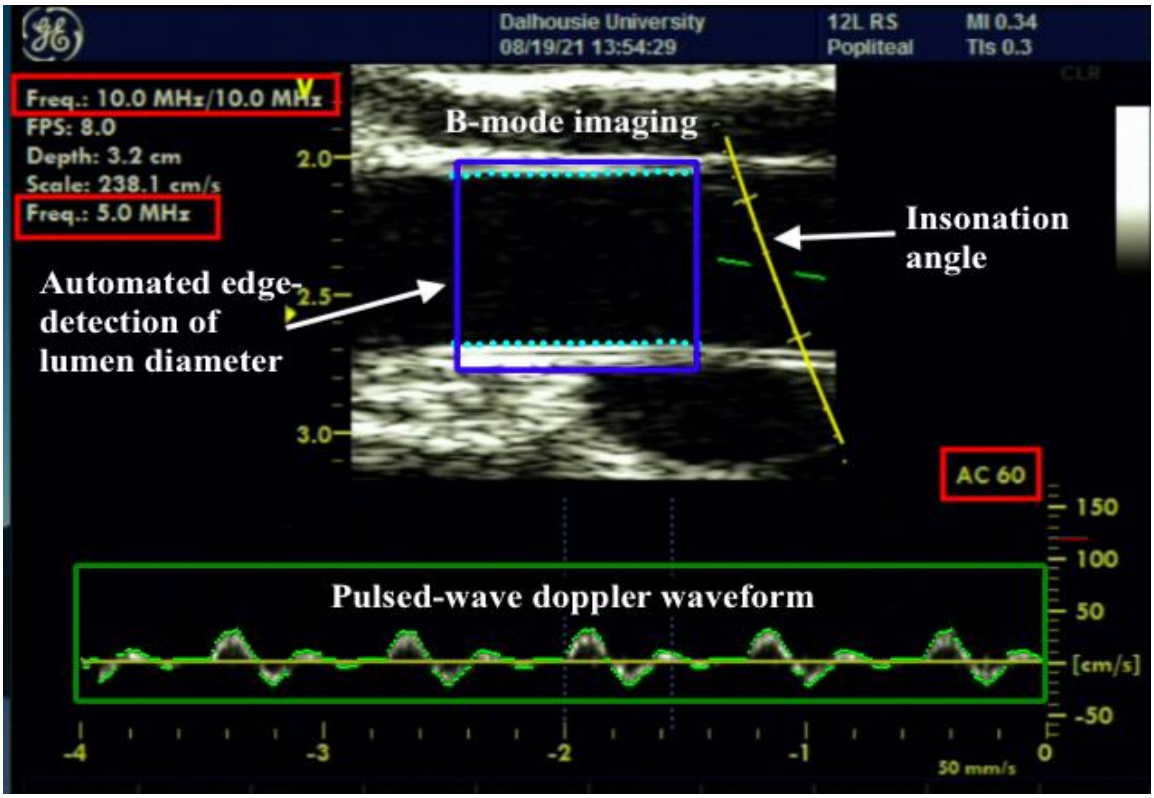


Figure 2-12. An image of the popliteal artery using Brightness-mode (B-mode: 10-MHz) for 2-dimensional imaging and pulsed-wave Doppler mode for quantifying red blood cell velocity. The dark blue box is the region of interest, and the two light blue dotted parallel lines are tracking the lumen artery diameter. The green and yellow lines within the artery represent the insonation angle corrected at 60° (AC 60) and the sample volume from which the red blood cell velocity is recorded from. Beat-by-beat red blood cell velocity is represented below within the green rectangle.

In pulsed-wave Doppler mode, the principle of evaluating RBCv is to measure the doppler frequency shift (Revzin et al., 2019). The probe transmits the ultrasound pulse at a transmitted frequency (f_t) that is reflected from the moving red blood cells within a vessel back to the probe at a received frequency (f_r). The difference between f_t and f_r is the Doppler frequency shift (Δf). The f_t is typically set at 5-MHz for the popliteal artery, while the f_r from stationary soft tissues does not change. However, when the direction of blood flow is moving toward the probe, the f_r will have a higher frequency than the f_t. When the direction of blood flow is moving away from the probe, the f_r will have a lower frequency than the f_t (Revzin et al., 2019).

The Doppler shift can also be expressed by the equation: $\Delta f = f_t - f_r = 2f_t \times$ blood velocity $\times \cos \theta \div C$; where: θ, angle of the insonation; C, speed of ultrasound (Figure 2-13). The angle between the direction of blood flow and the direction of the probe is the angle of the insonation (θ). B-mode echoes have greater intensity with 90°, but optimal pulsed-waved Doppler signals require 0° (Thijssen et al., 2019). Compared to 60°, the error of blood flow velocity is larger when the insonation angle increases to 70° and 80° (Harris, Nishiyama, Wray, & Richardson, 2010). However, there were no differences in blood flow velocity among the insonation angles at 40°, 50°, and 60° (Harris et al., 2010). Therefore, it is recommended that an insonation angle of ≤60° be used to ensure quality blood flow velocity recordings (Thijssen et al., 2019). For this project, the insonation angle was corrected to 60° (Figure 2-12), with the probe always held parallel to the skin (Figure 2-11). The speed of ultrasound (C) is a constant value of 1540 m/s. Hence, the Doppler frequency shift equation can be rearranged to calculate the velocity of moving blood: $RBCv = \Delta f \times C \div (2 \times f_t \times \cos \theta)$.

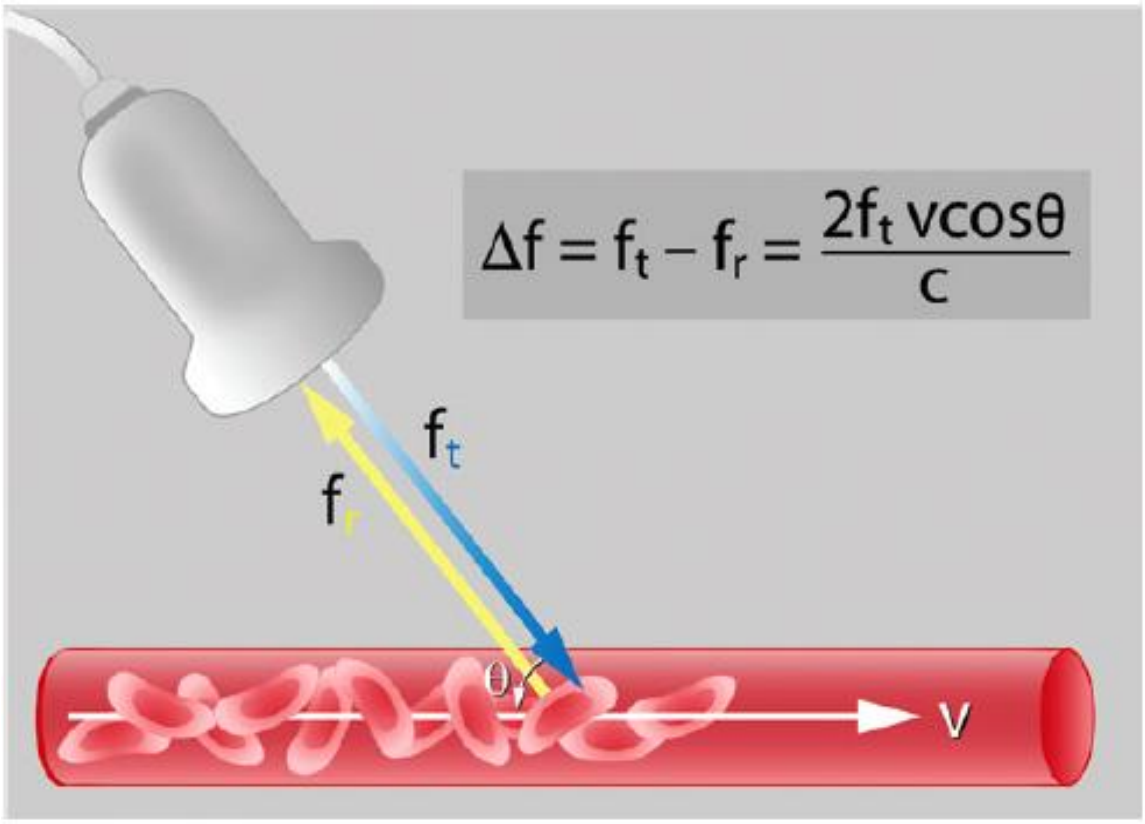


Figure 2-13. The principle of pulsed-wave Doppler to measure red blood cell velocity. Δf, doppler frequency; f_t, transmitted frequency (at 5 MHz); f_r, received frequency; θ, angle of the insonation; V, blood flow velocity; C, speed of ultrasound (1540 m/s).

2.4 Cardiovascular Impacts of Acute Prolonged Sitting

2.4.1 Systematic Hemodynamics

Previous studies (O'Brien et al., 2019; Restaino et al., 2015; Vranish et al., 2017) have observed that heart rate (HR) and MAP increase throughout a bout of uninterrupted prolonged sitting (≥3 hours). These responses may be a consequence of the increased blood pooling in the lower extremities during sitting (Credeur et al., 2019; Paterson et al., 2022). This would lead to a reduction in venous return and a decrease in stroke volume (SV), CO and MAP (Credeur et al., 2019; Paterson et al., 2022). The acute reduction in MAP is detected by baroreceptors, which causes reflex decreases in parasympathetic activity to the heart and increases in sympathetic activation to increase HR (Credeur et

al., 2019). Furthermore, reduced venous return leads to decreases in renal perfusion pressure, which stimulates the renin-angiotensin aldosterone system that contributes to an increase in MAP (Paterson et al., 2022).

O'Brien et al. (2019) conducted a 3-hour prolonged sitting study in 20 healthy young adults (age: 23 ± 2 yr) and found HR (males: 71 ± 9 beats•min^{-1}; females: 80 ± 7 beats•min^{-1}) and MAP (males: 89 ± 4 mmHg; females: 89 ± 6 mmHg) after 3-hours were higher than HR (males: 67 ± 9 beats•min^{-1}; females: 75 ± 7 beats•min^{-1}) and MAP (males: 84 ± 5 mmHg; females: 83 ± 4 mmHg) at baseline. Similarly, Vranish et al. (2017) studied 20 healthy young adults using a 3-hour prolonged sitting protocol and found HR and MAP increased during sitting (all, time effects only: $P \leq 0.01$). Restaino et al. (2015) used a 6-hour sitting protocol in 11 young males (age: 27 ± 1 yr) and found the highest HR at 6-hour (72 ± 0.8 beats•min^{-1}) compared to resting HR (63 ± 0.4 beats•min^{-1}). Additionally, Credeur et al. (2019) examined 20 adults (age: 26 ± 7 yr) and reported that HR increased during 3-hours of uninterrupted prolonged sitting, but HR decreased at post-sitting (60 ± 2 beats•min^{-1}) versus pre-sitting (65 ± 2 beats•min^{-1}) in the supine position ($P < 0.001$). They also observed no changes in MAP, systolic or diastolic blood pressure before, during, or after 3-hours of sitting (Credeur et al., 2019).

In contrast, two studies observed decreases in HR following a 4-hour uninterrupted prolonged sitting protocol (Carter et al., 2019; Kruse et al., 2018). Specifically, Carter et al. (2019) continuously recorded HR throughout the sitting period and measured MAP at baseline, 4-hours, and every 30 minutes during sitting in 15 younger adults. They indicated that HR (61 ± 10 beats•min^{-1}) and MAP (86 ± 9 mmHg) started to decrease after 1.5 h of sitting compared to HR (64±11 beats•min^{-1}) and MAP (87±8 mmHg) after

0.5 h of sitting (Carter et al., 2019). Furthermore, in 13 young adults, Kruse et al. (2018) observed that HR (70 ± 3 beats•min^{-1}) started to decrease at 1.0 h of sitting compared to baseline (73 ± 2 beats•min^{-1}) in the supine position (Kruse et al., 2018). The inconsistent findings in systematic hemodynamics might be due to different sitting time durations (e.g., 3 h, 4 h, 6 h sitting protocols), different measured time points (e.g., every 0.5 h, 1 h), and different pre- and post-sitting postures (e.g., pre-sitting in supine position, but post-sitting in the seated posture).

2.4.2 Lower-Limb Hemodynamics

Compared to an upper-limb artery (e.g., brachial artery), lower-limb arteries (e.g., popliteal artery, superficial femoral artery) are significantly affected by the exposure to acute bouts of prolonged sitting (Paterson et al., 2020). Popliteal (Kruse et al., 2018; Morishima et al., 2016, 2017; O'Brien et al., 2019; Restaino et al., 2015, 2016; Vranish et al., 2017) and superficial femoral artery (Carter et al., 2019) blood flow and/or RBCv decrease during prolonged sitting. Specifically, Kruse et al. (2018) measured resting PBF in the supine position and demonstrated that post-sitting PBF (91.2 ± 13.0 mL•min^{-1}) was lower than pre-sitting (132.3 ± 13.3 mL•min^{-1}, $P < 0.001$). Morishima et al. (2016) measured basal PBF at the end of each hour during 3 h of sitting in 11 healthy adults (age: 26±1 yr). In a separate study, this group measured PBF at 0 h, 0.5 h, 1.0 h, 2.0 h, and 3.0 h of sitting in 15 healthy adults (age: 26.7±0.5 yr) (Morishima et al., 2017). Besides, PBF started to reduce after sitting for 1 h (Morishima et al., 2016) and at the start of sitting (Morishima et al., 2017) compared to pre-sitting in supine position. In addition, Restaino et al. (2016) indicated that compared to pre-sitting (58.9 ± 11.2

mL•min^{-1}) in the supine position, PBF at the end of each hour during 3 h of sitting was lower (Restaino et al., 2016).

