

CONTRIBUTION OF ARTERIOLAR DISTENSION TO RAPID ONSET VASODILATION FOLLOWING A  
SINGLE CONTRACTION

By

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## Abstract

Oxygen consumption of the skeletal muscle increases at the onset of exercise. This increase in oxygen consumption requires an increase in oxygen delivery to match the demand in order to sustain exercise. Increased in oxygen delivery is predominantly achieved through increased vascular conductance to the exercising muscle. The rapid onset vasodilation response at the onset of exercise is traditionally thought to be achieved solely through mechanisms that act to reduce arteriolar smooth muscle tone. Contracting smooth muscle exerts an inward force, with blood pressure exerting an outward acting force, so when the vascular smooth muscle relaxes, it exerts less force, resulting in vasodilation. The elastic structure of arterioles also exerts an inward acting elastic recoil force. However, due to its elastic properties, resistance vessel walls are distensible with an increase in arteriolar transmural pressure. Distension could further contribute to an increase in conductance for blood flow when transmural pressure is increased (distension-mediated vasodilation). Whether distension-mediated vasodilation is increased when there is more active vasodilation (reduced inward acting force) is unknown. The purpose of the current study was to test the hypothesis that distension mediated vasodilation is increased with increased active vasodilation magnitude. We tested this hypothesis by increasing forearm blood flow (FBF) via a lower (LO, 20% maximal voluntary contraction, MVC) or higher (HI, 80%MVC) magnitude of immediate active vasodilation in humans (n=21) with a single handgrip contraction. This was done with the arm in an above-heart position, and also when the arm was moved from an above-heart to below-heart position during a single contraction where gravity increased transmural pressure in the arterioles. The increase in forearm vascular conductance (FVC) that was attributed to distensibility was calculated by subtracting the FVC response in the

above-heart position from the FVC response in the below-heart position. When transmural pressure was increased in the below-heart position, FBF and FVC was increased in both LO and HI conditions. However, the increase in FVC attributed to distension ( $FVC_D$ ) was not different between LO and HI conditions. These results demonstrate that  $FVC_D$  is not affected by active vasodilation magnitudes created in this study.

## Co-Authorship

This thesis presents the work of Taylor Liu in collaboration with her supervisor, Dr. Michael E. Tschakovsky.

Manuscript: Transmural pressure-mediated arteriolar distension magnitude following forearm contraction below vs. above heart level: dependence on active vasodilation magnitude?

Taylor Liu's contributions were as follows:

- Collaborated with Dr. Tschakovsky in the conception and development of the study protocol
- Independently conducted literature review for rationalization of the current hypothesis and interpretation of the findings
- Responsible for data acquisition, data analysis, and statistical analysis
- Collaborated with Dr. Tschakovsky in the interpretation of the data
- Writing of the manuscript with revisions based on feedback provided by Dr. Tschakovsky

Dr. Michael T. Tschakovsky's contributions in addition to the aforementioned were as follows:

- Principle generation of the study protocol
- Continuous feedback regarding proposal and manuscript writing, and building arm position change mechanism

Emmanuel T. Zangio's contributions were as follows:

- Assisted pilot testing of the protocol
- Aided in data collection

Abby K. Zedic's contributions were as follows:

- Assisted pilot testing of the protocol
- Aided in data collection

Stuart P. S. Mladen's contributions were as follows:

- Assisted pilot testing of the protocol
- Aided in data collection

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## List of Abbreviations

[Ca<sup>2+</sup>]<sub>i</sub> – Intracellular Calcium Concentration

AA – Above-Above

AB – Above-Below

ATP – Adenosine Triphosphate

Ca<sup>2+</sup> – Calcium

Cl<sup>-</sup> – Chlorine

eNOS – Endothelium Nitric Oxide Synthase

FAPP – Forearm Arterial Perfusion Pressure

FBF – Forearm Blood Flow

FV – Forearm Volume

FVC – Forearm Vascular Conductance

FVC<sub>D</sub> – Forearm Vascular Conductance Attributed to Distension

HI – High Magnitude of Activation

K<sup>+</sup> – Potassium

K<sub>IR</sub> channel – Inward Rectifying Potassium Channel

LO – Low Magnitude of Activation

MAP – Mean Arterial Pressure

MVC – Maximal Voluntary Contraction

Na<sup>+</sup> – Sodium

Na<sup>+</sup>/K<sup>+</sup> ATPase – Sodium Potassium Pump

RGD – Arginine-Glycine-Aspartate Peptide Chain

VGCC – Voltage Gated Calcium Channel

$\Delta FAPP_{1cc}$  – Change in Forearm Arterial Perfusion Pressure During the First Cardiac Cycle

$\Delta FBF_{1cc}$  – Change in Forearm Blood Flow During the First Cardiac Cycle

$\Delta FVC$  – Change in Forearm Vascular Conductance

$\Delta FVC_{1cc}$  – Change in Forearm Vascular Conductance During the First Cardiac Cycle

$\Delta FVC_D$  – Change in Forearm Vascular Conductance Attributed to Distension

## Chapter 1 – Introduction

During exercise, there is an increased adenosine triphosphate (ATP) consumption in the contracting muscle and sustainable exercise requires that this ATP demand is matched primarily via mitochondrial oxidative phosphorylation. This in turn requires an increase in oxygen delivery to the exercising muscle via an increase in muscle blood flow to support increased mitochondrial oxygen demand (Gonzalez-Alonso et al., 2008; Musch et al., 1987; Radegran, 1997). The increase in exercising muscle blood flow occurs in two phases. First, there is an immediate and rapid but incomplete increase that plateaus by 5-7 seconds, followed by a further increase starting at 15-20 s and reaching a new steady state (Saltin et al., 1998; Saunders et al., 2005). Increasing exercising muscle blood flow is accomplished primarily via dilation of muscle microvascular bed arterioles (resistance vessels) via reductions in arteriolar smooth muscle tone, with some contribution of elevated arterial blood pressure at higher exercise intensities (Joyner & Casey, 2015).

Feed forward vasodilatory mechanisms associated with muscle activation and mechanical compression of arterioles contribute to the immediate, rapid increase in exercising muscle blood flow (Clifford et al., 2006; Clifford & Tschakovsky, 2008; Crecelius et al., 2013). Feedback mechanisms linking metabolic demand and delivery of oxygen are thought to determine the steady state maintenance of blood flow in proportion to metabolic demand (Crawford et al., 2006; Gladwin, 2006). Simultaneously, sympathetic neural vasoconstrictor activity is also present, opposing the relaxation of arteriolar smooth muscle cells (Buckwalter & Clifford, 2001; Hamann et al., 2002), but the vasodilator mechanism effects, combined with exercising muscle substances that blunt sympathetic vasoconstriction, a phenomenon termed functional