Consistently, PBF has been shown to decrease during 3 h of sitting compared to the supine position (O'Brien et al., 2019: at 0.5 h, 1.0 h, 2.0 h, 3.0 h; Vranish et al., 2017: at 10-min, 1.0 h, 2.0 h, 3.0 h). When it comes to the superficial femoral artery, Carter et al. (2019) also observed reduced blood flow following uninterrupted sitting (baseline: 182 ± 67 mL•min^{-1}, at 4 h: 152 ± 56 mL•min^{-1}). These consistent findings of reduced blood flow and RBCv following an acute bout of prolonged sitting may contribute to sitting-induced lower-limb endothelium dysfunction.

2.4.3 Lower-Limb Endothelium-Dependent Vasodilation Responses

Relative (%) FMD has routinely been measured to quantify lower-limb endothelium-dependent vasodilation. A larger relative FMD represents better endothelium-dependent vasodilation and a healthier endothelium. Compared to pre-sitting FMD, decreased popliteal relative FMD after ≥3 h of uninterrupted prolonged sitting have been reported (Kruse et al., 2018; Morishima et al., 2016, 2017; O'Brien et al., 2019; Restaino et al., 2015, 2016; Vranish et al., 2017). Most studies measured pre- and post-sitting popliteal FMD in the supine position (Kruse et al., 2018; Morishima et al., 2016, 2017; Restaino et al., 2015, 2016; Vranish et al., 2017), but one study measured pre- and post-sitting popliteal FMD while participants remained in the seated posture (O'Brien et al., 2019).

Furthermore, other studies that examined superficial femoral artery FMD responses (Climie et al., 2018; Thosar, Bielko, Mather, et al., 2015; Thosar, Bielko, Wiggins, & Wallace, 2014) suggest that the magnitude of the decline in relative FMD was greatest

after ~1 h of uninterrupted sitting. Ballard et al. (2017) reported that a 1 h bout of uninterrupted sitting impaired endothelium function, characterized by a lower superficial femoral artery FMD (1.07 ± 0.53 %) compared to the seated baseline timepoint (2.58 ± 0.57 %).

In addition, the SR_{AUC} response declined after 1 h of uninterrupted sitting and then remained lower throughout 3-hours of sitting (Thosar et al., 2015, 2014). Restaino et al. (2016) also suggested that the sitting-induced decline in relative popliteal FMD may be due to a corresponding reduction in resting blood flow/SR. Specifically, they observed that relative FMD was augmented after sitting in a foot/lower leg bathed in 42°C water (pre-sitting FMD: 7.2 ± 1.5%; post-sitting FMD: 11 ± 1.5%) compared to non-heated control leg (pre-sitting FMD: 6.9 ± 1.5%; post-sitting FMD: 2.9 ± 1.5%). Importantly, this was because SR decreased in the non-heated leg but increased in the heated leg during sitting (Restaino et al., 2016).

Moreover, Morishima et al. (2016) asked 11 participants (age: 26 ± 1 yr) to intermittent fidget one leg during sitting, but had the contralateral leg remain still throughout a 3 h sitting period. Compared to the non-fidgeting leg (pre-sitting popliteal FMD: 4.3 ± 0.9%; post-sitting popliteal FMD: 1.8 ± 0.9%), the fidgeting leg had a similar pre-sitting popliteal FMD (3.6 ± 0.9%) that increased post-sitting (6.8 ± 0.9%). The fidgeting leg also had a correspondingly greater increase in popliteal SR than the non-figeting leg during sitting (Morishima et al., 2016). This evidence suggests that minimizing (or increasing) PBF/SR may prevent the decrease in lower-limb artery relative FMD after sitting.

Walsh et al. (2017) found that the mean popliteal SR in a straight leg (i.e., 0° knee-flexion) during the first 2-hours of side-lying was higher than that in the contralteral bent leg (i.e., 90° knee-flexion; see Figure 2-14). Furthermore, the relative popliteal FMD in the staight leg after 3 h of side-lying was higher than that in the knee bent leg. These data suggest that having a straighter knee-flexion angle may protect popliteal endothelium-dependent vasodilation responses following prolonged sitting, through a preservation of SR.

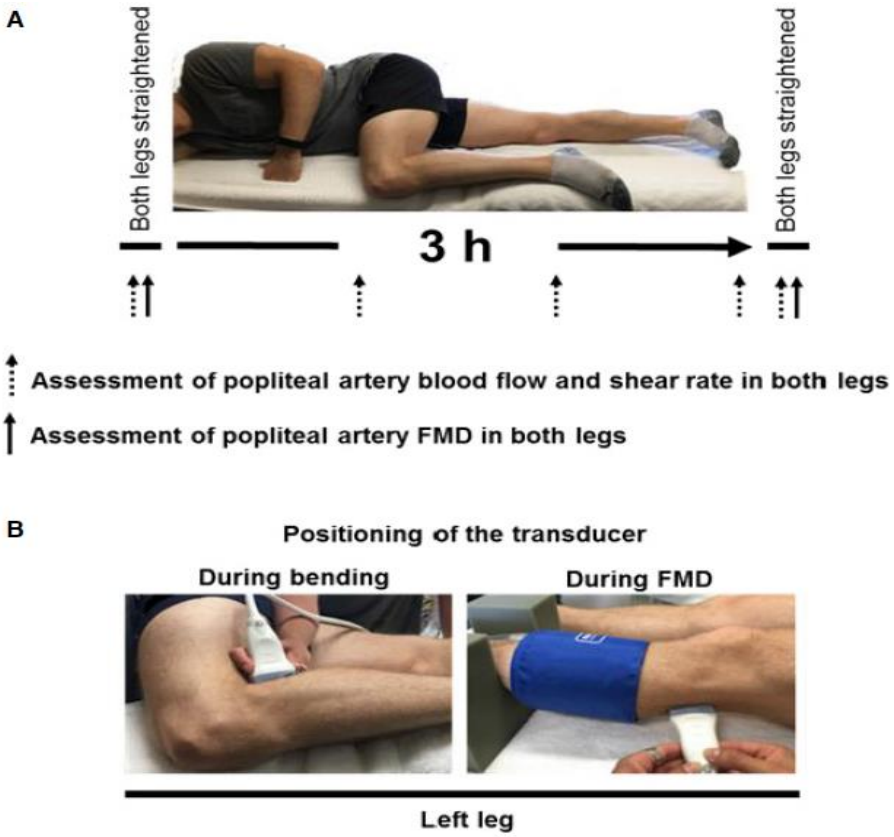


Figure 2-14. Experimental design. (A) Popliteal artery blood flow, shear rate, and flow-mediated dilation (FMD) were assessed in both legs at pre- and post-3 h of side-lying with one leg (and hip) bent at ~90°, while the contralateral leg and hip remained straight. Pre- and post-measures were performed in the supine position with both legs straightened. The post-measures were initiated immediately (within 5 min) after reestablishing the supine position. The order of measures was randomized between legs within each subject. During the 3 h side-lying period, popliteal blood flow and shear rate were assessed in both the straight and bent legs at 1-, 2-, and 3 h. (B) Positioning of the Doppler ultrasound transducer during leg bending measurements and FMD assessments (Walsh et al., 2017).

2.5 Impact of Knee-Flexion Angles on Popliteal Artery

Compared to upper-limb arteries, lower-limb arteries are more negatively affected by exposure to acute bouts of prolonged sitting (Climie et al., 2018; Thosar et al., 2014). Furthermore, atherosclerosis and PAD are more likely to occur in lower-limb arteries, including the popliteal artery (Jadidi et al., 2021; Watt, 1965). The popliteal artery undergoes a larger mechanical deformation during knee flexion, including axial and radial compression, bending, as well as twisting (Desyatova et al., 2017; Poulson et al., 2018). The anatomy of the superficial femoral and popliteal arteries in a bent versus straight leg are indicated in Figure 2-15. Knee flexion-induced mechanical deformation during sitting (and the correspondingly larger reductions in PBF/SR) may explain why the popliteal artery develops more vascular diseases than the superficial femoral artery (Watt, 1965). In addition, fibrous plaque resulting in atherosclerosis and PAD was found in the popliteal artery, but not in the superficial femoral artery, from the same 38-year-old male donor (Figure 2-16; Jadidi et al., 2021).

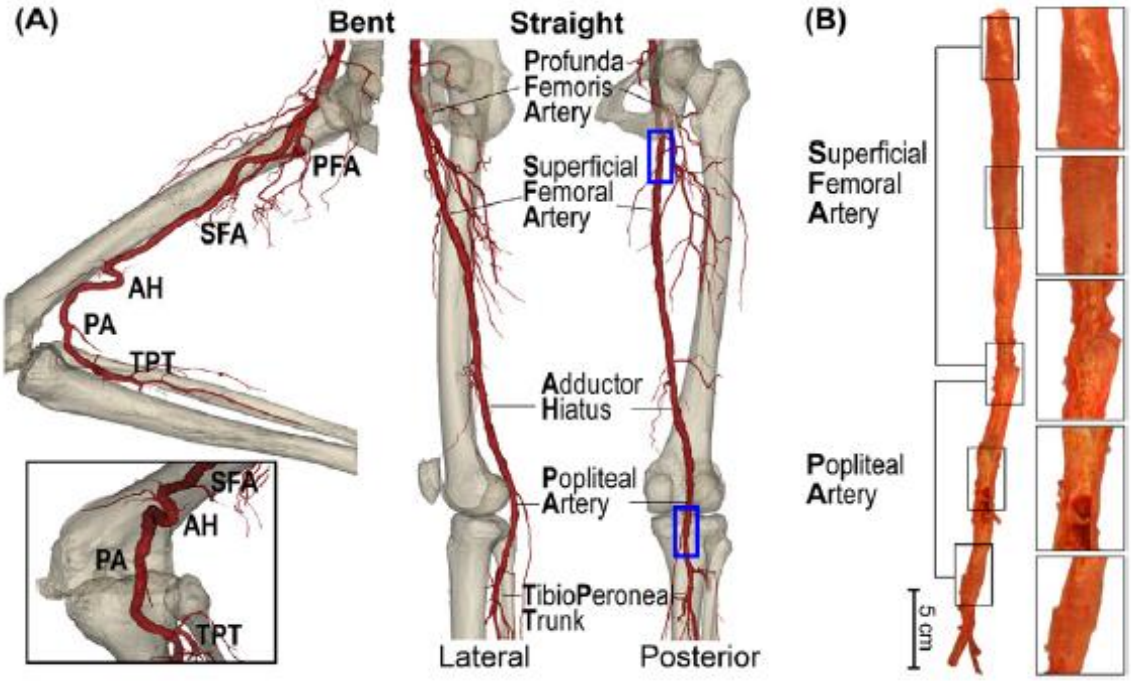


Figure 2-15. Mechanical deformation of the superficial femoral artery (SFA) and popliteal artery (PA) from a bent (left) versus straight leg (right). The segment between the adductor hiatus (AH) and the PA behind the knee experiences severe deformations during knee flexion. Panel B, the longitudinal-opened view of the SFA and PA from a 79-year-old male (Jadidi et al., 2021). FPA, femoropopliteal artery; SFA, superficial femoral artery; PA, popliteal artery; AH, adductor hiatus; PFA, profunda femoris artery; TPT, tibioperoneal trunk.

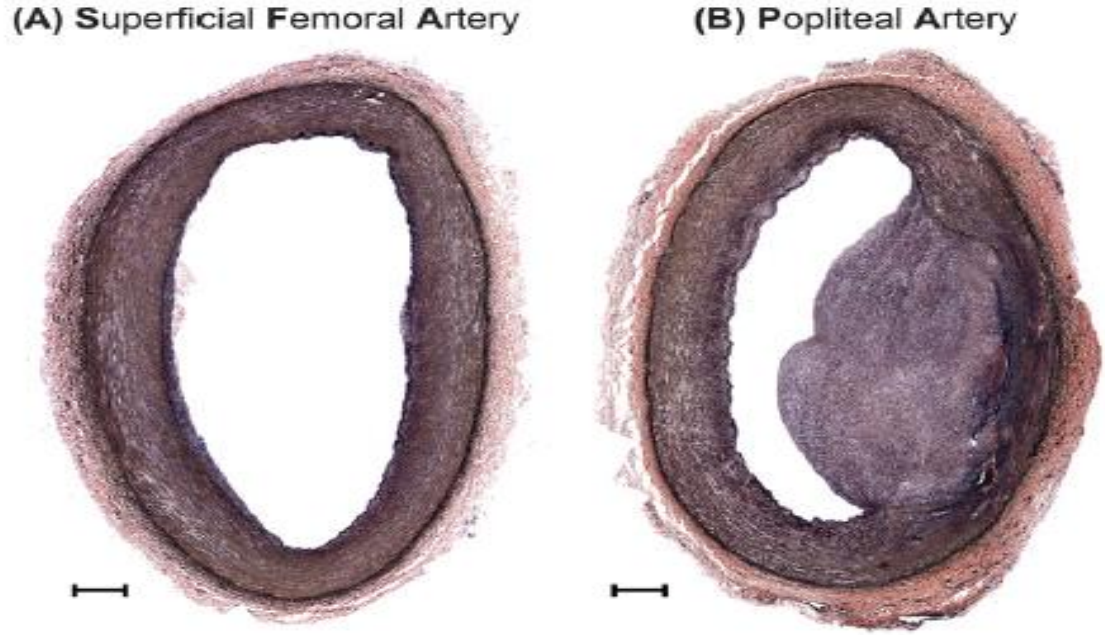


Figure 2-16. Fibrous plaque was not found in (A) superficial femoral artery but in the popliteal artery (B) of a 38-year-old male donor (Jadidi et al., 2021).

Specifically, PBF is significantly decreased during knee flexion (McDaniel, Ives, & Richardson, 2012). As highlighted above, Walsh et al., (2017) conducted a 3 h side-lying protocol with one leg bent (90° knee-flexion) while the contralateral leg was straight (0° knee flexion) in 12 healthy adults (Figure 2-14). These authors demonstrated that PBF and SR were reduced in the bent leg during the first 2 h of lying but remained unchanged in the straight leg. In addition, the bent lower leg experienced markedly reduced relative FMD responses (pre FMD: 6.3 ± 1.2% vs. post FMD: 2.8 ± 0.91%; $P < 0.01$) than the straight leg (pre FMD: 5.6 ± 1.1% vs. post FMD: 7.1 ± 1.2%; $P < 0.24$) (Walsh et al., 2017). Importantly, the side-lying protocol used by Walsh et al. (2017) also involved having the hip flexed to ~90° in the bent leg (Figure 2-14). As such, upstream 'kinking' of the profunda femoris artery may have impacted blood flow/SR response in the downstream popliteal artery (i.e., the independent impact of knee flexion angle was not assessed in this study). Furthermore, Walsh et al. (2017) did not assess the impact of knee flexion angle in the seated position, which is associated with more unhealthy vascular outcomes, increased cardiovascular disease risk and all-cause mortality (Katzmarzyk et al., 2009; Thorp et al., 2011). The present study will attempt to address some of these limitations. Specifically, by using the seated posture, the independent impact of knee flexion angle can be assessed (i.e., hip angle will also be flexed during sitting) in a more ecologically valid/relevant posture.

2.6 Objectives and Hypotheses

The first objective of this study was to uncover the acute effects of sitting at various knee-flexion angles (0°, 45°, 90°) on PBF and SR. The second objective was to determine the prolonged effects of sitting (~2.5 h) at these knee-flexion angles on PBF, SR, and

relative FMD. It was hypothesized that both acute and prolonged sitting-induced impairments to PBF, SR, and relative FMD would be the greatest after sitting with a 90° knee-flexion angle, less after sitting at 45° knee-flexion angle, and the least after sitting at 0° knee-flexion angle.

Chapter 3: Methods

3.1 Participants

Eight young, healthy, non-obese, non-smoking participants (4 females) were recruited via word of mouth (see Table 4-1 for descriptive characteristics). All females were not pregnant or breastfeeding and self-reported having a regular menstrual cycle (~28 days). To minimize the potential confounding influence of endogenous or exogenous sex hormones on vascular function (Thijssen et al., 2019), females were tested in the early-follicular phase of their natural menstrual cycle (i.e., days 1-5 following onset of menstruation; $n = 2$), or during the placebo/inactive phase of monophasic oral contraceptive pill ($n = 1$). There was one female using a copper intrauterine device.

Participants provided written informed consent to participate in the study and completed a Health History Questionnaire (Appendix A) to confirm study eligibility and the Canadian Society for Exercise Physiology - Get Active Questionnaire (Appendix B) to be cleared for an incremental, maximal exercise test (see below for details). The experimental design and protocols were approved by the Dalhousie Health Sciences Research Ethics Board (REB file#: 2020-5278; Appendix C).

3.2 Experimental Measures and Analyses

3.2.1 Systematic Hemodynamic Measurements

Heart rate was determined from successive cardiac intervals obtained from lead II of a bipolar electrocardiogram recording. Continuous, non-invasive blood pressure was recorded via photoplethysmography from the left index or middle finger (Portapres; Finapres Medical Systems, Amsterdam, the Netherlands). Due to equipment malfunction, Portapres recordings ($n = 7$) were not obtained from one participant. An automated patient vital signs monitor (Carescape v100, General Electric Healthcare, Mississauga, ON, Canada) was used to record brachial artery systolic (SBP) and diastolic pressure

(DBP), which were used to perform a 'physiological' calibration of the Portapres waveform.

The electrocardiogram (1000 Hz) and Portapres (200 Hz) recordings were continuously sampled via a commercial data acquisition system (PL3508 PowerLab 8/53; ADInstruments, Sydney, Australia). All signals were displayed in real-time and analyzed offline using dedicated software (LabChart, version 8.1.16, ADInstruments, Sydney, Australia). Beat-by-beat SBP and DBP were determined from the Portapres recordings as the highest and lowest within beat values, respectively. Mean arterial pressure was calculated as ⅓SBP + ⅔DBP. Stroke volume ($mL \bullet beat^{-1}$) was estimated from the Portapres waveform using the non-invasive cardiac output extension in LabChart (ADInstruments, Sydney, Australia). Cardiac output ($L \bullet min^{-1}$) was calculated as the product of HR and SV. Total vascular conductance ($mL \bullet min^{-1} \bullet mmHg^{-1}$) was determined as the quotient of CO and MAP. All systemic hemodynamic measures were collected at all time points throughout study design (Figure 3-2), at least 2-min of stable recordings were analyzed.

3.2.2 Popliteal Artery Measurements

All popliteal measurements were recorded via duplex ultrasonography. Specifically, popliteal lumen diameter was imaged proximal to the bifurcation at, or slightly above, the popliteal fossa (i.e., behind the knee) using a 12-MHz multi-frequency linear array transducer connected to a high-resolution ultrasound machine (Vivid i, General Electric Healthcare, Mississauga, ON, Canada). Simultaneous RBCv was recorded at a pulsed-frequency of 5-MHz and corrected with an insonation angle of 60°, which remained constant throughout the study. The pulsed-wave sample volume was

adjusted for each participant to ensure that the entire lumen diameter was included, as recommended in published guidelines (Thijssen et al., 2019). Ultrasound probe placement was marked on the skin to ensure that the same portion of the artery was imaged for all serial measurements (see below for details).

Prior to the FMD assessment, a pressure cuff attached to a rapid inflation system (E20 and AG101, Hokanson®, Bellevue, WA) was positioned around the largest circumference of the calf, ~10 cm distal to the popliteal fossa. Peak calf circumference (cm) was measured and marked on the skin to ensure consistency of cuff placement for repeated tests. The FMD test began with a 2-min baseline recording of lumen diameter and RBCv. The pressure cuff was then rapidly inflated to 250 mmHg for 5-min to produce a temporary period of distal ischemia (i.e., no blood flow) in the calf and foot. Following cuff deflation, lumen diameter and RBCv recordings continued for an additional 5-min to quantify the resulting reactive hyperemia/shear stress stimulus and capture the peak vasodilatory response.

Video signals from the ultrasound were exported to an external laptop via a video graphics array converter (Epiphan Systems Inc., VGA 2 USB, Ottawa) for offline analysis. Lumen diameter and mean RBCv were then analyzed using customized commercial automated edge-detection and wall-tracking software (Cardiovascular Suite 3.6.1, Quipu, Pisa, Italy). Mean RBCv was calculated as the numerical sum of antegrade (forward moving) and retrograde (backward moving) portions of the Doppler blood velocity waveform. Absolute FMD (mm) was calculated as the difference between the peak and baseline diameters. Relative FMD was determined using the equation: FMD (%) = [(post-cuff deflation peak diameter – baseline diameter) ÷ baseline diameter ×

100%]. Popliteal blood flow ($mL \cdot min^{-1}$) was calculated as [mean RBCv ($cm \cdot s^{-1}$) × 60 (s/min) × π × (lumen diameter ÷ 2)^2 (cm^2)]. Shear rate (s^{-1}) was calculated as [8 × mean RBCv ($cm \cdot s^{-1}$)] ÷ baseline diameter (cm)]. Subsequently, the SR_{AUC} stimulus for the FMD response was quantified between the start of cuff deflation to the time that peak dilation occurred. The time-to-peak dilation was also recorded.

To control for potential inter-individual differences in the magnitude of the SR_{AUC} responses following cuff deflation, it has been recommended that relative FMD be adjusted for SR_{AUC} via an analysis of covariance (ANCOVA) if the following statistical assumptions are met: 1) the relationship between relative FMD and SR_{AUC} is linear, and 2) the y-intercept for the regression slope of this relationship is zero (Atkinson et al., 2009; Pyke & Tschakovsky, 2007). Although a weak linear relationship between relative FMD and SR_{AUC} ($r = 0.32$, $P = 0.026$) was observed (Appendix F), the y-intercept was greater than zero (2.26, 95% CI: [1.12, 3.40], $P < 0.001$). As such, relative FMD responses were not normalized to SR_{AUC}.

Allometric scaling has also been recommended to account for the potential influence of baseline diameter on FMD responses if the following statistical assumptions are met: 1) the unstandardized β-coefficient for the relationship between the natural log of both the peak and baseline diameters deviates from 1, and 2) the unstandardized β-coefficient has an upper level 95% confidence intervals (CI) <1 (Atkinson & Batterham, 2013). However, the FMD diameters in our sample ($\beta = 0.99$, $P < 0.001$, 95% CI: [0.925, 1.003]) did not meet these assumptions. As such, FMD responses were not allometrically-scaled to baseline diameter.

3.2.3 Habitual Activity Monitoring

Participants' habitual physical activities were collected using an activPAL monitor to quantify their sedentary time and/or patterns (e.g., frequency of sedentary breaks), as well as stride-based physical activity at different intensities (light-, moderate- and vigorous-intensity) in the free-living environment. These data were collected for descriptive purposes only and not used for any other analyses. The activPAL inclinometer has been validated as an accurate method of measuring habitual physical and sedentary activities (Bassett et al., 2014; Lyden, Kozey Keadle, Staudenmayer, & Freedson, 2012; Sellers, Dall, Grant, & Stansfield, 2016). Participants wore the activPAL monitor (PAL Technologies Ltd, Glasgow, UK) on the mid-line of the right thigh, one-third of the way between the hip and the knee, 24 hours per day for 7 days. The monitor was waterproofed using a nitrile finger cot and secured to the skin via transparent medical adhesive (Tegaderm^{TM}, 3M, London, ON, Canada). As such, participants were able to shower and participate in water-based activities. Participants self-reported the time they fell asleep, time they woke up, and time spent in other exercise activities (e.g., swimming, cycling, resistance training) to aid with the analysis of waking time and engagement in non-stepping-based forms of physical activity (Appendix D). None of the participants reported any non-stepping-based physical activities. The sleep log was used to help confirm awake time and quantify sedentary time and patterns (e.g., total sedentary time, time spent in prolonged sedentary bouts, sedentary breaks).

The activPAL data were sampled at 20 Hz, averaged over 15-second intervals, and analyzed using a customized LabVIEW program (LabVIEW 2013; National Instruments, Austin, TX). The LabVIEW program summarized daily averages of time spent standing,

and engaged in sedentary patterns (Johns, Frayne, Goreham, Kimmerly, & O'Brien, 2020; Wu et al., 2021). Inter-observer reliability for these sedentary outcomes were previously determined in 21 participants (120 total days) and reported as follows: total sedentary time (R=0.94, P<0.001; mean error: 9 ± 58 mins•day^{-1}; absolute mean errors of 19 ± 56 mins•day^{-1}), sedentary breaks (R=0.99, P<0.001; mean error: 0.03 ± 0.19 breaks•hour^{-1}; absolute mean error: 0.08 ± 0.20 breaks•hour^{-1}), and total time in sedentary bouts >1-hour (R=0.93, P<0.001, mean error: 3 ± 55 mins•day^{-1}, absolute mean error: 15 ± 53 mins•day^{-1}). Stepping-based physical activity intensities were determined using height-based step rate thresholds as previously reported (O'Brien, Johns, Fowles, & Kimmerly, 2020). Specifically, average step rate thresholds for light-, moderate- and vigorous-intensity physical activity thresholds were <102 steps/min, 102-135 steps/min, and >135 steps/min, respectively. Inter-instrument reliability using the height-based step rate thresholds for these physical activity outcomes were previously determined in 32 participants (192 total days) and reported as follows: time spent in light- (R=0.98, P<0.001; mean error: 1.8 ± 6.7 mins•day^{-1}), moderate- (R=0.99, P<0.001; mean error: -0.3 ± 3.2 mins•day^{-1}), and vigorous- (R=0.99, P<0.001; mean error: -0.2 ± 1.3 mins•day^{-1}) intensity physical activity.

3.2.4 Assessment of Aerobic Fitness

There is some controversy in the literature regarding whether having higher aerobic fitness provides a protective effect (i.e., a smaller sitting-induced decreases in lower leg FMD) (Morishima, Tsuchiya, Ueda, Tsuji, & Ochi, 2020), a greater sitting-induced decline in FMD (Liu et al., 2021,) or no impact on FMD (Garten et al., 2019). As such, we assessed aerobic fitness in our participants as both an additional descriptive measure,

as well as to address future questions regarding the potential impact of aerobic fitness on sitting-induced reductions in popliteal FMD. However, this analysis was not included in the current thesis.

To assess aerobic fitness, participants were equipped with a telemetry-based chest strap HR monitor (Polar H9, Kempele, FI) and either a full-face mask or mouthpiece and nose clip to determine the volume rates of oxygen consumption ($\dot{V}O_2$) and carbon dioxide ($\dot{V}CO_2$) production via a breathing tube connected in series to a mixing chamber-based commercial metabolic system (TrueOne 2400, Parvo Medics Inc., Sandy, UT). The HR data were recorded in real-time on the metabolic system. Participants sat on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) and had the seat height adjusted to ensure a slight knee bend (~10-20° knee-flexion) when the pedals were at the lowest position.