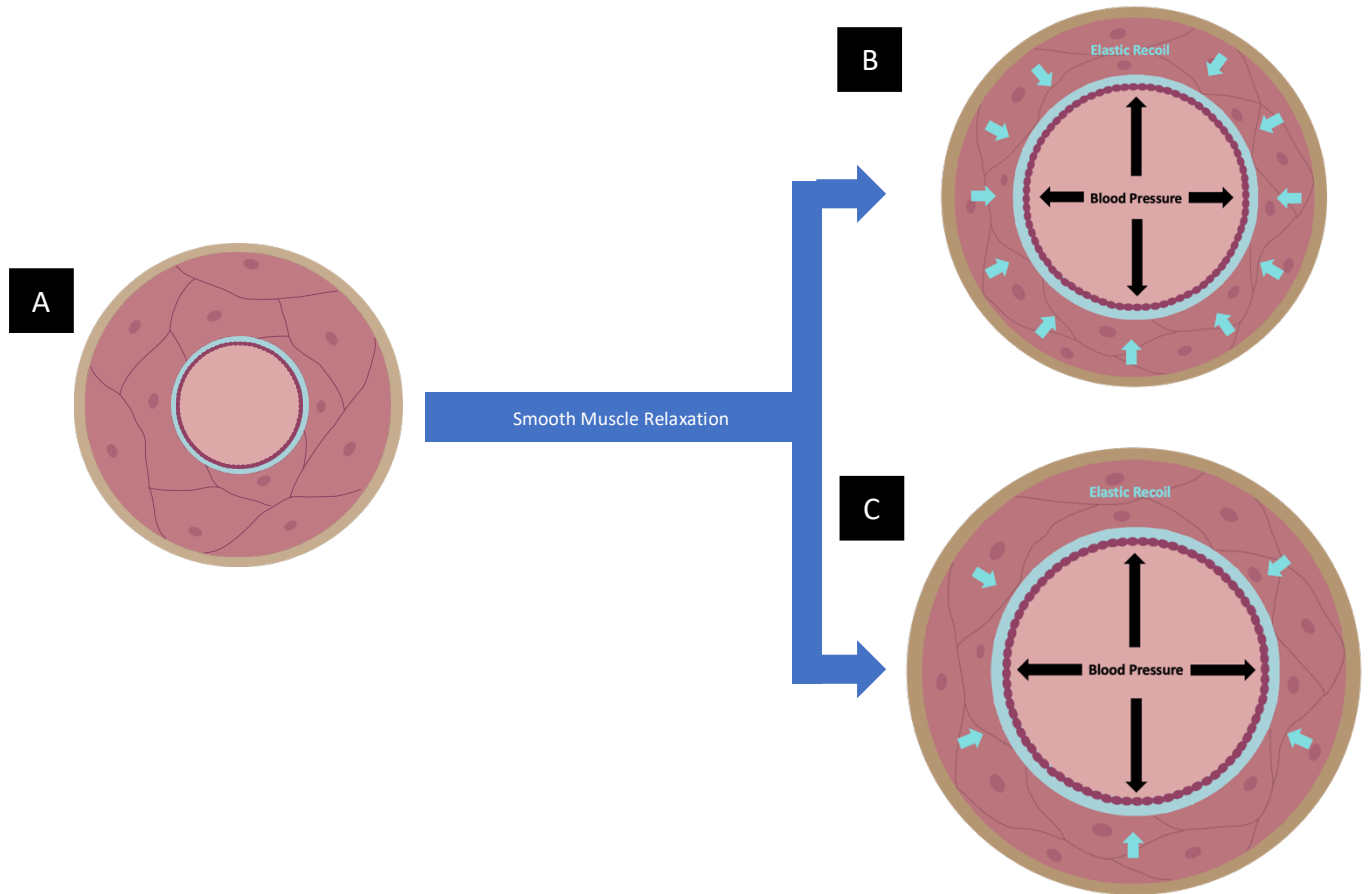
sympatholysis (Gliemann & Carter, 2018; Remensnyder et al., 1962) outweigh sympathetic vasoconstriction and allow vasodilation-mediated increases in oxygen delivery to match increased metabolic demand.

At present, only active vasodilation (arteriole dilation resulting from mechanisms reducing smooth muscle tone) is considered in determining oxygen delivery:demand matching in exercise. However, resistance vessels can be mechanically distended with increases in transmural pressure (Jasperse et al., 2015; Koller & Bagi, 2004). Findings from our laboratory support the hypothesis that added distension of resistance vessels when transmural pressure is increased – henceforth referred to as “passive” vasodilation – may contribute to the observed increase in muscle blood flow in the exercising forearm when it is repositioned from a lower (above heart) to higher (below heart) transmural pressure environment (Lynn et al., 2020; Walker et al., 2007). Specifically, Walker et al. (2007) demonstrated an increase in forearm blood flow during rhythmic forearm exercise when the forearm was moved from above to below heart level. This increase in forearm blood flow was more than what could be explained by the increase in arterial perfusion pressure with limb repositioning, resulting in an increased calculated forearm vascular conductance. Considering the arm was moved from the low to high perfusion pressure environment during a contraction in steady state exercise where vasodilatory effects had stabilized, the immediate increase in conductance that was observed upon release of contraction when the forearm was moved below heart level must have been due to a mechanical distension of resistance vessels.

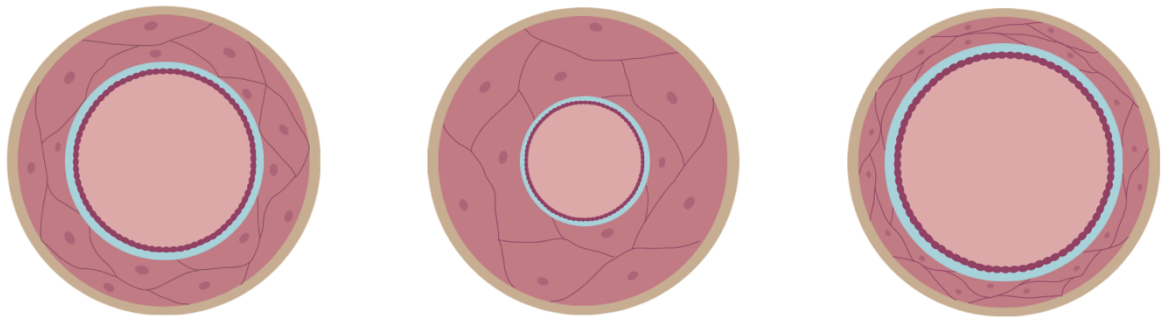
This excess forearm blood flow that could not be accounted for by the increased perfusion pressure or active vasodilatory signals when moving the forearm from above to below heart level during contraction was also observed by Lynn et al. (2020) in the response to a single forearm muscle contraction. Thus, it appears that an increase in local arteriolar transmural pressure of ~30 mmHg, as occurred in these studies, can create significant distension in actively vasodilated arterioles, and this occurs even with arterioles that have just initiated active vasodilation in response to muscle contraction.

This arteriolar “distensibility” is the arteriole wall’s capacity to stretch in response to an increase in pressure from the inside and reflects the vessel wall contributors to inward acting recoil force. Figure 1 illustrates the hypothesis that for a given reduction in smooth muscle tone (active vasodilation), an increase in transmural pressure may create a greater magnitude of dilation of resistance vessels. The cross-sectional area of a resistance vessel is dependent on the balance between inward and outward acting forces. Inward acting forces are derived from 1) vascular smooth muscle contraction and 2) vessel wall recoil force that stem from the properties of the vessel’s extracellular matrix’ structural components – collagen and elastin. The outward acting force is arteriolar blood pressure. Transmural pressure is the pressure in the lumen of the vessel relative to the pressure outside the vessel, which is referenced as 0 in the absence of any added external compressive forces, so arteriolar blood pressure represents transmural pressure. Thus, relaxation of smooth muscle tone reduces inward acting force resulting in “active” vasodilation until inward acting recoil force increases as the vessel walls are stretched, such that inward acting recoil force and smooth muscle tone once again balance outward acting force of arteriolar blood pressure.

Although findings by Walker et al. (2007) and Lynn et al. (2020) indicate arteriolar distension as a contributor to increased exercising muscle blood flow when local transmural pressure is increased, it is not known whether the magnitude of rapid onset vasodilation observed in response to a single forearm contraction is dependent on how “distensible” resistance vessels are. The purpose of this study is therefore to test the hypothesis that passive distension-mediated dilation with increased transmural pressure is greater when active vasodilation is greater. If arteriolar distensibility contributes to total vasodilation magnitude, its quantification could provide an important index for future research exploring the contributions of arteriolar distention in oxygen delivery demand matching across a range of active vasodilation.



**Figure 1.** A) Arteriole with basal vasomotor tone B) Arteriole with less distensibility for a given smooth muscle relaxation resulting in a small increase in luminal cross-sectional area C) Arteriole with greater distensibility for the same smooth muscle relaxation resulting in a larger increase in luminal cross-sectional area



**Figure 2.** Changes in vascular smooth muscle tone alter luminal cross-section. From left to right: arteriole with basal vasomotor tone; arteriole with contracting vascular smooth muscle, constricting the arteriole and decreasing the luminal cross-sectional area; arteriole with relaxed vascular smooth muscle, dilating the arteriole resulting in increased luminal cross-sectional area

## Chapter 2 – Literature Review

In this section I will introduce rapid vasodilation at the onset of exercise, discuss mechanisms responsible for this vasodilation, and provide a more detailed assessment of recent findings regarding transmural pressure mediated resistance vessel distension as a contributor to the magnitude of vasodilation observed in an exercising muscle vascular bed.