The incremental and maximal exercise test started with a 5-min warm-up at a light workload (50W). Following the warm-up period, the workload was increased to 70W and gradually increased 20 $W \bullet min^{-1}$ until voluntary exhaustion. Ratings of perceived exertion were determined every 2-min using the Borg 6-20 scale (Borg, 1982). The exercise test was terminated if the participant experienced a sudden decrease in HR (<30 $beats \bullet min^{-1}$) or cycling pedaling cadence (<40 $revolutions \bullet minute^{-1}$) for longer than 15 seconds or at the participant's own discretion. Participants provided a cue to indicate when they had approximately 1-min remaining in the test. A final rating of perceived exertion measurement was collected at this time. After the completion of the test, the workload was rapidly reduced to the warm-up level for a 5-min cool-down period.

For the test to be considered a maximal effort (i.e., $\dot{V}O_2max$), participants had to achieve a plateau in oxygen consumption (<1.5 $mL \cdot kg^{-1} \cdot min^{-1}$) despite an increase in workload. In summary, 5/8 participants achieved a plateau in oxygen consumption. For those that did not achieve a $\dot{V}O_2max$, a $\dot{V}O_2peak$ was qualified if ≥2 of the following secondary criteria were achieved: 1) maximal HR ≥ 90% of age-predicted maximal HR (i.e., 208 - 0.7 × age) (n = 8/8) (Tanaka, Monahan, & Seals, 2001), 2) a maximal respiratory exchange ratio ≥ 1.10 (n = 8/8), or 3) a maximal rating of perceived exertion >17 (n = 8/8). In conclusion, every participant met ≥2 of these criteria at all time points. Since not all participants achieved a plateau in $\dot{V}O_2$, $\dot{V}O_2peak$ was the term used to describe aerobic fitness in this study.

Peak oxygen consumption was presented in both absolute units ($L \cdot min^{-1}$), as well as relative to body mass ($mL \cdot kg^{-1} \cdot min^{-1}$). All $\dot{V}O_2$ and HR data were averaged every 15-s over the duration of the test, with peak values considered as the greatest consecutive 30-s average.

3.4 Study Design

Participants visited the laboratory on 3 separate days. The first 2 days involved a randomly assigned prolonged sitting protocol (see below). At the end of Day 2, participants were instrumented with the activPAL monitor. The assessment of aerobic fitness was conducted the day they returned the monitor to the lab (Day 3).

Twenty-four hours prior to each experimental day, participants abstained from the engagement in vigorous physical activity. They also avoided the consumption of alcohol and caffeinated products for 12 h before each visit, arrived well-rested (~8 h of sleep), well-hydrated, and consumed their last meal ~6 h beforehand. The FMD test can be

influenced by certain foods and nutritional supplements (Thijssen et al., 2019). As such, all participants avoided the consumption of foods containing saturated fats, polyphenols (e.g., chocolate), high levels of vitamin C (e.g., citrus fruits), as well as antioxidant supplements (e.g., vitamins A, C and/or E) for 12 h before Days 1 and 2. To avoid confounding influences on cardiovascular function associated with diurnal variation (Thijssen et al., 2019), Days 1 and 2 were scheduled at the same time of day.

All lab experimental days were performed in a temperature-controlled room kept at ~21°C. To confirm study eligibility on Day 1, participant height (stadiometer) and weight (physician's scale) were measured to the nearest 0.5-cm and 0.1-kg, respectively (Health-O-Meter, McCook, IL, USA). Body mass index ($kg \bullet m^{-2}$) was calculated to confirm study eligibility (non-obesity: body mass index ≤ 30 $kg \bullet m^{-2}$) because obesity can negatively influence FMD (Ne et al., 2017). Participants sat for a minimum of 5-min before brachial artery blood pressure was recorded from the upper left arm using the automatic vital signs monitor (Carescape v100, General Electric Healthcare, Mississauga, ON, Canada).

On Days 1 and 2, participants went to the bathroom first, and then were instrumented for the electrocardiogram and Portapres recordings before resting in the prone position (i.e., lying on their front) to record 2-min of resting PBF in each leg. They moved to the seated position to complete a randomly assigned prolonged sitting protocol (Figure 3-1). At least 2-min of stable beat-by-beat systematic hemodynamic and PBF data were averaged during the prone resting condition, at the start of the baseline FMD tests, as well as 0.5 h, 1.0 h, and 2.5 h after baseline FMD assessments (i.e., during sitting).

There were 3 knee-flexion angles [0° (straight leg), 45°, 90°] assigned between each leg over Days 1 and 2 (i.e., 1 knee flexion angle per leg per day). On each day, 1 leg was always assigned a knee-flexion angle of 45°, by which to compare against the other 2 knee-flexion angles. The 45° knee flexion angle was chosen as the repeated angle assigned to both legs on each day because it is in between the two extreme knee-flexion angles investigated (i.e., 0° and 90°). Given this constraint, all possible combinations of leg, day, and knee-flexion angle are summarized in Figure 3-1. The order each leg was assessed on each day is included in parentheses. Knee-flexion angles were positioned by the researcher via a goniometer. A protocol was randomly assigned to each participant through blindly drawing a random number from 1 to 8.

Two participants were randomly assigned sitting protocol 2. One participant was scheduled between 1-4:30 pm and had a 36-day break between sitting sessions, while the other was scheduled between 8-11:30 am had and 1-day break between sitting sessions. Two participants were assigned protocol 3, one scheduled between 11-2:30 pm with a 1-day break between sitting sessions, while the other participant was scheduled between 10:30-2 pm and had a 2-day break between sitting sessions.

Protocol 1	Left leg (1)	Right leg (2)
Day 1	45°	0°
Day 2	90°	45°

Protocol 2	Left leg (1)	Right leg (2)
Day 1	45°	90°
Day 2	0°	45°

Protocol 3	Left leg (1)	Right leg (2)
Day 1	0°	45°
Day 2	45°	90°

Protocol 4	Left leg (1)	Right leg (2)
Day 1	90°	45°
Day 2	45°	0°

Protocol 5	Right leg (1)	Left leg (2)
Day 1	45°	0°
Day 2	90°	45°

Protocol 6	Right leg (1)	Left leg (2)
Day 1	45°	90°
Day 2	0°	45°

Protocol 7	Right leg (1)	Left leg (2)
Day 1	0°	45°
Day 2	45°	90°

Protocol 8	Right leg (1)	Left leg (2)
Day 1	90°	45°
Day 2	45°	0°

Figure 3-17. Details regarding the 8 possible randomly assigned knee-flexion angle protocols. The numbers in parentheses indicate the order that each leg performed the flow-mediated dilation assessment.

As dictated by the randomly assigned protocol, their left and right legs were positioned into the respective knee-flexion angles. The baseline FMD assessments were performed with the leg positioned at the assigned knee-flexion angle unless the leg had to be passively moved to accommodate an acceptable high-quality image of the popliteal artery (n = 2). Participants had platforms positioned underneath their feet to avoid any passive muscle contractions. The baseline FMD test commenced for leg 1 at the assigned knee-flexion angle, while leg 2 was positioned at 0° knee-flexion (i.e., straight) to minimize sitting-induced reductions in PBF and FMD (Walsh et al., 2017). After the leg 1 assessment, the leg 2 FMD test was immediately performed at its assigned knee-flexion angle. During the prolonged seated period, participants were permitted to read books and/or watch videos but remained awake throughout. Upper limb movements were permitted. At 0.5 h and 1.0 h after each baseline FMD test, 5-min popliteal lumen diameter and RBCv recordings were collected. The FMD assessments were then repeated for each leg after sitting for ~2.5 hours (same order as baseline testing). Calf circumferences for both legs were measured during the prone resting, as well as at the 0.5 h, 1.0 h, and 2.5 h sitting time points after baseline FMD assessments. Figure 3-2 provides an overview of the study design for Days 1 and 2. Figure 3-3 shows representative pictures of the set up during FMD (A) and blood flow (B) assessments.

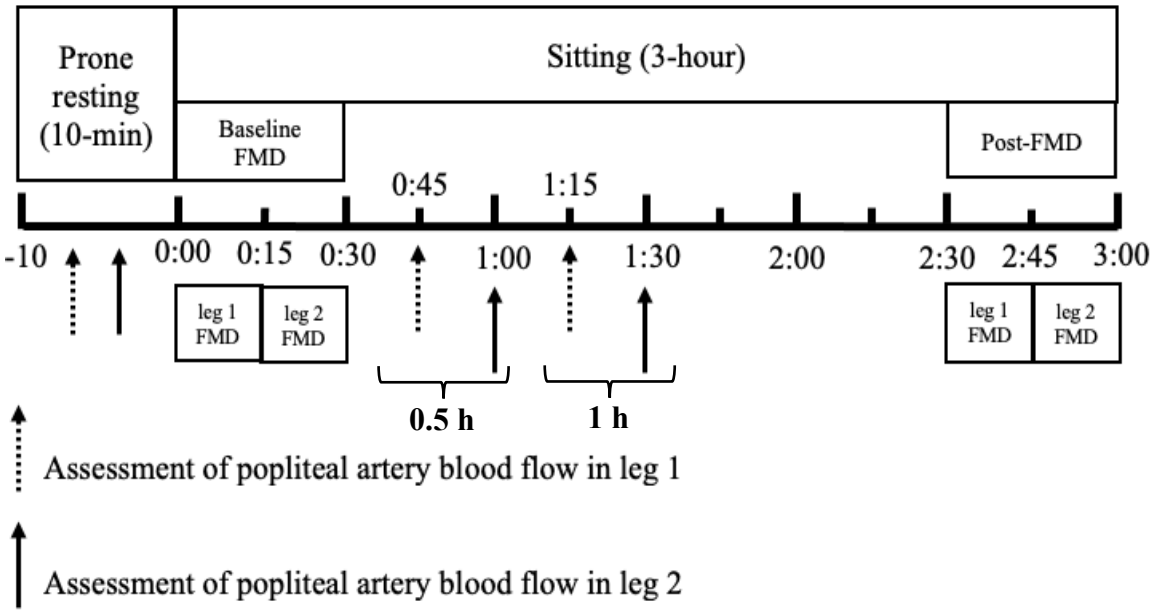


Figure 3-18. Study design. Each participant was randomly assigned to a prolonged sitting protocol, which included the leg order of ultrasound measures (see Figure 17 for details of these protocols). They first completed a prone resting period, which included a 2-min resting popliteal artery blood flow recording in each leg. The leg 1 baseline flow-mediated dilation (FMD) test was performed at the assigned knee-flexion angle while the opposite leg (leg 2) was positioned at 0° knee-flexion (i.e., straight). The leg 2 baseline FMD assessment at the assigned knee-flexion angle occurred immediately after the leg 1 baseline FMD was completed. Popliteal artery blood flow in each leg was assessed at 0.5- and 1.0-h after the corresponding baseline FMD tests. The post-sitting FMD tests occurred in the same order as the baseline assessments, starting ~2.5-hour after baseline testing in each leg.

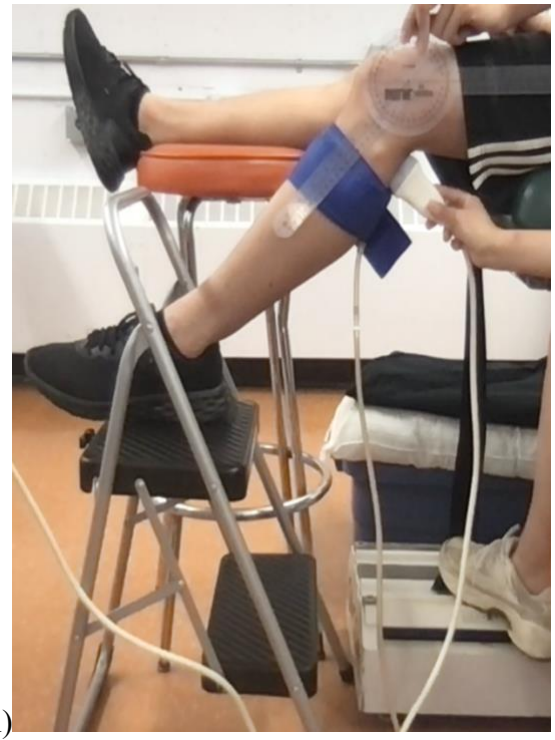
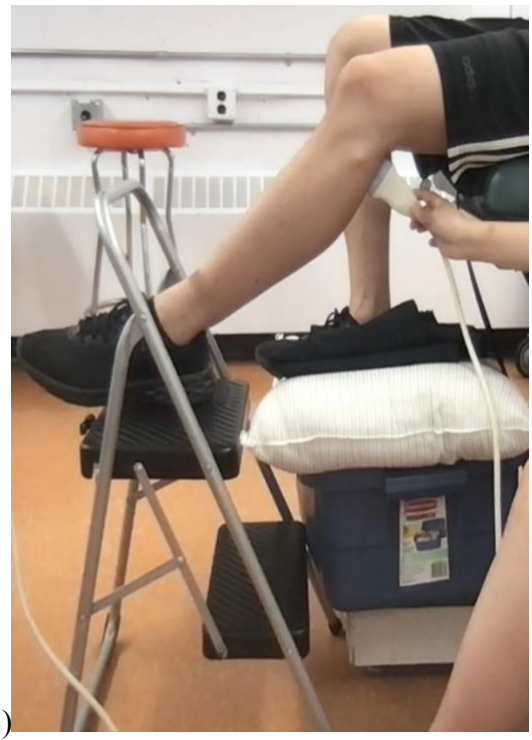
(A)  (B) 

Figure 3-19. Set up and positioning of the Doppler ultrasound transducer during FMD (A) and blood flow (B) assessments. The example in panel A was positioned participant's left leg at 45° and right leg at 0°, and in panel B was positioned participant's right leg at 90°.

3.5 Statistical Analysis

Systemic hemodynamic (i.e., calf circumference, HR, SV, CO, SBP, DBP, MAP, TVC) and popliteal outcomes (i.e., lumen diameter, mean RBCv, PBF, SR, relative FMD, SR_{AUC}) were considered as dependent variables. All dependent variables were normally distributed based on the results of a Shapiro-Wilk test.

3.5.1 Control Test to Determine Between-Day Differences in Systemic Hemodynamics

A Visit (Day1, Day 2) × Time (Prone Rest, Baseline, 0.5 h, 1.0 h, and 2.5 h of sitting) repeated-measures analysis of variance (RM-ANOVA) was performed on all variables except calf circumference (no baseline sitting assessment). These RM-ANOVAs did not uncover a main effect of Visit, or any interaction effects (all, $P \geq 0.069$) for the systemic hemodynamic variables. As such, the time course of these outcomes is

reported as the average of Days 1 and 2. Subsequently, a one-way ANOVA was performed to determine any main effects of time.

3.5.2 Control Test to Determine Between-Day Differences in Popliteal Outcomes During Prone Resting

For data collected during the Prone Resting time point, a Visit (Day 1, Day 2) × Leg (Left, Right) RM-ANOVA was conducted on the popliteal outcomes (i.e., lumen diameter, mean RBCv, PBF, SR) and calf circumference as an internal control measure to determine whether participants were in a similar resting 'physiological state' prior to sitting. In addition, the changes in calf circumferences (i.e., Δ calf circumference from Prone Resting) were calculated at the 0.5 h, 1.0 h and 2.5 h time points during sitting for each knee-flexion angle.

3.5.3 Control Test to Determine Between-Day Differences in Popliteal Outcomes at 45˚ Knee Flexion

Furthermore, RM-ANOVAs were performed for lumen diameter, mean RBCv, PBF, and SR [Visit (Day 1, Day 2) × Time (Prone Rest, Baseline, 0.5 h, 1.0 h, and 2.5 h of sitting)], as well as for relative FMD and SR_{AUC} [Visit (Day 1, Day 2) × Time (Baseline, 2.5 h of sitting), and calf circumference [Visit (Day 1, Day 2) × Time (Prone Rest, 0.5 h, 1.0 h, and 2.5 h of sitting)] and Δ calf circumference [Visit (Day 1, Day 2) × Time (0.5 h, 1.0 h, and 2.5 h of sitting)]. These RM-ANOVAs were conducted to determine whether there were any main effects of Visit, or any interaction effects on popliteal outcomes between the right versus left legs while positioned at the 45° knee flexion angle. In brief, no main effect of Visit (Leg) or interaction effects were observed

(all, $P \geq 0.062$). As such, data recorded from both legs were averaged together to represent the 45° knee flexion condition.

3.5.4 Statistical Tests to Determine Primary Objectives

To determine the acute impact of sitting at different knee-flexion angles on PBF and SR (Objective 1), a one-way ANOVA was performed on these variables from data recorded during the Baseline FMD assessments (i.e., the start of sitting).

To uncover the prolonged effects of sitting at different knee-flexion angles on popliteal function (Objective 2), RM-ANOVAs were performed for PBF and SR [Knee Flexion Angle (0°, 45°, 90°) × Time (Baseline, 0.5 h, 1.0 h, and 2.5 h of sitting)], as well as for relative FMD [Knee Flexion Angle (0°, 45°, 90°) × Time (Baseline, 2.5 h of sitting).

For all ANOVAs, the variance of differences was assessed using Mauchly's test of sphericity and the Greenhouse-Geisser correction factor to the degrees of freedom used if assumptions of sphericity were violated. Bonferroni *post-hoc* testing was used for pairwise comparisons if significant interactions were identified. All statistical analyses were completed in SPSS (Version 25.0. IBM Corp., Armonk, NY). Data were reported as means ± standard deviations (SD), and statistical significance were accepted as $P < 0.05$.

Chapter 4: Results

4.1 Descriptive Characteristics, Habitual Activity, and Aerobic Fitness

Participants' descriptive characteristics, habitual activity, and aerobic fitness measures are presented in Table 4-1. Habitual activity measures suggested that this population (on average) did not meet sedentary activity guidelines (<8 hour•day^{-1}) but met physical activity guidelines (moderate-to-vigorous physical activity ≥150 min•week^{-1}). The average moderate-to-vigorous physical activity level was 426±212 min•week^{-1}.

Table 4-1. Participant descriptive characteristics, habitual activity, and aerobic fitness.

Variable	Participants (n = 8, 4♀)
Descriptive Characteristics	
Age, yr	24 ± 2
Stature, m	1.71 ± 0.09
Body mass, kg	65.8 ± 10.1
Body mass index, kg•m^{-2}	22.3 ± 1.9
Habitual Activity	
Daily step count, steps•day^{-1}	12148 ± 3604
Sedentary time, hour•day^{-1}	8.8 ± 1.4
Sedentary breaks, breaks•hour^{-1}	3.2 ± 1.1
Prolonged sitting (≥ 1 hour) bouts, bouts•day^{-1}	2.1 ± 0.6
Standing time, hour•day^{-1}	6.1 ± 1.5
Light physical activity, min•day^{-1}	72 ± 14
Moderate physical activity, min•day^{-1}	49 ± 22
Vigorous physical activity, min•day^{-1}	12 ± 11
Aerobic Fitness	
Absolute $\dot{V}O_2$peak, L•min^{-1}	2.6 ± 0.8
Relative $\dot{V}O_2$peak, mL•kg^{-1}•min^{-1}	39.8 ± 9.0
Peak aerobic power output, watts	218 ± 52
Peak heart rate, beats•min^{-1}	190 ± 13
Peak age-predicted heart rate, beats•min^{-1}	191 ± 1
Peak respiratory exchange ratio	1.2 ± 0.1
Peak rating of perceived exertion	19 ± 0

Data presented as means ± SD. $\dot{V}O_2$peak, peak oxygen consumption.

4.2 Systematic Hemodynamic Responses to Sitting

As shown in Table 4-2, main effects (all, $P \leq 0.016$) were observed for HR, SV, DBP, MAP, and TVC between the Prone Rest, Baseline, 0.5 h, 1.0 h, and 2.5 h sitting time points. HR increased ($P=0.027$) after 1.0 h of sitting from the Prone Rest level. SV decreased from Prone Rest at 0.5 h, 1.0 h, and 2.5 h of sitting (all, $P \leq 0.041$). DBP and MAP increased from Prone Rest after 2.5 h of sitting (both, $P \leq 0.005$). TVC decreased after 2.5 h of sitting versus Prone Rest ($P=0.004$).

4.3 Popliteal Hemodynamic Responses to Sitting

4.3.1 Prone Resting Condition

As shown in Table 4-3, there were no main and/or interaction effects on lumen diameter, mean RBCv, PBF, SR, as well as calf circumference at Prone Rest (all, $P \geq 0.060$). Thus, on average, participants were in a similar resting 'physiological state' prior to sitting on Days 1 and 2.

4.3.2 Acute Effects of Knee-Flexion Angle on Popliteal Hemodynamics

As shown in Table 4-4, there were no effects of knee-flexion angle on lumen diameter and SR (both, $P \geq 0.082$) during the baseline FMD assessment at the start of sitting. However, acute effects of sitting at the different knee-flexion angles were observed for mean RBCv and PBF (all, $P \leq 0.010$). Specifically, mean RBCv at 45° and 90° were lower (all, $P \leq 0.029$) than when sitting with straight legs (0° knee flexion). Furthermore, PBF was greater ($P=0.021$) sitting with a 0° knee flexion angle versus at 90°.

Table 4-2. Systemic hemodynamic responses to prolonged sitting.

Measure		Prolonged Sitting				One-way ANOVA
	Prone Rest	Baseline	0.5 h	1.0 h	2.5 h	
Heart rate, beats•min^{-1}	61±5	66±6	68±7	69±6*	68±8	P=0.005
Stroke volume, mL•beat^{-1}	68±3	60±8	55±7*	50±12*	55±8*	P<0.001
Cardiac output, L•min^{-1}	4.1±0.4	4.0±0.6	3.8±0.6	3.5±0.8	3.7±0.5	P=0.016
Systolic blood pressure, mmHg	111±5	116±9	118±12	118±9	120±10	P=0.007
Diastolic blood pressure, mmHg	62±5	70±7	69±6	70±9	72±7*	P=0.001
Mean arterial pressure, mmHg	78±5	85±7	85±7	86±9	88±7*	P<0.001
Total vascular conductance, mL•min^{-1}•mmHg^{-1}	52±7	47±7	45±8	41±11	42±6*	P=0.003

Data were averaged from Days 1 and 2 and presented as means ± SD. Data were analyzed using a one-way analysis of variance (ANOVA). Pairwise comparisons for one-way ANOVA are presented. Baseline refers to data collected during the first FMD test conducted while in the seated position. *, P<0.05 vs. Prone Rest.

Table 4-3. Comparison of popliteal hemodynamic and calf circumference outcomes between legs in the Prone Rest condition on Days 1 and 2.

Measures	Left Leg	Right Leg	RM-ANOVA
Lumen diameter, mm			Day: *P*=0.505
Day 1	5.0±0.9	5.3±0.8	Leg: *P*=0.139
Day 2	5.0±0.9	5.2±0.8	Interaction: *P*=0.835
Mean RBCv, cm•s^{-1}			Day: *P*=0.634
Day 1	4.4±1.2	4.1±1.0	Leg: *P*=0.299
Day 2	4.6±1.1	4.5±0.7	Interaction: *P*=0.711
PBF, mL•min^{-1}			Day: *P*=0.843
Day 1	57±30	58±30	Leg: *P*=0.702
Day 2	54±19	57±18	Interaction: *P*=0.791
SR, s^{-1}			Day: *P*=0.398
Day 1	71±17	62±12	Leg: *P*=0.060
Day 2	77±26	71±17	Interaction: *P*=0.730
Calf Circumference, cm			Day: *P*=0.489
Day 1	35.5±1.8	35.7±1.8	Leg: *P*=0.517
Day 2	35.4±1.5	35.4±1.8	Interaction: *P*=0.381

Data presented as means ± SD. Mean RBCv, mean red blood cell velocity; PBF, popliteal blood flow; SR, shear rate. Data were analyzed using a Day × Leg repeated-measures analysis of variance (RM-ANOVA) to check if, on average, participants were at a consistent resting level before starting the prolonged sitting protocol.

4.3.3 Impact of Knee-Flexion Angle During Prolonged Sitting

Mean RBCv at 45° (2.5±0.6 cm•s^{-1}) and 90° (2.4±0.6 cm•s^{-1}) were not different (*P*=1.000) but both were lower than mean RBCv at 0° (3.2±0.8 cm•s^{-1}; all, *P*≤0.017). Mean RBCv decreased from baseline (3.3±0.7 cm•s^{-1}) at 0.5 h (2.6±0.7 cm•s^{-1}), 1.0 h (2.4±0.6 cm•s^{-1}), and 2.5 h (2.4±0.7 cm•s^{-1}) of sitting (all, *P*≤0.002). There were no differences in mean RBCv at 0.5 h, 1.0 h, 2.5 h of sitting between the different knee-flexion angles (all, *P*≥0.575).

Similarly, PBF at 45° (33 ± 10 mL•min^{-1}) and 90° (30 ± 12 mL•min^{-1}) were not different (*P*=1.000) from each other but were both lower than PBF at 0° (42 ± 15 mL•min^{-1}; all, *P*≤0.026). PBF at baseline (43 ± 15 mL•min^{-1}) was greater at 0.5 h (34 ± 13 mL•min^{-1}), 1.0 h (31 ± 11 mL•min-1), and 2.5 h (31 ± 11 mL•min-1) of sitting (all,

$P \leq 0.020$). There were no differences in PBF between 0.5 h, 1.0 h, and 2.5 h of sitting (all, $P \geq 0.169$).

Shear rate at 45° (38 ± 13 s^{-1}) was not different ($P=1.000$) than 90° (38 ± 12 s^{-1}) but was lower than 0° (50 ± 16 s^{-1}; $P=0.012$). There was also a trend for SR at 90° to be lower than at 0° ($P=0.059$). Shear rate decreased from baseline (51±15 s^{-1}) after 0.5 h (40 ± 14 s^{-1}), 1.0 h (37 ± 12 s^{-1}), and 2.5 h (38 ± 15 s^{-1}) of sitting (all, $P \leq 0.007$). There were no differences in SR between 0.5 h, 1.0 h, and 2.5 h of sitting (all, $P=1.000$).

Calf circumference from Prone Rest (35.2 ± 1.7 cm) was unchanged during the 0° knee flexion angle condition (all, $P \geq 0.911$) at 0.5 h (35.5 ± 1.5 cm), 1.0 h (35.4 ± 1.8 cm), and 2.5 h (35.3 ± 1.7 cm) of sitting. In contrast, calf circumference at 45° knee-flexion angle increased (all, $P \leq 0.007$) at 0.5 h (36.5 ± 1.8 cm), 1.0 h (36.8 ± 1.9 cm), and 2.5 h (37.1 ± 1.9 cm) of sitting. Calf circumference at 90° knee-flexion were not different ($P=0.216$) at 0.5 h (36.9 ± 2.0 cm) and 1.0 h (37.2 ± 2.0 cm) but increased ($P \leq 0.031$) after 2.5 h of sitting (37.5 ± 2.0 cm). The increases in calf circumference were not different (all, $P \geq 0.150$) between the 45° and 90° knee-flexion angles but were both greater than at 0° through all time points (all, $P \leq 0.044$).