### ***Rapid Onset Vasodilation***

At the onset of exercise, there is an immediate increase in muscle blood flow to support the increase in metabolic demand. This rapid response has been shown to occur within the first second following a single contraction (Credeur et al., 2015; Tschakovsky et al., 1996), and even before the release of a 1 second contraction (Turturici et al., 2012). As muscle blood flow is a product of both vascular conductance and the pressure gradient for flow across a capillary network, increases in flow are achieved through an increase in conductance, a greater pressure gradient, or both. A larger pressure gradient across the muscle bed is thought to be achieved through the muscle pump effect (Tschakovsky et al., 1996), where contraction-induced compression acts to empty venules. This would increase the pressure gradient from the arterial side to the venous side by reducing the pressure in the venous compartment. However, there is evidence that the muscle pump does not contribute to exercise hyperemia without vasodilation. In a study conducted by (Hamann et al., 2003), dog hindlimb arterioles were maximally dilated via adenosine infusion before and during treadmill exercise. At the onset of exercise, hindlimb blood flow did not increase; conversely blood flow did increase with saline infusion. Similarly, infusion of high dose potassium to cause steady state vasoconstriction abolished contraction induced hyperemia (Hamann et al., 2004). Further, peak hyperemic response following a

contraction typically occurs 3-4 cardiac cycles post-contraction (Kirby et al., 2007). If the muscle pump was a main contributor to the rapid hyperemic response, its effect would be maximized during the first cardiac cycle (Clifford & Tschakovsky, 2008), indicating that vasodilation plays a larger role. Traditionally, vasodilation is thought to be achieved through the relaxation of the smooth muscle that lines the vessel wall. Mechanisms that act to relax the vascular smooth muscle at the onset of exercise have been linked to muscle activation via skeletal muscle fibre potassium ( $K^+$ ) release (Armstrong et al., 2007; Crecelius et al., 2013; Mohrman & Sparks, 1974b) or the compression of arterioles by the force of a muscle contraction (Clifford et al., 2006; Kirby et al., 2007; Mohrman & Sparks, 1974a).

### ***Skeletal Muscle Activation-Dependent Mechanisms of Rapid Onset Vasodilation***

This section will assess the evidence for rapid onset vasodilation mechanisms initiated by the activation of skeletal muscle contraction.

#### ***Skeletal Muscle Fibre $K^+$ Release in Response to Skeletal Muscle Activation***

A proposed mechanism for the rapid onset vasodilation at the start of exercise is via skeletal muscle action potential-mediated  $K^+$  release into the interstitial space. Interstitial  $K^+$  increases from rest to exercise (Frandsen et al., 2000; Nordsborg et al., 2003), and increases in proportion to exercise intensity (Green et al., 2000; Juel et al., 2000).  $K^+$  is a vasodilator at concentrations below 20 mM, which are observed in the interstitial space of exercising muscle (Quayle et al., 1997). Infusion of  $K^+$  at physiological concentrations has been shown to increase

muscle blood flow at rest (Juel et al., 2007). This study also demonstrated that resting leg blood flow increased with increasing infusion rates.

Activated skeletal muscle fibres are a source of interstitial  $K^+$  that is released during the repolarization phase of an action potential (Clifford, 2007; Hopkins, 2006) and acts on the smooth muscle cells in the tunica media of arterioles to cause active vasodilation (Siegel et al., 1992). The amount of  $K^+$  released from the muscle is dependent on how much the skeletal muscle fibres are activated (Green et al., 2000; Sejersted & Sjogaard, 2000). Armstrong et al. (2007) demonstrated a significant decrease in transverse arteriolar dilation response with skeletal muscle fibre voltage-gated  $K^+$  channel inhibition across a large range of stimulus frequencies. These data demonstrate that inhibition of skeletal muscle fibre  $K^+$  release into the interstitial space blunts the rapid vasodilatory response to a contraction. Further, Burns et al. (2004) demonstrated similar results while measuring smooth muscle cell membrane potential. In isolated cremaster muscle, superfusate of 20 mM of potassium chloride induced hyperpolarization of the smooth muscle cell membrane. The hyperpolarization of the smooth muscle cell by  $\sim -10$  mV preceded dilation of the arterioles. Following this initial observation, small spikes in smooth muscle membrane potential and small oscillations in arteriolar diameter occurred concurrently; thus, demonstrating a tight association between muscle activation and dilation. Hyperpolarization and increased dilation were attenuated with  $Na^+/K^+$  ATPase and  $K_{IR}$  channel blockers, respectively.

$K^+$  released from skeletal muscle causes vasodilation via inward rectifying  $K_{IR}$  channels in the smooth muscle (Crecelius et al., 2014; Hearon & Dinunno, 2017; Quayle et al., 1996; Quayle et al., 1997; Zaritsky et al., 2000). When  $K^+$  is released from the exercising muscle, it accumulates in the interstitial space and hyperpolarizes the smooth muscle cell membrane. This

hyperpolarization is achieved through two mechanisms. Firstly, increased interstitial potassium activates  $\text{Na}^+/\text{K}^+$ -ATPase pumps in the smooth muscle membrane (Quayle et al., 1997) which have a net positive charge on the outside of the cell, leading to hyperpolarization. Secondly, increased interstitial potassium creates a rightward shift of the current-voltage relationship for  $\text{K}_{\text{IR}}$  channels (Quayle et al., 1997). A rightward shift means that for a given flow of potassium into the cell, a higher smooth muscle membrane potential is required. Ultimately, this leads to an increase in the resting outward  $\text{K}^+$  current through  $\text{K}_{\text{IR}}$  channels leading to hyperpolarization (Quayle et al., 1997). Smooth muscle membrane hyperpolarization closes voltage-gated  $\text{Ca}^{2+}$  channels, reducing  $\text{Ca}^{2+}$  ion flow into the cell (Jackson, 2005; Ledoux et al., 2006; Quayle et al., 1997). As calcium is essential to muscle contraction (Bárány, 1996), reducing intracellular calcium leads to smooth muscle relaxation and active vasodilation (Jackson, 2005).

In a more recent animal study, Armstrong et al. (2007) observed a significant increase in dilation of transverse arterioles of hamster cremaster skeletal muscle one second after a range of stimulus frequencies. They also demonstrated an attenuation in rapid onset dilation of the observed arterioles with the blockade of  $\text{Na}^+/\text{K}^+$  ATPase pumps and  $\text{K}_{\text{IR}}$  channels in addition to the aforementioned voltage-gated  $\text{K}^+$  channels in the skeletal muscle cell membrane, respectively. These data convey the important role of  $\text{K}^+$  in mediating vasodilation.

Crecelius et al. (2014) demonstrated  $\text{K}_{\text{IR}}$  channel activation accounts for ~50% of the increase in forearm blood flow at the onset of rhythmic handgrip exercise. In this study, saline (control), barium chloride ( $\text{K}_{\text{IR}}$  channel inhibitor), and ouabain ( $\text{Na}^+/\text{K}^+$  ATPase pump inhibitor) were infused into the forearm prior to five minutes of forearm contractions. Trials with barium chloride infusion – with and without accompaniment of ouabain – demonstrated a significant

reduction in forearm vascular conductance and blood flow compared to control. Co-infusion of barium chloride and ouabain did not differ significantly from barium chloride infusion alone. These data effectively demonstrate that  $K_{IR}$  channels are important regulators in rapid onset vasodilation.

Similarly, a study conducted by Terwoord et al. (2020) demonstrated (a) increased forearm vascular conductance with increased dose of infused potassium chloride – an exogenous source of  $K^+$  and  $K_{IR}$  channel activator, (b) that infusion of barium chloride reduced forearm vascular conductance by ~70% with the highest dose administered, and (c) inhibition of  $K_{IR}$  channels reduced forearm vascular conductance by 180% during the first cardiac cycle after an increase in work load. This reduction was less pronounced with subsequent contractions and the same pattern was seen from rest to exercise. These researchers further explored the relationship between  $K_{IR}$  channel activation and greater muscle fibre recruitment independent of exercise intensity. Greater muscle recruitment for a given exercise intensity would result in more  $K^+$  being released from skeletal muscle, providing an endogenous source of interstitial  $K_{IR}$ . The high muscle fibre recruitment condition exhibited a 25% increased  $K_{IR}$  efflux from the skeletal muscle compared to the low fibre recruitment condition. Despite this increase in interstitial  $K_{IR}$ , there was no significant change in forearm vascular conductance with barium chloride blockade; conversely, the control condition did demonstrate a significant change in conductance. These data demonstrate that  $K_{IR}$  channels play a large role in the mediation of rapid onset vasodilation, especially at the initiation of exercise or increased exercise demand from a steady state.