Table 4-4. Popliteal hemodynamic and calf circumference responses to prolonged sitting.

Measures	Prolonged Sitting				One-way ANOVA (Baseline)	Pairwise Comparisons (Acute Sitting)	RM-ANOVA (Prolonged Sitting)	Pairwise Comparisons (Prolonged Sitting)
	Baseline	0.5 h	1.0 h	2.5 h				
Lumen diameter, mm								
0°	5.4±1.0	5.2±1.1	5.2±1.0	5.3±1.0			Angle: P=0.614	
45°	5.3±0.7	5.4±0.7	5.3±0.8	5.2±0.8	P=0.174	-	Time: P=0.954	-
90°	5.1±0.9	5.3±0.8	5.1±0.8	5.2±0.8			Interaction: P=0.310	
Mean red blood cell velocity, cm•s^{-1}								
0°	3.8±0.3	3.2±0.8	2.8±0.7	2.8±0.7			Angle: P<0.001	0° vs. 45°: P=0.001
						0° vs. 45°: P=0.029	(partial η^2 = 0.690)	0° vs. 90°: P=0.017
45°	3.1±0.8	2.4±0.3	2.1±0.3	2.2±0.5	P=0.009		Time: P<0.001	Baseline vs. 0.5 h: P=0.002
						0° vs. 90°: P=0.016	(partial η^2 = 0.781)	Baseline vs. 1.0 h: P<0.001
								Baseline vs. 2.5 h: P=0.002
90°	2.9±0.7	2.2±0.5	2.2±0.5	2.1±0.7			Interaction: P=0.723	
							(partial η^2 = 0.080)	
Popliteal blood flow, mL•min^{-1}								
0°	53±15	40±15	36±12	37±12			Angle: P=0.002	0° vs. 45°: P=0.018
							(partial η^2 = 0.579)	0° vs. 90°: P=0.026
45°	40±9	33±10	28±10	28±8	P=0.010	0° vs. 90°: P=0.021	Time: P<0.001	Baseline vs. 0.5 h: P=0.020
							(partial η^2 = 0.783)	Baseline vs. 1.0 h: P=0.002
								Baseline vs. 2.5 h: P=0.001
90°	36±15	29±12	28±12	27±10			Interaction: P=0.410	
							(partial η^2 = 0.130)	
Shear rate, s^{-1}								
0°	58±13	51±16	45±16	46±18			Angle: P=0.009	0° vs. 45°: P=0.012
						-	(partial η^2 = 0.597)	0° vs. 90°: P=0.059
45°	49±16	36±7	32±7	36±13	P=0.082		Time: P<0.001	Baseline vs. 0.5 h: P=0.004
							(partial η^2 = 0.684)	Baseline vs. 1.0 h: P=0.004
								Baseline vs. 2.5 h: P=0.007
90°	47±15	34±9	35±9	34±13			Interaction: P=0.620	
							(partial η^2 = 0.077)	
Δ Calf Circumference, cm								
0°		0.3±0.5	0.2±0.8	0.1±0.7			Angle: P<0.001	
					-	-	(partial η^2 = 0.919)	
45°	-	0.9±0.3*	1.3±0.5^{*a}	1.6±0.3^{*ab}			Time: P=0.024	-
							(partial η^2 = 0.414)	
90°		1.2±0.4*	1.5±0.5*	1.8±0.6^{*a}			Interaction: P=0.039	
							(partial η^2 = 0.388)	

Data presented as means ± SD. Baseline sitting data (lumen diameter, mean RBCv, PBF, SR) were analyzed using one-way analysis of variance (ANOVA) to determine the acute effects of sitting at various knee-flexion angles on popliteal hemodynamics (Objective 1). Data during prolonged sitting (lumen diameter, mean RBCv, PBF, SR, Δ Calf Circumference) were analyzed using a 2-factor (Knee-Flexion Angle × Time) repeated-measures analysis of variance (RM-ANOVA) to determine the prolonged effects of sitting at the different knee-flexion angles on popliteal hemodynamics (Objective 2), and partial η^2 was used to calculate effect size. Only statistically significant pairwise comparisons observed from the RM-ANOVA are presented. *, P<0.05 vs. 0°; [a], P<0.05 vs. 0.5 h; [b], P<0.05 vs. 1.0 h.

4.4 Popliteal Flow-Mediated Dilation Responses to Sitting

In Figure 4-1A, only a main effect of time (P=0.005) was observed for popliteal relative FMD responses between baseline and 2.5 h of sitting. However, there were no differences in the sitting-induced reduction in popliteal relative FMD between the three knee-flexion angles (P=0.958).

At baseline (pre-sitting), there were no differences in SR_{AUC} among the three knee-flexion angles (all, $P \geq 0.254$). As displayed in Figure 4-1B, an interaction effect (P=0.020) was observed for SR_{AUC} among the three knee-flexion angles between baseline and 2.5 h of sitting. Specifically, the SR_{AUC} at 0° knee flexion was not different (P=0.457) between pre- (13839±7970 a.u.) and post-sitting (15346±8383 a.u.). The post-sitting SR_{AUC} during the 45° knee-flexion angle (6407±4276 a.u.) was lower (P=0.006) than pre-sitting (9218±4880 a.u.). Similarly, post-sitting SR_{AUC} during the 90° knee flexion angle condition (6784±4750 a.u.) was also lower (P=0.016) than baseline (10128±5033 a.u.). However, there were no differences in sitting-induced declines in SR_{AUC} between the three knee-flexion angles ($P \geq 0.074$). After 2.5 h of sitting, SR_{AUC} responses were similar (P=1.000) between the 45° and 90° knee-flexion angles, which were both lower (both, $P \leq 0.023$) than after sitting with straight legs (0° knee-flexion angle).

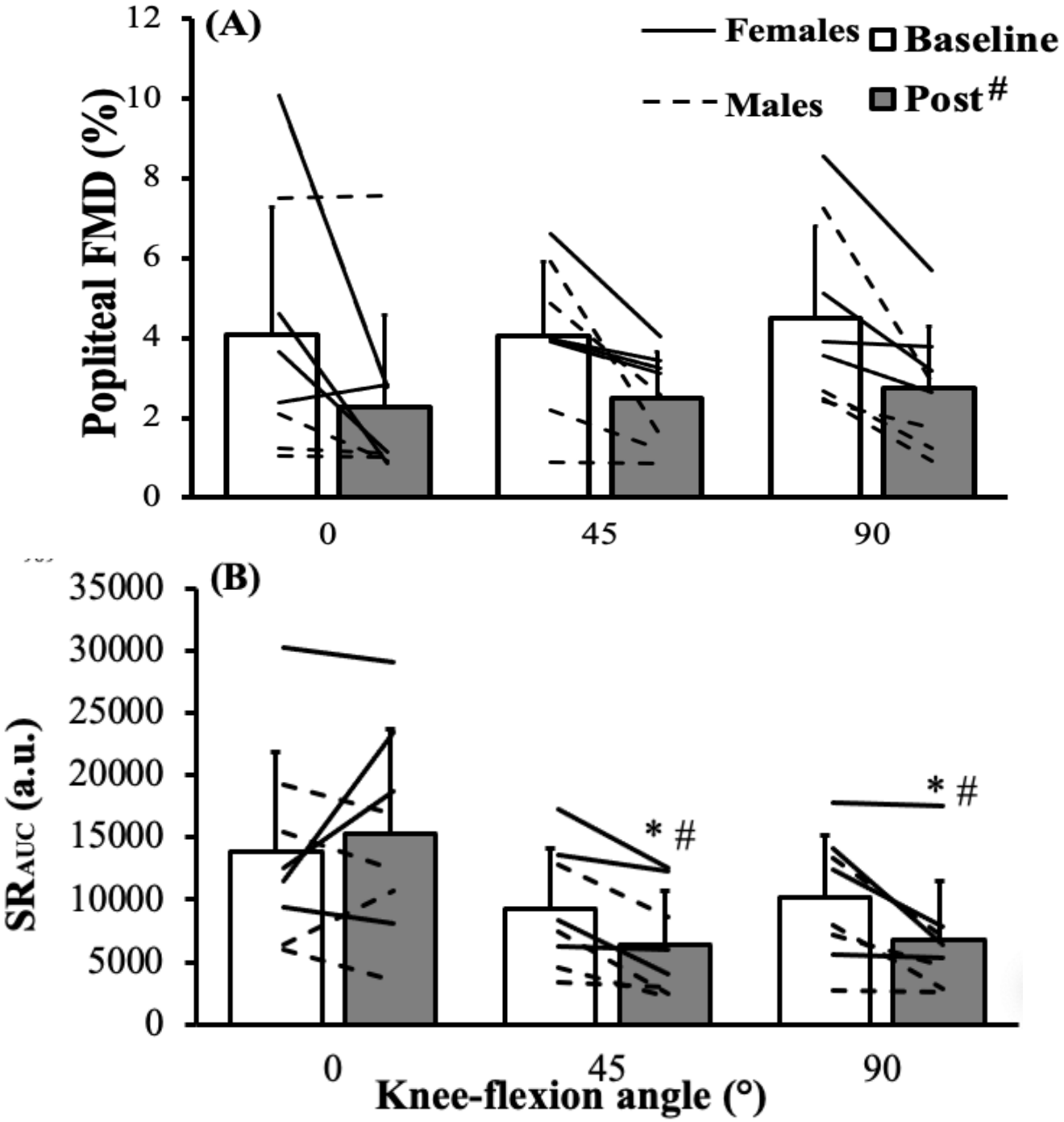


Figure 4-20. Group (Means ± SDs) and individual (lines) (A) popliteal flow-mediated dilation (FMD) and (B) shear rate area under the curve (SR_{AUC}) responses to prolonged sitting between knee-flexion angles (0°, 45°, 90°). There was no main effect of knee flexion angle (P=0.674) or an interaction effect (P=0.959) on relative FMD outcomes. However, there was a main effect of time (P<0.005). For SR_{AUC}, there was both a main effect of knee flexion angle (P=0.019), as well as an interaction effect (P=0.020), but no main effect of Time (P=0.139). Data were analyzed using a Knee Flexion Angle × Time repeated-measures analysis of variance (RM-ANOVA). a.u., arbitrary units. *, P<0.05 versus 0°; #, P<0.05 versus Baseline.

Chapter 5: Discussion

There were 2 main objectives to the current project: 1) to determine the acute effects of sitting at various knee-flexion angles (0°, 45°, 90°) on PBF and SR, and 2) to uncover the prolonged effects of these knee-flexion angles on PBF, SR, and relative FMD after ~3 h of sitting. The proposed hypotheses were that both acute and prolonged sitting-induced impairments on PBF, SR, and relative FMD would be the greatest after sitting at 90° knee-flexion angle, less after sitting at 45° of knee-flexion, and the least after sitting at 0° of knee-flexion. At the start of sitting (Baseline, Table 4-4), the predicted acute effects of knee-flexion angles were observed on PBF, but not SR. Furthermore, PBF during the 90° knee-flexion angle condition was the lowest compared to 0° and 45° knee-flexion angles. During prolonged sitting (Table 4-4), only main effects of knee-flexion angle and time, but no interaction effects, were observed on PBF and SR. Regardless of the time, PBF and SR at the 0° knee-flexion angle condition was the greatest compared to 45° and 90° knee-flexion angles. Regardless of the knee-flexion angle, PBF and SR were impaired after as little as ~0.5 h of sitting with popliteal FMD reductions evident after ~2.5 h. However, the sitting-induced reduction in relative FMD was similar for all knee flexion angles investigated. Therefore, these findings partially supported the proposed hypotheses.

5.1 Systematic Hemodynamic Responses to Sitting

Systematic hemodynamics were unchanged during sitting but varied versus the Prone Rest timepoint (Table 4-2). Compared to Prone Rest, HR was higher after 1.0 h of sitting, SV started to decrease after 0.5 h of sitting, DBP and MAP were higher after 2.5 h of sitting, and TVC was lower after 2.5 h of sitting. Due to the increased blood pooling in

the lower extremities during sitting, venous return reduces leading to a decrease in SV and MAP (Credeur et al., 2019; Paterson et al., 2022). Baroreceptors detect the acute reduction in MAP to cause reflex decreases in parasympathetic activity and increases in sympathetic activation to increase HR and further increase MAP (Credeur et al., 2019). What's more, the acute reduction MAP stimulates renin secretion through renin-angiotensin-aldosterone system and then drives an increase in MAP (Paterson et al., 2022).

The systematic hemodynamic responses to sitting in the present study are consistent with other studies. Two studies (Credeur et al., 2019; Vranish et al., 2017) using a 3 h prolonged sitting protocol in healthy young adults observed increased HR at the end of sitting compared to pre-sitting in the supine position. O'Brien et al. (2019) conducted a 3 h sitting protocol in healthy young adults and found that SV started to decrease after 0.5 h of sitting in females (51 ± 9 mL•beat^{-1}) and after 2 h of sitting in males (56 ± 10 mL•beat^{-1}) compared to a seated baseline period (females: 53±10 mL•beat^{-1}; males: 59±10 mL•beat^{-1}). However, CO did not change in response to sitting in either females or males (O'Brien et al., 2019). Kruse et al. (2018) using 4 h sitting protocol in young adults did not observe any changes to SBP. In contrast, O'Brien et al. (2019) observed increased DBP and MAP during 3-h sitting, and two other studies (Carter et al., 2019; Vranish et al., 2017) reported that MAP at 3 h of sitting was higher than that at pre-sitting in supine position. In addition, O'Brien et al. (2019) demonstrated that TVC at the end of sitting (females: 44±11 mL•min^{-1}•mmHg^{-1}; males: 44±10 mL•min^{-1}•mmHg^{-1}) was lower than that at the start of sitting (females: 48±13 mL•min^{-1}•mmHg^{-1}; males: 47±10 mL•min^{-1}•mmHg^{-1}).

5.2 Acute Effects of Knee-Flexion Angle on Popliteal Hemodynamics

Related to objective 1, there were acute effects of knee-flexion angles on PBF at the start of the sitting protocol (Baseline; Table 4-4). Specifically, baseline PBF with the legs straight (0° knee flexion) was greater than when the knees were flexed at 90°. Although two participants' legs were moved to ~60° to get a clear image of the popliteal artery when the knees were flexed at 90°, this might have underestimated the 'true' decrease in blood flow at this knee flexion angle. There was no significant difference in baseline popliteal SR (P=0.082) among three knee-flexion angles, but SR with the legs straight was higher than when sitting started at either 45° or 90° knee-flexion angle. At the start of a prolonged side-lying protocol, Walsh et al. (2017) reported similar PBF and SR in both the straight (0° knee-flexion angle: PBF: 65 ± 9 mL•min^{-1}; SR: 58 ± 6 s^{-1}) and bent legs (90° knee-flexion angle: PBF: 62 ± 9 mL•min^{-1}; SR: 54 ± 3 s^{-1}). However, their baseline measures were collected with participants in the supine position with both legs straight. Thus, the findings from the present study suggest that sitting with a more flexed knee angle (with a consistently flexed hip) may incrementally reduce resting PBF.

5.3 Prolonged Effects of Knee-Flexion Angle on Popliteal Hemodynamics

Specific to objective 2, there were prolonged effects of knee-flexion angles on PBF and SR (Table 4-4) following a 3 h bout of sitting. As with the baseline outcomes, PBF and SR remained higher throughout sitting during the 0° knee-flexion angle condition compared to both the 45° and 90° conditions. This implies that PBF responses to sitting might be impaired with a minimum 45° knee-flexion angle. Whether or not these findings can be extrapolated to other positions or other lower-limb arteries is unclear. However, Walsh et al. (2017) reported that compared to straight leg, both PBF

and SR were reduced in a 90° bent leg during 3 h of side-lying. McDaniel et al. (2012) observed that femoral blood flow also decreased when the knee was positioned from straight to bent (80° knee-flexion angle). Most previous studies that conducted prolonged sitting using an ~90° knee-flexion angle also reported a decrease in PBF (Kruse et al., 2018; Morishima et al., 2016, 2017; O'Brien et al., 2019; Restaino et al., 2015, 2016; Vranish et al., 2017) or superficial femoral blood flow (Carter et al., 2019; Tremblay, Stimpson, Murray, & Pyke, 2019) during sitting. Accordingly, even sitting with straight legs was associated with progressive declines in PBF and SR, which is likely to be attributed to sustained inactivity of the lower limbs and/or 'kinking' of arteries upstream from the popliteal or superficial femoral due to hip flexion (e.g., common femoral or profunda femoris arteries).

The knee-flexion angle might not solely cause the impairments of popliteal responses during prolonged sedentary activity. Baseline PBF and SR markedly decreased ~0.5 h after sitting at the assigned knee flexion angles (~45 minutes in the seated posture), which remained at these lower levels throughout the remainder of the sitting protocol (Table 4-4). These findings were consistent with two studies that reported PBF and SR started to decrease after ~1 h, compared to baseline measurements performed in the supine position (Morishima et al., 2016; Morishima, Tsuchiya, Padilla, & Ochi, 2020). Other studies have suggested that reductions in PBF and SR may occur even earlier (≤10 minutes) into a prolonged bout of sitting (Morishima et al., 2017; Restaino et al., 2016; Vranish et al., 2017). O'Brien et al. (2019) have also reported that PBF and SR start to decrease after 0.5 h of sitting, versus baseline measurements also assessed in the

seated position. As such, the duration of sitting may be more important than the knee-flexion angle on sitting-induced reductions in PBF and SR.

5.4 Prolonged Effects of Knee-Flexion Angle on Popliteal FMD Responses

Prolonged effects of knee-flexion angle were not observed on popliteal relative FMD (Figure 4-1A). However, the SR_{AUC} response (Figure 4-1B) following a 3 h bout of sitting was altered. Popliteal relative FMD decreased similarly after 2.5 h of sitting across all knee flexion angles. Sitting-induced impairments in FMD responses have been well established (Paterson et al., 2020). However, prolonged sitting-induced impairments (at ~90° knee-flexion angle) to lower-limb artery endothelial function can be prevented by a variety of interruption strategies (i.e., sedentary breaks), including aerobic exercise (Carter et al., 2019; Kruse et al., 2018; Morishima et al., 2016; Thosar, Bielko, Mather, et al., 2015), resistance activity (Climie et al., 2018), and standing (Kruse et al., 2018). Accordingly, longer, uninterrupted bouts of sitting is a significant factor related to lower-limb artery health deficits, especially reduced blood flow and shear stress as a primary factor underlying endothelial dysfunction (Morishima et al., 2016; Restaino et al., 2016; Walsh et al., 2017). However, the results from the present study challenge the view that the magnitude of sitting-induced reductions in PBF/SR produce corresponding decreases in popliteal FMD impairments.

There were interaction effects of knee-flexion angle and time on popliteal SR_{AUC} (Figure 4-1B) following prolonged sitting. Interestingly, at the 0° knee-flexion angle, there was no difference in SR_{AUC} between baseline and post-sitting FMD assessments. Similar post-sitting SR_{AUC} responses were observed after sitting in the 45° and 90° knee-flexion angles, which were both lower than sitting with straight legs. However, Walsh et

al. (2017) observed decreased popliteal relative FMD (assessed in the supine position) in a bent, but not a straight leg, following 3 h of side-lying without any corresponding between-leg differences in the SR_{AUC} response. The inconsistent findings could be because the different postures between Walsh et al. (2017; Figure 2-14, side-lying posture with 90° angle at the hip and the knee while extended at the contralateral hip and knee) and the present study (sitting posture with constant hip-flexion angles ~90°). Thus, repeated bouts of prolonged knee bent sitting at ≥45° might perturb popliteal endothelial function due to reduced SR_{AUC} occurring immediately distal to the site of arterial bending.

5.5 Limitations and Strengths

There are some limitations to recognize from this study. First, only 8 participants were included, but the inclusion of multiple knee-flexion angles and time points provided relatively robust results to investigate the impact of knee-flexion angles on popliteal artery responses to prolonged sitting. Based on the partial η^2 (0.130) of the PBF interaction, the estimated sample to achieve statistical power was 15 participants (assuming a two-tailed alpha 0.05 with a power value of 0.8). Based on the partial η^2 (0.077) of the popliteal SR interaction effect, the estimated sample was 24 participants (assuming a two-tailed alpha of 0.05 with a power value of 0.8). Second, this study required healthy young adults to sit for 3 h with minimal lower-limb movement on a laboratory chair. This overly strict study design is likely not an ecologically valid representation of how most people accumulate their habitual sitting time in a free-living environment. Based on objective measures of sedentary time, it has reported that most people tend to spend 1-2 hours during prolonged sedentary bouts and break up prolonged

sedentary periods every 20-30 minutes (O'Brien, Wu, Petterson, Frayne, & Kimmerly, 2022). However, this study could not distinguish between different sedentary postures (e.g., sitting versus lying) (O'Brien et al., 2022). Third, the present study did not figure out how often people would habitually sit at various knee-flexion angles in a free-living environment.

One major strength of this study is that this is the first study looking at the impacts of knee-flexion angle on lower-limb artery responses to an acute prolonged sitting. Although Walsh et al. (2017) also investigate the impacts of knee-flexion angle on popliteal artery health, they performed in a prolonged side-lying down with one leg bent at a 90° angle at the hip and the knee while the contralateral leg extended at a 0° angle at the hip and the knee (i.e., straight leg). This might not be able to independently observe the impacts of knee-flexion angle on popliteal artery health due to different hip-flexion angles. However, in the present study, we examined the impacts of knee-flexion angle on popliteal artery responses to sitting position always at 90° hip-flexion angle. Therefore, our study can find out the acute and prolonged impacts of knee-flexion angle on PBF and FMD responses to sitting independent of hip-flexion angle. Moreover, findings from this study may help inform future sedentary guidelines-related public health recommendations. Specifically, people consider sitting with straight legs and to not extend prolonged sitting time ≥45 minutes. In addition, a clinical implication from these results may suggest that wheelchair-bound individuals try to extend their legs when possible.

5.6 Perspectives

Due to the 90° hip-flexion angle during sitting with different knee-flexion angles in this study, further research could be performed while sitting at various hip-flexion (e.g., reclined versus upright sitting) and knee-flexion angles. This is because prolonged side-lying with both the hip and knee extended at 0° did not impair popliteal endothelial function (Walsh et al., 2017). Moreover, for all those studies (Kruse et al., 2018; Morishima et al., 2016, 2017, 2020a, 2020b; Restaino et al., 2015, 2016; Vranish et al., 2017; Walsh et al., 2017) with post-sitting measurements in supine position (i.e., both the hip and knee extended at 0°), PBF slightly increased at post-sitting compared to PBF at the end of sitting. In contrast, the present study found sitting-induced impairments of popliteal endothelial function with consistent ~90° hip-flexion angles were not impacted by knee-flexion angles. In addition, future studies aimed at determining the effects of knee-flexion angle on popliteal artery health during prolonged sitting specifically should also consider sitting durations that reflect habitual sedentary patterns, which are not currently known.

The population group in this study was a healthy, young active sample. The results might not be able to extrapolate to other populations with risk factors to develop lower-limb PAD. Taylor et al. (2020) conducted a 7 hour uninterrupted sitting (knee-flexion at 90°) in 20 adults with type 2 diabetes and measured superficial femoral artery blood flow and FMD at 0, 1, 3.5, 4.5, and 6.5–7 h. However, femoral blood flow and FMD did not change during sitting compared to 0 h. Due to limited studies investigating popliteal artery health during sitting in people with risk factors to develop lower-limb

PAD, future studies should investigate the effects of knee-flexion angle on popliteal artery health during prolonged sitting in risky population to develop PAD.

Furthermore, it is also worth studying what the most frequent knee-flexion angle used by people when they sit. The activPAL, a tri-axial inclinometer, has been frequently utilized to measure sedentary behavior among researchers (Edwardson et al., 2017) and has been validated to classify body posture (O'Brien, Wu, Petterson, Bray, & Kimmerly, 2022). To quantify more detailed sedentary postures, a dual-monitor method has been used that incorporates a second activPAL monitor either on the anterior torso (Bassett et al., 2014; Smits et al., 2018) or on the anterior tibia (Martin, Kenney, Pratt, & Granat, 2015). These methods exhibited high validity (classification accuracy >90%, sensitivity & specificity >80%) to broadly differentiate sitting from lying. In addition, placing 3 monitors on the torso, thigh and calf accurately distinguish between kneeling and squatting postures (sensitivity: 88-99%, specificality: 98-99%) in a semi-structured condition (Hendriksen, Korshøj, Skotte, & Holtermann, 2020). Accordingly, the use of multiple activPALs has shown promise in characterizing broad body positions (e.g., sitting versus lying versus kneeling). Three activPALs positioned simultaneously on the torso, thigh, and tibia may identify sitting versus lying (from torso and thigh monitors) and may predict knee-flexion angles during sitting (from thigh and tibia monitors) when people are conducting everyday activities of daily living.

Chapter 6: Conclusion

Sitting with straight legs may induce smaller acute and prolonged sitting-induced reductions in PBF than when the knees are flexed at 45° or 90°. However, long periods of uninterrupted sitting, even with straight legs, still causes reductions in PBF, SR and impairments to endothelial-dependent vasodilation. Future studies should recruit a larger sample and consider a more reflective study design to better characterize habitual sitting patterns in a free-living environment to create laboratory-based protocols that better reflect the amount of time (and frequency of breaks), as well as knee flexion angles that most people use during sitting periods.

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Appendix A: Health History Questionnaire

AGE:

PARTICIPANT I.D. (Completed by Research Team)

Instructions to calculate your body mass index (BMI). If your BMI is greater than 30 kg/m^2 you will not be eligible to participate in the study.

1. What is your approximate weight in kilograms? _____
   To convert from pounds to kilograms, multiply by 0.454
2. What is your approximate height in meters? _____
   To convert from inches to meters, multiple by 0.0254
3. Please calculate your approximate BMI:

$$\text{BMI (kg/m}^2) = \frac{\text{kg}}{(\text{m})*(\text{m})} = \text{\_\_\_\_\_} = \text{\_\_\_\_\_}$$

These questions are designed to determine your eligibility for the study. If you answer 'Yes" to any question you will not be able to participate in the study.