It has been demonstrated in knockout mice with gene deletion of two subtypes of  $K_{IR}$  channels that these channels are essential in  $K^+$  mediated vasodilation (Zaritsky et al., 2000).

Zaritsky et al. (2000) demonstrated an inability in knockout mice who did not express  $K_{IR}$  channel subtypes 2.1 and 2.2 for cerebral arteries to dilate in response to increased extracellular  $K^+$ . Conversely, wild type mice cerebral arteries with gene expression of all  $K_{IR}$  channel subtypes did demonstrate dilation in response to extracellular  $K^+$ . This study further demonstrated that across a range of membrane potentials from -100 mV to +40 mV that wild type mice demonstrated a current-voltage curve for  $K_{IR}$  channels that closely resembles the normal curve in adult rat models (Quayle et al., 1993) and porcine models (Quayle et al., 1996). Knockout mice demonstrated no current across the entire membrane potential range.  $K_{IR}$  channels are activated by extracellular  $K^+$  to cause active vasodilation. The inability of knockout mice to display any vasodilation in response to increase extracellular  $K^+$  demonstrate that without  $K_{IR}$  channels,  $K^+$  is unable to elicit a vasodilatory response.

#### *Compression of Arterioles Resulting From Force of Skeletal Muscle Contraction*

It is known that during muscle contractions, intramuscular pressure increases with increasing contraction intensity (Sadamoto et al., 1983; Sejersted et al., 1984), subsequently creating an increase in extravascular pressure and a decrease in transmural pressure (Mohrman & Sparks, 1974a). This increased extravascular pressure mechanically compresses the vasculature that exists along the muscle fibres. Both vascular smooth muscle and the endothelium are known to be mechanosensitive (Clifford & Tschakovsky, 2008; Davis & Hill, 1999; Koller & Bagi, 2002). In response to changes in transmural pressure, smooth muscle elicits changes in myogenic tone (Davis & Hill, 1999); meanwhile, endothelial cells cause vasodilation in response to changes in intraluminal shear stress (Koller & Bagi, 2002; Kuo et al., 1990). Immediate mechanosensitive

vasodilation – defined as vasodilation occurring within one second of compression or contraction release – has been demonstrated in animal models (Clifford et al., 2006; Turturici et al., 2012), and in the human forearm (Kirby et al., 2007; Tschakovsky et al., 2004; Tschakovsky et al., 1996).

Compression induced vasodilation was thought to increase skeletal muscle blood flow at the onset of exercise through the muscle pump effect (Tschakovsky et al., 1996), by which downstream venous pressure is reduced, creating a larger pressure gradient for blood flow. However, it seems that immediate hyperemia relies on smooth muscle relaxation rather than the muscle pump (Clifford & Tschakovsky, 2008; Hamann et al., 2004). Clifford et al. (2006) clearly shows that both the vascular smooth muscle and endothelial lining contribute to compression induced vasodilation. In this study, rat soleus feed arteries were isolated in vitro and compressed with 600 mmHg pressure pulses to mimic the extravascular pressure experienced by arteries during maximal contractions in vivo. These researchers demonstrated a significant increase in immediate dilation to different compression patterns (a single 1-sec compression, a single 5-sec compression, or five 1-sec compressions separated by a period of 1-sec). When the endothelium was removed, all compression patterns continued to demonstrate an immediate increase in dilation despite the response being attenuated by ~50% compared to trials with the endothelium intact. The attenuation of response in the absence of the endothelium was of similar magnitude across compression patterns. This suggests that compression pattern does not alter the relative contribution of smooth muscle and the endothelium to compression-mediated vasodilation. It also suggests that there may be mechanosensitive mechanisms exclusive to smooth muscle and the endothelium in eliciting vasodilation in response to compression.

Resistance vessels respond to increases in intraluminal pressure with constriction; meanwhile, decreases in intraluminal pressure as seen with mechanical compression results in vasodilation (Davis & Hill, 1999). The myogenic mechanisms by which the vasoconstriction response to an increase in pressure is well described; conversely, the inverse remains largely unknown. It is thought that the myogenic vasodilation response is achieved, at least in part, via attenuation of the mechanisms by which myogenic vasoconstriction is achieved. The myogenic response to pressure induced mechanical changes is achieved through mechanosensitive ion channels on the vascular smooth muscle and cell surface integrin binding to ligands in the extracellular matrix (Davis & Hill, 1999; Hill et al., 2001). Mechanosensitive ion channels are thought to be regulated through deformation signals via the cytoskeleton of the vascular smooth muscle cell (Davis & Hill, 1999). Increases in intraluminal pressure activate nonselective cation channels that may either increase in influx of  $\text{Na}^{2+}$  or  $\text{Ca}^{2+}$ , efflux of  $\text{Cl}^-$ , or reduced  $\text{K}^+$  efflux (Davis & Hill, 1999). Depolarization of the cell membrane is ultimately achieved. This in turns activates voltage gated calcium channels to allow more calcium entry into the cell, leading to an increase in myogenic tone (Davis & Hill, 1999; Hill et al., 2001; Hong et al., 2020).

Additionally, integrins are thought to play a considerable role in the myogenic response (Davis, 2012; Hong et al., 2020; Osol, 1995). Integrin activation ultimately also regulates intracellular calcium concentrations and thus myogenic tone. Vascular smooth muscle cell surface integrins bind to vasoactive ligands in the extracellular matrix, such as ligands containing an arginine-glycine-aspartate (RGD) peptide chain (D'Angelo et al., 1997; Davis et al., 2001). D'Angelo et al. (1997) demonstrated that RDG-integrin binding induced a vasodilatory response, and that dilation was associated with a reduction in intracellular calcium concentration.

Regulation of intracellular calcium is achieved through specific integrin binding that modulates voltage gated calcium channels (Hill et al., 2001).

Similarly, the endothelium is also mechanosensitive and causes dilation in response to intraluminal shear stress (Clifford & Tschakovsky, 2008; Kuo et al., 1990), and deformation of the endothelial cells upon compression (Koller & Bagi, 2002; Sun et al., 2001). This dilation is thought to be achieved through nitric oxide release, a potent vasodilator (Gilligan et al., 1994; Kuo et al., 1991; Trinity et al., 2012; Wray et al., 2011). Sun et al. (2001) demonstrated that endothelial deformation via increased extracellular pressure from 0 to 75 mmHg on isolated arterioles resulted in increased nitric oxide release and increased vasodilation. Removal of the endothelium and inhibition of endothelium nitric oxide synthase (eNOS) significantly reduced dilation in response to increased extracellular pressure, respectively.

In summary, exercise hyperemia occurs at the onset of exercise in response to a single contraction (Credeur et al., 2015; Tschakovsky et al., 1996) and can even be observed before the end of a 1 second contraction (Turturici et al., 2012). Rapid onset vasodilation is initiated by skeletal muscle activation through skeletal muscle action potential mediated potassium release and mechanical deformation of resistance vessels via muscular contraction. Upon activation, skeletal muscle releases potassium into the interstitial space and causes hyperpolarization of both the endothelium and the vascular smooth muscle cell to cause vasodilation (Siegel et al., 1992). Mechanical deformation of both the endothelium and the vascular smooth muscle by contracting skeletal muscle is sensed via several mechano-transduction pathways including cell-surface integrin-ligand binding and mechanosensitive ion channels (Koller & Bagi, 2002; Osol, 1995). Through intracellular signalling pathways, mechanoreceptors on the endothelium act to

release nitric oxide. Meanwhile, mechanoreceptors in the vascular smooth muscle function to reduce intracellular calcium levels to reduce myogenic tone (Davis & Hill, 1999). Mechanisms for rapid onset vasodilation reduce vascular smooth muscle tone to achieve increased vascular conductance are well studied. However, the mechanical distension of arterioles as a potential contributor to vasodilation remains unknown. The following section will introduce and explore passive mechanical distension of arterioles as a contributor to vasodilation for a given vascular smooth muscle relaxation.