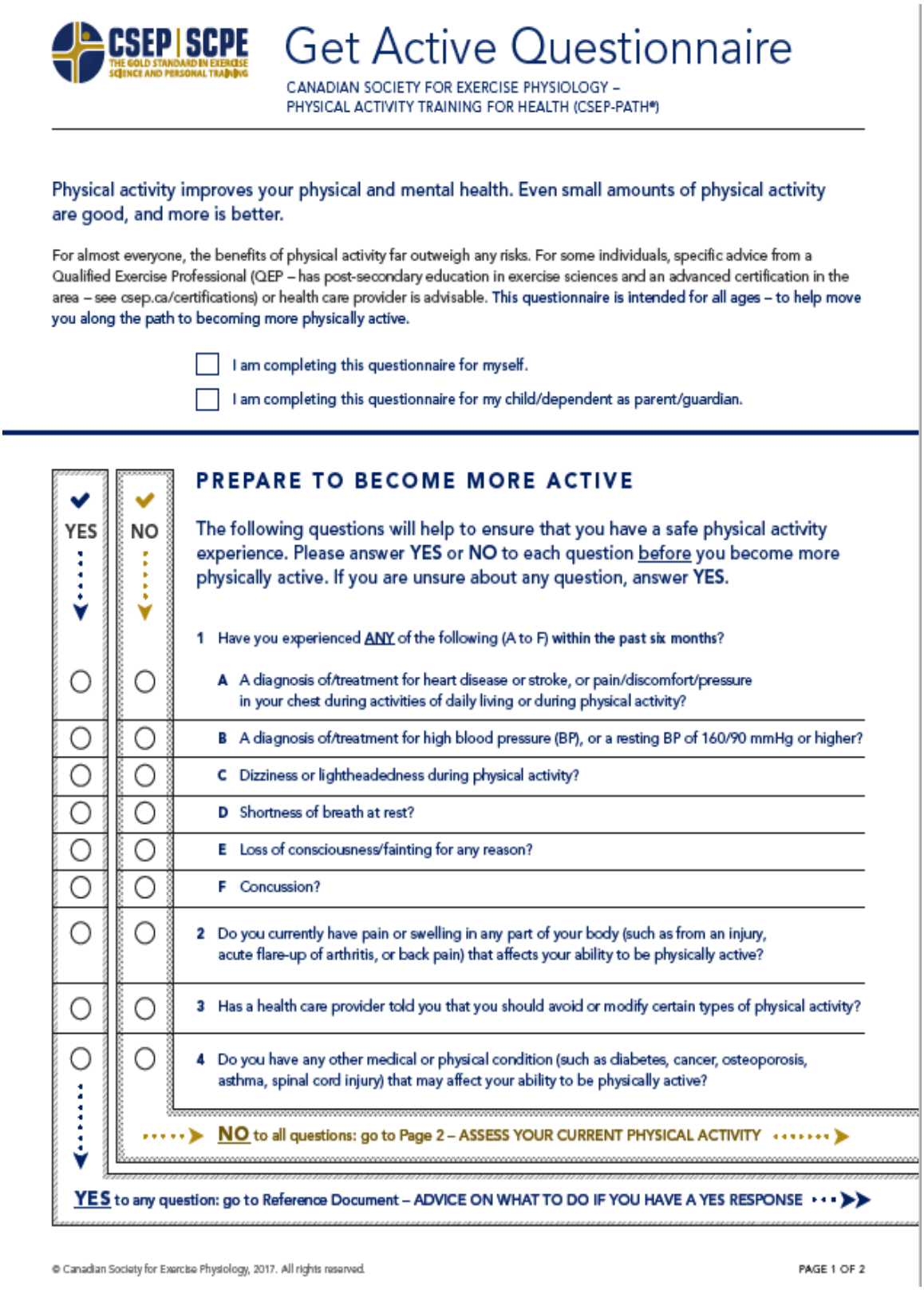
1.	Have you smoked or consumed any nicotine or marijuana containing products daily within the past 6 months?	Yes	No
2.	Are you younger than 18 or older than 30?	Yes	No
3.	Do you have a history of fainting or dizziness during sitting or standing?	Yes	No
4.	Are you allergic to Tegaderm™ clear, medical adhesive dressing?	Yes	No

For females only:

If you feel uncomfortable providing information regarding your menstrual or oral contraceptive pill phase to a male researcher, we can arrange for a female researcher to gather this information.

5.	Is there a possibility that you may be pregnant and/or are you breastfeeding?	Yes	No
6.	Do you have an irregular menstrual cycle? (i.e., not menstruating consistently within the first 7 days of a regular menstrual cycle).	Yes	No

Appendix B: CSEP-PATH Get Active Questionnaire



CSEP | SCPE
THE GOLD STANDARD IN EXERCISE SCIENCE AND PERSONAL TRAINING

Get Active Questionnaire

CANADIAN SOCIETY FOR EXERCISE PHYSIOLOGY –
PHYSICAL ACTIVITY TRAINING FOR HEALTH (CSEP-PATH®)

Physical activity improves your physical and mental health. Even small amounts of physical activity are good, and more is better.

For almost everyone, the benefits of physical activity far outweigh any risks. For some individuals, specific advice from a Qualified Exercise Professional (QEP – has post-secondary education in exercise sciences and an advanced certification in the area – see csep.ca/certifications) or health care provider is advisable. **This questionnaire is intended for all ages – to help move you along the path to becoming more physically active.**

☐ I am completing this questionnaire for myself.

☐ I am completing this questionnaire for my child/dependent as parent/guardian.

PREPARE TO BECOME MORE ACTIVE

The following questions will help to ensure that you have a safe physical activity experience. Please answer **YES** or **NO** to each question before you become more physically active. If you are unsure about any question, answer **YES**.

YES	NO	
		1 Have you experienced ANY of the following (A to F) within the past six months?
○	○	**A** A diagnosis of/treatment for heart disease or stroke, or pain/discomfort/pressure in your chest during activities of daily living or during physical activity?
○	○	**B** A diagnosis of/treatment for high blood pressure (BP), or a resting BP of 160/90 mmHg or higher?
○	○	**C** Dizziness or lightheadedness during physical activity?
○	○	**D** Shortness of breath at rest?
○	○	**E** Loss of consciousness/fainting for any reason?
○	○	**F** Concussion?
○	○	2 Do you currently have pain or swelling in any part of your body (such as from an injury, acute flare-up of arthritis, or back pain) that affects your ability to be physically active?
○	○	3 Has a health care provider told you that you should avoid or modify certain types of physical activity?
○	○	4 Do you have any other medical or physical condition (such as diabetes, cancer, osteoporosis, asthma, spinal cord injury) that may affect your ability to be physically active?

NO to all questions: go to Page 2 – ASSESS YOUR CURRENT PHYSICAL ACTIVITY

YES to any question: go to Reference Document – ADVICE ON WHAT TO DO IF YOU HAVE A YES RESPONSE

PAGE 1 OF 2

Get Active Questionnaire

ASSESS YOUR CURRENT PHYSICAL ACTIVITY

Answer the following questions to assess how active you are now.

1 During a typical week, on how many days do you do moderate- to vigorous-intensity aerobic physical activity (such as brisk walking, cycling or jogging)? ____ DAYS/WEEK

2 On days that you do at least moderate-intensity aerobic physical activity (e.g., brisk walking), for how many minutes do you do this activity? ____ MINUTES/DAY

For adults, please multiply your average number of days/week by the average number of minutes/day: ____ MINUTES/WEEK

Canadian Physical Activity Guidelines recommend that adults accumulate at least 150 minutes of moderate- to vigorous-intensity physical activity per week. For children and youth, at least 60 minutes daily is recommended. Strengthening muscles and bones at least two times per week for adults, and three times per week for children and youth, is also recommended (see csep.ca/guidelines).

GENERAL ADVICE FOR BECOMING MORE ACTIVE

Increase your physical activity gradually so that you have a positive experience. Build physical activities that you enjoy into your day (e.g., take a walk with a friend, ride your bike to school or work) and reduce your sedentary behaviour (e.g., prolonged sitting).

If you want to do **vigorous-intensity physical activity** (i.e., physical activity at an intensity that makes it hard to carry on a conversation), and you do not meet minimum physical activity recommendations noted above, consult a Qualified Exercise Professional (QEP) beforehand. This can help ensure that your physical activity is safe and suitable for your circumstances.

Physical activity is also an important part of a healthy pregnancy.

Delay becoming more active if you are not feeling well because of a temporary illness.

DECLARATION

To the best of my knowledge, all of the information I have supplied on this questionnaire is correct.
If my health changes, I will complete this questionnaire again.

I answered NO to all questions on Page 1

Sign and date the Declaration below

I answered YES to any question on Page 1

Check the box below that applies to you:

- ☐ I have consulted a health care provider or Qualified Exercise Professional (QEP) who has recommended that I become more physically active.
- ☐ I am comfortable with becoming more physically active on my own without consulting a health care provider or QEP.

Name (+ Name of Parent/Guardian if applicable) [Please print]	Signature (or Signature of Parent/Guardian if applicable)	Date of Birth

Date	Email (optional)	Telephone (optional)

With planning and support you can enjoy the benefits of becoming more physically active. A QEP can help.

☐ Check this box if you would like to consult a QEP about becoming more physically active.
(This completed questionnaire will help the QEP get to know you and understand your needs.)

Appendix C: Research Ethics Board Letter of Approval

Derek Kimmerly

From: ethics@dal.ca
Sent: February 12, 2021 9:45 AM
To: Derek Kimmerly
Cc: Research Ethics
Subject: REB # 2020-5278 Letter of Approval

DALHOUSIE UNIVERSITY
Research Services

Health Sciences Research Ethics Board
Letter of Approval

February 12, 2021

Derek Kimmerly
Health\School of Health and Human Performance

Dear Derek,

REB #: 2020-5278
Project Title: The impact of knee flexion angle during prolonged sitting on popliteal artery blood flow and endothelial-dependent function

Effective Date: February 12, 2021
Expiry Date: February 12, 2022

The Health Sciences Research Ethics Board has reviewed your application for research involving humans and found the proposed research to be in accordance with the Tri-Council Policy Statement on *Ethical Conduct for Research Involving Humans*. This approval will be in effect for 12 months as indicated above. This approval is subject to the conditions listed below which constitute your on-going responsibilities with respect to the ethical conduct of this research.

Effective March 16, 2020: Notwithstanding this approval, any research conducted during the COVID-19 public health emergency must comply with federal and provincial public health advice as well as directives from Dalhousie University (and/or other facilities or jurisdictions where the research will occur) regarding preventing the spread of COVID-19.

Sincerely,

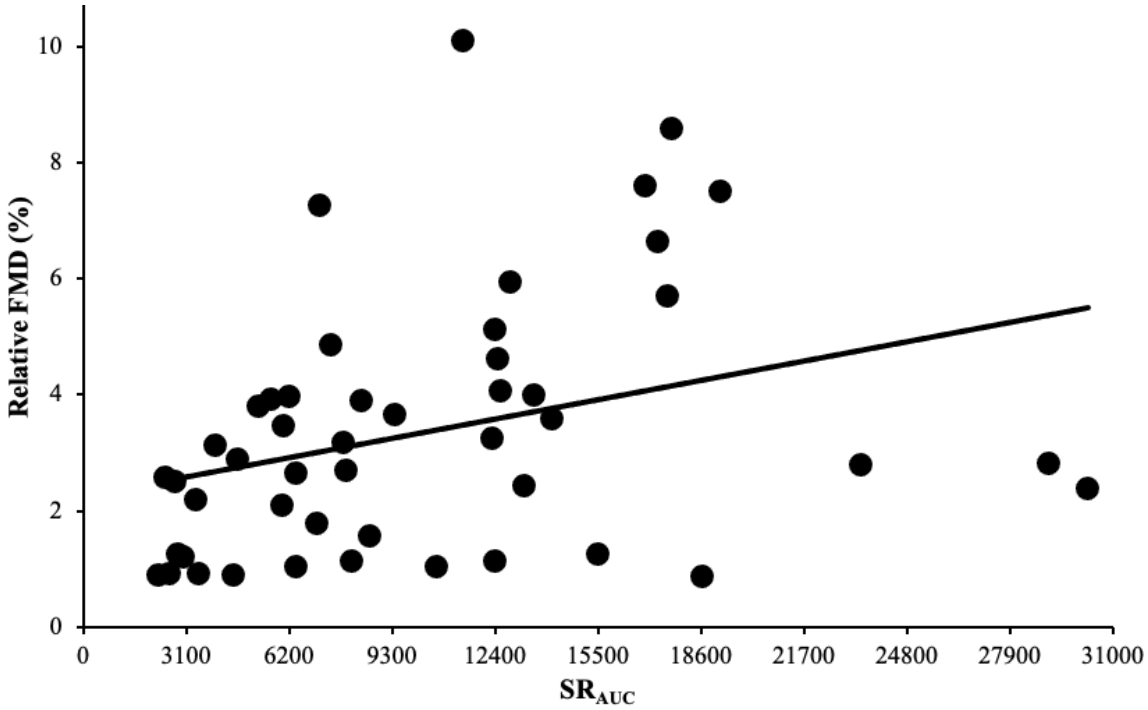
Dr. Lori Weeks, Chair

Post REB Approval: On-going Responsibilities of Researchers

Appendix D: Sleeping Time and Device Wear Time

Date	Time you fell asleep	Time you woke up	Other Exercise Activities (Start/End Times)
Example: July 20th, 2020	*2:30pm*	*3:30 pm*	*Bicycled (8:00-8:20am)*
July 20th, 2020	*11:15 pm*	*7:00 am*	Swimming (5:00-6:00 pm)

Appendix E: Supplemental Figure 1



Supplemental Figure 1. Scatterplot of the relationship (r=0.32, P=0.026) between relative popliteal flow-mediated dilation (FMD) and shear rate area under the curve (SR_{AUC}). The y-intercept was 2.26% (95% confidence interval: [1.12, 3.40]) and greater than zero (P<0.001). As such, relative FMD responses were not normalized to SR_{AUC}.