### ***Passive Distension-Mediated Vasodilation***

While active vasodilation is the means by which forearm vascular conductance can be altered, resistance vessel walls are elastic and can therefore be distended with increased transmural pressure (Borgstrom et al., 1981; Davis & Sikes, 1990; Jasperse et al., 2015). This distension would also increase the conductance for blood flow. In this section I will review evidence that such a distension response can contribute substantially to the increase in blood flow in exercising muscle when arterial blood pressure is increased, what characteristics of vessel wall structure could impact how much distension occurs for a given increase in arterial blood pressure, and what potential implications resistance vessel distensibility might have for understanding exercising muscle oxygen delivery demand matching and how it may be compromised or enhanced.

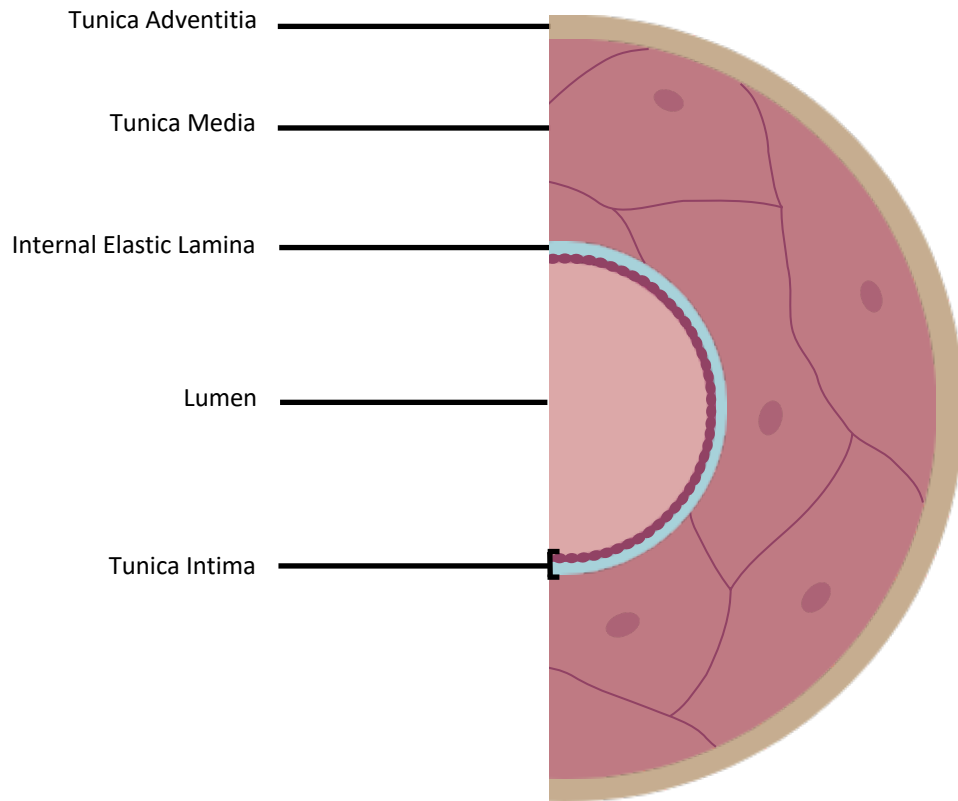
### ***Increasing Arterial Transmural Blood Pressure – Impact on Exercising Muscle Blood Flow***

Research from our laboratory has demonstrated resistance vessel capacity for passive vasodilation in response to increases in transmural pressure when moving the forearm from an above to a below heart position. This passive vasodilation is indicated by observed increases in blood flow beyond what could be explained by the change in perfusion pressure when active vasodilatory signals are held constant.

Walker et al. (2007) had participants perform submaximal steady state handgrip exercise (30% MVC with a 2 sec contraction/2 sec relaxation duty cycle) with the experimental arm positioned above heart level. After 5 minutes of exercise, during a 2 second contraction, the arm was swiftly lowered into a below heart position. The repositioning of the arm increased hydrostatic pressure contributing to local forearm arterial blood pressure. As the arm was repositioned during a contraction, metabolic demand and active vasodilatory signals associated with muscle activation remain constant while the local transmural pressure and perfusion pressure increased. Thus, the instantaneous effect of this on forearm blood flow could be observed. It was observed that the first cardiac cycle following arm position change showed an immediate increase in forearm blood flow. This increase in blood flow was out of proportion to the increase in local perfusion pressure, resulting in an immediate increase in calculated local vascular conductance. This increase in vascular conductance was attributed to the distension of arterioles in response to the increase in transmural pressure. However, subsequent cardiac cycles demonstrated rapid restoration of vascular conductance while blood flow remained elevated, likely demonstrating typical myogenic vasoconstriction response to increases in transmural pressure (Davis & Hill, 1999).

Further, Lynn et al. (2020) conducted a similar study in which a single 2 sec forearm handgrip contraction was initiated in an environment with lower forearm arterial perfusion pressure and lower forearm transmural pressure (above heart level). During the contraction, the arm either remained in the above heart position or the arm was lowered into a below heart position. The below heart position created an environment of higher arterial perfusion pressure and higher transmural pressure. When the contraction was released in the below heart position, it resulted in a greater post-contraction forearm blood flow and forearm vascular conductance during the first cardiac cycle. These researchers did attribute an increase in hydrostatically induced perfusion pressure due to arm position change as a small contributing factor to the observed increased flow. It was assumed that the contraction emptied the venules, creating a venous pressure of 0 mmHg. This assumption maximizes the contribution of the pressure gradient to the increase in flow as the veins quickly begin to fill post-contraction. Like the Walker et al. (2007) paper, active vasodilatory magnitude remained constant throughout the single contraction; thus, it is expected that the increase in perfusion pressure would fully explain the increase in forearm blood flow and that forearm vascular conductance would remain the same regardless of end-of-contraction arm position. However, this was not the case. Forearm vascular conductance was significantly greater when the contraction was released below heart compared to above heart. Thus, hydrostatic change in perfusion pressure explained some but not all the increase in forearm blood flow. The excess blood flow that could not be explained by the increased perfusion pressure was attributed to the greater forearm vascular conductance and the greater conductance was attributed to the transmural pressure-mediated distension of the arterioles.

Distensibility of resistance vessels is dependent on the structural components of the vessel wall itself but has yet to be considered a contributor to the observed vasodilation during exercise. Arteriolar vessel wall structure is comprised of three main layers – listed from closest to farthest from the lumen: tunica intima, tunica media, and the tunica adventitia (Rahman & Siddik, 2022). These layers are visualized in Figure 3. The tunica intima consists of the endothelial lining which is supported by a thin layer of connective tissue. An elastic tissue layer – the internal elastic lamina – separates the tunica intima from the tunica media. The internal elastic lamina contains junctions by which endothelial derived vasodilation substances and hyperpolarizing signals can be passed to the smooth muscle found in the tunica media (Kirby et al., 2013; Rahman & Siddik, 2022). The arteriolar tunica media consists of alternating layers of connective tissue and 1-3 layers of vascular smooth muscle cells, (Martinez-Lemus, 2012; Rahman & Siddik, 2022). In addition to smooth muscle, the tunica media also consists of ground substance, and the extracellular matrix and is separated from the outermost layer by the external elastic lamina. The outermost layer is the tunica adventitia; it houses nerve bundles where the adventitia borders the tunica media that can conduct vasodilatory and vasoconstricting signals to the vascular smooth muscle (Martinez-Lemus, 2012; Rahman & Siddik, 2022).



**Figure 3.** Schematic of the different layers that comprise the arteriolar vessel wall.

At any given time, a resistance vessel's diameter is achieved by a balance between vasodilatory and vasoconstrictor influences on smooth muscle tone. Vasodilation magnitude is impacted by smooth muscle relaxation and vessel distensibility; conversely, constriction is achieved through smooth muscle contraction and inward recoil forces exerted by vessel walls. This inward elastic recoil force stems from the structural properties of the extracellular matrix and occurs in response to pulsatile blood pressure (Green et al., 2014; Rahman & Siddik, 2022). The structural integrity of the extracellular matrix is contingent on its composition of both collagen and elastin content. Collagen provides mechanical strength and stability due to its right-handed triple helix structure (Ramachandran & Kartha, 1954, 1955; Shoulders & Raines,

2009). Each strand of this structural protein consists of a repetition of a pattern of three amino acids, the third amino acid residue always being glycine (Shoulders & Raines, 2009). Glycine being the smallest of the essential amino acids allows for an extremely tightly bound triple-helix structure. Further, glycine's chemical structure allows for a ladder formation of hydrogen bonds within the triple helix (Pauling & Corey, 1951). This tightly bound triple-helix structure further stabilized by the strongest known chemical bond – hydrogen bonds – gives collagen its rigid property. Conversely, while collagen provides strength and rigidity to the vasculature, elastin provides resilience to mechanical strain (Lopez Barreiro et al., 2021; Wang et al., 2021). Elastin consists of tropoelastin subunits that adopt a random coil formation and are cross-linked together which gives elastin its spring like property (Vrhovski & Weiss, 1998).

In summary, passive distension of arterioles contributes to the magnitude of hyperemia that occurs in response to a single contraction when transmural pressure is increased (Lynn et al., 2020). In the studies conducted by Lynn et al. (2020) and Walker et al. (2007), both manipulated exercising arm position from above to below heart during a contraction. This ensured that active vasodilatory magnitude remained the same between arm positions. Both studies observed an increase in forearm blood flow and calculated forearm vascular conductance following arm position change to a higher transmural pressure environment. This increase in vascular conductance was attributed to the mechanical distension of the resistance vessels. Mechanical distension relies on the structural properties of the components that make up resistance vessels, specifically collagen and elastin. Collagen exists to provide mechanical strength and rigidity to the vessel while elastin provides resilience to mechanical stretching of the vessel (Lopez Barreiro et al., 2021; Wang et al., 2021). However, it remains unknown if the

magnitude of distension-mediated increase in forearm vascular conductance during the rapid onset vasodilation response to a single contraction changes with active vasodilation magnitude.

## **Problem Statement**

During exercise, greater muscle blood flow is observed following a single contraction when the arm is in the below position compared to the above heart position despite active vasodilatory signals remaining constant (Tschakovsky et al., 2004; Tschakovsky et al., 1996). Evidence from our laboratory support the idea that passive vasodilation of resistance vessels plays a role in post-contraction hyperemia below compared to above heart level (Lynn et al., 2020; Walker et al., 2007). While these studies demonstrated evidence for passive vasodilation, its relationship with active vasodilation magnitude has yet to be explored. Increased active vasodilation reduces vasotone, thus reducing the inward acting force of the vascular smooth muscle and perhaps allows for greater distension of the arteriolar wall in response to an increase in transmural pressure. Thus, we hypothesize that under conditions of increased active vasodilation, passive vasodilation will be greater. Exploring the relationship between active and passive vasodilation may help us to identify an index of contribution of resistance vessel distensibility to the observed vasodilation during exercise.

## **Chapter 3 – Manuscript**

**Transmural pressure-mediated arteriolar distension magnitude following forearm contraction  
below vs. above heart level: dependence on active vasodilation magnitude?**

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## ABSTRACT

Blood flow is a product of perfusion pressure (PP) and vascular conductance. When moving the arm from a low transmural pressure (TP) environment (limb above-heart) to a high TP environment (limb below-heart) during forearm handgrip exercise, there is an immediate increase in forearm blood flow (FBF, mL/min). This increase exceeds the PP increase, resulting in an increased calculated forearm vascular conductance ( $\Delta FVC$ , mL/min/100mmHg). We have previously attributed this to TP-mediated arteriolar distension. It remains unknown whether the magnitude of distension due to increased TP is dependent on the magnitude of active vasodilation. 21 participants (11 female) in supine position performed single, 2-sec handgrip contractions with the arm above-heart (low pressure) and the arm moved from above to below heart during contraction to increase TP upon contraction release (high pressure). Both conditions were performed under a low (20% maximal voluntary contraction; MVC) and a high (80% MVC) active vasodilation magnitude. We measured FBF (Doppler and echo ultrasound) and forearm arterial perfusion pressure (FAPP; heart level arterial blood pressure via finger photoplethysmography adjusted for hydrostatic column differences between arm positions;  $FAPP_{above}$ ,  $FAPP_{below}$ ). FVC for the first cardiac cycle following contraction was calculated as  $FBF/FAPP$  (ml/min/100 mmHg). Distension-mediated dilation with increased TP was calculated as  $FVC_{below} - FVC_{above}$ . As expected,  $\Delta FVC$  increased more from baseline in 80% vs. 20% MVC (above:  $107.0 \pm 63.2$  vs.  $38.76 \pm 34.2$ ,  $P < 0.0001$ ; below:  $164.2 \pm 77.1$  vs.  $98.04 \pm 58.7$ ,  $P < 0.0001$ ). Within both contraction intensities,  $\Delta FVC$  increased more below vs. above heart level (20%:  $98.01 \pm 58.7$  vs.  $38.76 \pm 34.2$ ,  $P < 0.0001$ ; 80%:  $164.2 \pm 77.1$  vs.  $107.0 \pm 63.2$ ,  $P < 0.0001$ ). However, the arteriolar distension-mediated increase in FVC with arm position change was not

different in 80% MVC vs. 20% MVC ( $57.2 \pm 36.8$  vs.  $59.23 \pm 33.4$ ,  $P > 0.05$ ). In conclusion, the magnitude of distension due to increased TP is independent of the magnitude of active vasodilation within the range tested in this study, suggesting factors other than smooth muscle tone determine TP-mediated distension magnitude.

## INTRODUCTION

At the onset of exercise, there is an increase in mitochondrial oxidative phosphorylation to produce sufficient adenosine triphosphate (ATP) to sustain exercise. This requires an increase in oxygen delivery to the exercising muscle to match the increase in mitochondrial oxygen demand (Gonzalez-Alonso et al., 2008; Musch et al., 1987; Radegran, 1997). This is achieved through increased muscle blood flow. Increasing exercising muscle blood flow is predominantly achieved via dilation of the muscle microvasculature by reducing vascular smooth muscle tone; although, elevated arterial pressure provides some contribution at higher exercise intensities (Joyner & Casey, 2015).

At present, reduction of vascular smooth muscle tone is the primary mechanism by which we understand local determination of oxygen delivery:demand matching at the onset of exercise. Initial relaxation of the vascular smooth muscle is achieved through feed forward mechanisms associated with muscle activation and mechanical compression of arterioles, contributing to the rapid increase in exercising muscle blood flow (Clifford et al., 2006; Clifford & Tschakovsky, 2008; Crecelius et al., 2013). However, resistance vessels are elastic and can therefore be distended with an increase in transmural pressure (Borgstrom & Grande, 1979; Groot et al., 2013; Jasperse et al., 2015; Koller & Bagi, 2002; Trinity et al., 2012). Our previous studies have demonstrated evidence for distension mediated arteriolar dilation and its contribution to the observed increase in muscle blood flow in the exercising forearm when the arm is repositioned from a lower (above-heart) to a higher (below-heart) transmural pressure environment (Lynn et al., 2020; Walker et al., 2007). These studies demonstrated an increase in forearm blood flow (FBF) during rhythmic forearm exercise (Walker et al., 2007) or single contraction exercise (Lynn et al., 2020) when the

forearm was moved from above to below heart level. This increase in FBF was greater than what could be explained by the increase in perfusion pressure between arm positions, resulting in an increase in calculated forearm vascular conductance (FVC). As the arm was repositioned during a contraction in which the active vasodilatory effects remain the same, the immediate increase in FVC during the first cardiac cycle following the release of the contraction following arm position change is attributed to the mechanical distension of the microvasculature.

Arteriolar distensibility reflects the structural components that make up the vessel wall, primarily the collagen and elastin components predominantly found in the tunica media. The elastic nature of the vessel walls provides an inward acting elastic recoil force that opposes transmural pressure that is produced by blood volume exerting outward force on vessel walls. Vascular smooth muscle adds an additional inward force, and the magnitude of this force is dependent on smooth muscle tone. When vascular smooth muscle is more constricted, the inward forces are larger; conversely, when the vascular smooth muscle is more relaxed, the inward forces are less. However, the relationship between the inward force provided by vascular smooth muscle tone and distensibility of arterioles remains unknown.

Therefore, the purpose of this study is to test the hypothesis that distension mediated forearm vascular conductance ( $FVC_D$ ) with increased transmural pressure is greater when active vasodilation magnitude is also greater. To test this hypothesis, muscle blood flow and hydrostatic column perfusion pressure were measured in a low and high transmural pressure environment with a low and high active vasodilation magnitude. Should arteriolar distensibility contribute to total vasodilation magnitude, it may provide an index for future research exploring the contributions of arteriolar distension and vasodilation in oxygen delivery:demand matching.

## METHODS

### ***Experimental Protocol***

*Sample Size Calculation.* A *a priori* sample size calculation based on previous research (Tschakovsky et al., 2004) for the primary outcome – forearm vascular conductance – suggested that a sample size of 18 will be sufficient to detect a statistically significant medium effect size ( $d = 0.5$ ), assuming a predicted power of 80% and a type 1 error rate of 5%. Estimating a 20% drop out rate, 22 participants were to be tested.

*Participants.* 22 participants recruited from the student population at Queen’s University, Kingston, Ontario, Canada began this study. One was excluded as the data collection was interrupted by a fire alarm and the participant was unable to return to baseline values post-alarm. Therefore, 21 (11 female, 10 male) participants completed this study. Eligible participants were between the ages of 18 and 29, were non-smokers, were not taking any medication with possible vasoactive effects, and were without history of cardiovascular and metabolic disease. Eligible participants identified with Tier 0, Tier 1, or Tier 2 of the physical activity and performance framework developed by McKay et al. (2022). Participants were normotensive and had a body mass index between 18.5 and 24.9. Each participant signed consent to the experimental procedures approved by the Queen’s University Health Sciences Research Ethics Board (Kingston, Ontario, Canada), which conforms to the standards set in the Declaration of Helsinki, after receiving a verbal and written description of the experimental protocol and potential risks at a screening/familiarization session. Participants filled out a medical screening form to ensure eligibility for participation. Participants were instructed to do the following prior to testing:

refrain from engaging in upper body strength training 24 hours before, refrain from consuming alcohol and nicotine 12 hours before, refrain from caffeine 12 hours before, and refraining from eating 4 hours before.

*Experimental Design & Conditions.* This study was a within-participant design in which all participants completed a minimum of three single forearm handgrip contractions (dependent on data viability) at a low intensity (20% maximal voluntary contraction; MVC) and a high intensity (80% MVC) for all conditions (above-heart, and below-heart) for a total of 6 exercise blocks as depicted in Figure 4. Contractions were held for two seconds, guided by an auditory and visual metronome. Contraction force was visible to the participant in real time, along with a visual target to ensure the desired contraction force was achieved. All condition blocks for a given exercise intensity were performed consecutively to reduce the learning curve experienced to hit a specific target intensity. Order of exercise intensity performed, and arm position conditions was randomized and counterbalanced to eliminate order bias.

*Experimental Arm Position.* Handgrip exercise was always completed with the right forearm. The right arm was supinated and extended at the elbow. The arm was approximately 45 degrees away from the body and fashioned to a sliding armrest on a board that could be rapidly lowered via a pulley system. The wrist was strapped to the sliding armrest to prevent rotational movement of the forearm during contractions. The sliding armrest allowed the arm to remain extended as the arm was moved from an above heart to a below heart position. The midpoint between the two medial muscle bellies of the flexor digitorum superficialis – using guidelines by (Bickerton et al.,

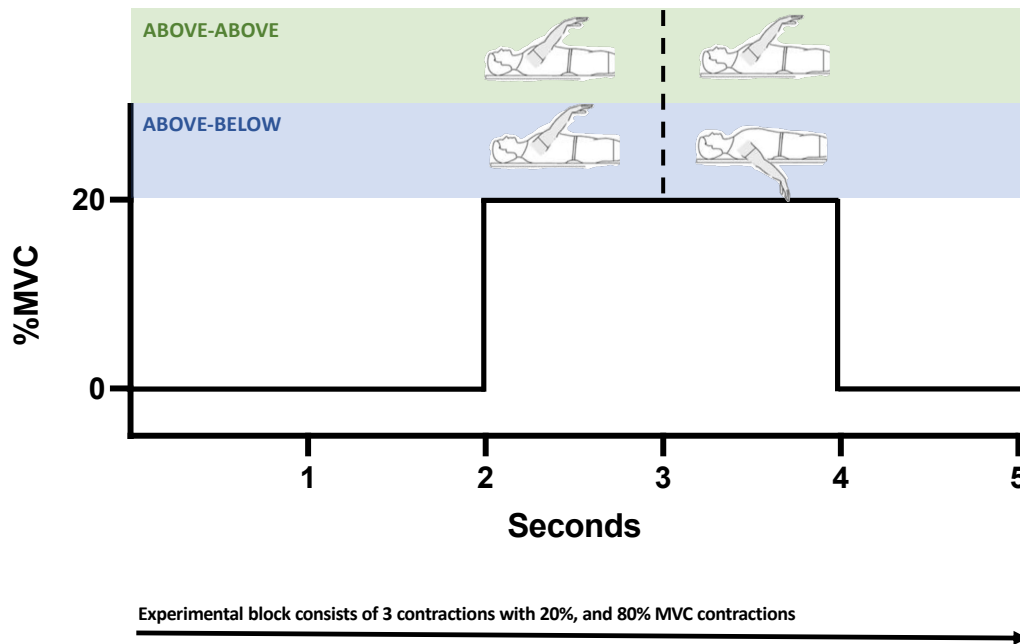
1997) – was used as a reference point to measure the change in height between the above and below heart positions. This measurement was later used to account for the contribution of hydrostatic pressure on arterial pressure when calculating forearm blood flow and forearm vascular conductance.

*Maximal Voluntary Contractions.* Prior to the test protocol, participants completed three two-second MVCs spaced one minute apart or until mean blood velocity returned to baseline values. The MVC displaying the largest force output was deemed the participant's true MVC and was used to calculate relative single contraction intensities.

*Above-Above Heart Protocol.* Following a 10-minute rest period in the supine position with the experimental arm in the above heart position, a two second handgrip contraction was performed at the target exercise intensity. The arm remained in the above heart position for the entirety of the block. Contractions will be separated by one minute of rest or until mean blood velocity returned to baseline values. Contractions were performed until a minimum of three trials with high quality mean blood velocity signal were obtained before cessation of the exercise block. This above-above heart protocol provides the magnitude of dilation due to active vasodilation for a given exercise intensity without the effect of distension.

*Above-Below Heart Protocol.* A two second handgrip contraction was performed at the target exercise intensity with the experimental arm in the above heart position. Upon initiation of the contraction, a research assistant rapidly lowered the arm below heart level prior to the release

of the contraction. With the help of gravity, this change in arm position increased the arteriolar transmural pressure. Ten seconds following the release of the contraction, the arm was returned to the above heart position. Thereafter, time between contractions from the above-above protocol remain the same for the above-below heart protocol. All contractions at a single intensity will be performed consecutively; thus, a total of 6 contractions (three contractions per protocol) at a certain intensity will be completed before moving onto the next intensity. This design was employed to reduce the learning curve in achieving a target intensity when jumping from one intensity to the next.



**Figure 4.** Protocol Schema. Depicted are the two different exercise conditions: above-above heart, and above-below heart. The starting position is the position of the arm prior to a single two second forearm contraction. The arm is moved or not moved during the two second contraction, indicated by the end position. Contractions are performed at 20% and 80% MVC.

### ***Instrumentation & Data Acquisition***

All data acquisition on a PowerLab/8<sub>SP</sub> using Labchart Software (ADInstruments, Colorado Springs, CO) will be at 200 Hz.

*Mean Blood Velocity.* Brachial artery blood velocity of the experimental arm was continuously measured beat by beat with a flat 4 MHz pulsed Doppler probe (Model 500V 131 Transcranial Doppler, Multigon industries, Mt. Vernon, NY) attached to the skin superficial to the brachial artery ~3cm proximal to the antecubital fossa. The analog voltage signal representing mean blood velocity analog signal to a PowerLab/8<sub>SP</sub> (ADInstruments, Colorado Springs, CO) and converted to cm/s in LabChart 7 software (ADInstruments, Colorado Springs, CO) using a two-point calibration based off of a previously determined calibration curve for a range of insonation angles. The insonation angle of the brachial artery relative to the Doppler probe was measured using echo ultrasound to provide an image of the artery orientation relative to the skin surface which corrects insonation angle relative to a parallel-to-skin position (Vivide I; GE Medical Systems, London, ON, Canada) and brachial artery mean blood velocity was adjusted for each participant.

*Mean Arterial Blood Pressure.* A finger photoplethysmograph cuff (Finometer MIDI; Finapres Medical Systems) was placed on the middle phalange of the left middle finger to continuously measure mean arterial blood pressure (MAP) beat by beat. The left arm and hand rested at heart level to obtain heart level arterial blood pressure.

*Handgrip Force Output.* Force of handgrip contractions were obtained using a force transducer connected to a PowerLab/8sp. The force transducer was calibrated using two-point calibration based on a previously determined calibration curve with voltages associated with known weights.

*Brachial Artery Diameter.* Echo ultrasound with a probe operating at 13 MHz in 2D mode was used to image the brachial artery in the above and below heart positions before and after conducting the testing protocol. The probe was positioned at the site of the Doppler probe. Upon obtaining a clear image of the brachial artery using echo ultrasound, brachial artery diameter was then measured with Measurements from Arterial Ultrasound Imaging (MAUI, Hedgehog Medical) using automated edge detection.

*Forearm Blood Flow.* Blood flow to the forearm was calculated by the following equation:

$$FBF = MBV (cm/s) \cdot \left[ \pi \left( \frac{\text{brachial artery diameter (cm)}}{2} \right)^2 \right] \cdot 60 (s/min)$$

where Forearm Blood Flow (FBF, mL/min) is the product of MBV and the cross-sectional area of the brachial artery.

*Forearm Arteriolar Perfusion Pressure.* Forearm arteriolar perfusion pressure (FAPP) is the MAP value adjusted for the hydrostatic pressure depending on the height (h; cm) of the arm above or below heart level. FAPP was calculated by the following equation:

$$FAPP = MAP \pm \left( h \cdot 0.75 \frac{mmHg}{cm} \right)$$

*Forearm Vascular Conductance.* Forearm Vascular Conductance (FVC) was calculated by the following equation:

$$FVC = \left[ \frac{FBF(mL/min)}{FAPP(mmHg)} \right] \cdot 100$$

where FVC (mL/min/100mmHg) is the quotient when Forearm Blood Flow (FBF) is divided by FAPP.

*Forearm Vascular Conductance due to Distension.* The forearm vascular conductance due to distension (FVC<sub>D</sub>) was isolated with the following equation:

$$FVC_D = FVC_{\text{ABOVE-BELOW HEART}} - FVC_{\text{ABOVE-ABOVE HEART}}$$

*Forearm Contraction Impulse.* To account for small participant error in achieving the target force and/or holding the contraction for exactly two seconds, the forearm contraction impulse was used and calculated via a LabChart 7 channel created to calculate the integral of the force output channel, providing the area under the curve for each two second contraction.

*Forearm Volume.* Forearm volume was measured using a water displacement technique. Using a cylindrical tube with a spout at the top, the tube was filled with water up to the spout. The hand was submerged into the water up to the radial styloid. The water displaced from hand volume spilled out of the spout and was not accounted for. The forearm was then submerged up to the medial humeral epicondyle. The water displaced from forearm submersion was collected from the spout into a graduated cylinder and represented forearm volume.

### ***Data Analysis***

Baseline data was averaged over the 10 seconds prior to the initiation of a handgrip contraction. Post-contraction response data was analyzed for the first full cardiac cycle unaffected by the preceding handgrip contraction. All three trials for each condition were averaged to characterize the individual response per participant. Change in FBF ( $\Delta\text{FBF}$ ) was calculated as the difference between baseline FBF and the first measurable cardiac cycle following the release of a handgrip contraction. Similarly, change in FVC ( $\Delta\text{FVC}$ ) was calculated as the difference between baseline FVC and the first measurable cardiac cycle following the release of a handgrip contraction.  $\Delta\text{FVC}_D$  was calculated as the difference between the FVC response of the first cardiac cycle in the above-above condition and the FVC response of the first cardiac cycle in the above-below condition.

Local FAPP was defined as the MAP in the forearm. FAPP was calculated by adjusting MAP for the hydrostatic pressure of blood related to the difference in height between the forearm and the heart ( $\Delta h$ , cm) depending on arm position. Using the aforementioned equation, the adjusted value is added to MAP when the arm is in the below-heart position and subtracted from MAP when the arm is in the above-heart position.

### ***Statistical Analysis***

To test the hypothesis that distension mediated forearm vascular conductance will be greater in the 80% MVC condition compared to the 20% MVC condition, a paired t-test was used. A two-way repeated measures ANOVA (arm position x contraction intensity) was used to assess main effects of arm position and contraction intensity on mean FVC, FBF, and FAPP. A repeated measures approach was used because all participants participated in all conditions. The

Bonferroni method was used for post-hoc analysis of differences between arm positions and across active vasodilation magnitudes.

## RESULTS

### *Participants*

See Table 1 for participant characteristics.

**Table 1.** Age and anthropometric measures.

	Female	Male	Combined
Age (years)	21.1 ± 2.2	22.6 ± 2.2	21.8 ± 2.3
Height (cm)	166.1 ± 5.2	178.5 ± 6.4	172.0 ± 8.5
Weight (Kg)	62.1 ± 10.4	78.4 ± 10.9	69.9 ± 13.3
BMI (Kg/m <sup>2</sup> )	22.5 ± 3.3	24.6 ± 2.8	23.5 ± 3.2
Forearm Volume (mL)	768.2 ± 164.9	1155 ± 222.4	952.4 ± 273.9

Values are means ± SD. BMI, Body Mass Index.

### *Was the force integral consistent between conditions?*

Force output was not consistent between the above-above (AA) condition and the above-below (AB) arm positions ( $F(1, 20) = 7.722$ ,  $P = 0.0116$ ). Post-hoc test determined that force output was not different between the AA arm position ( $12.29 \pm 6.0$  Kg•s) compared to the AB arm position ( $11.70 \pm 5.9$  Kg•s,  $P > 0.9999$ ) in the 20% MVC condition (see Table 2). However, in the 80% MVC condition force output was significantly different between the AA arm position ( $48.05 \pm 25.1$  Kg•s) compared to the AB arm position ( $45.54 \pm 25.1$  Kg•s,  $P = 0.0021$ ).

**Table 2.** Quantification of target force integral and force integral achieved.

Female	20%MVC Target Force AUC		80%MVC Target Force AUC	
	8.0 ± 1.6		36.1 ± 7.3	
	20%MVC AA Actual Force AUC	20%MVC AB Actual Force AUC	80%MVC AA Actual Force AUC	80%MVC AB Actual Force AUC
	8.3 ± 2.2	8.2 ± 2.5	31.8 ± 8.9	30.7 ± 8.6
Male	20%MVC Target Force AUC		80%MVC Target Force AUC	
	16.5 ± 4.6		74.1 ± 20.63	
	20%MVC AA Actual Force AUC	20%MVC AB Actual Force AUC	80%MVC AA Actual Force AUC	80%MVC AB Actual Force AUC
	16.7 ± 5.7	15.56 ± 6.2	65.9 ± 25.2	61.9 ± 27.55*
Combined	20%MVC Target Force AUC		80%MVC Target Force AUC	
	12.0 ± 5.4		54.2 ± 24.4	
	20%MVC AA Actual Force AUC	20%MVC AB Actual Force AUC	80%MVC AA Actual Force AUC	80%MVC AB Actual Force AUC
	12.3 ± 6.0	11.7 ± 5.9	48.1 ± 25.1	45.5 ± 25.1*

Values are means ± SD. AUC, Area Under the Curve (Kg•s); MVC, Maximal Voluntary Contraction; AA, Above-Above condition; AB, Above-Below condition. \*Significantly different from the AA condition for the same exercise intensity, P < 0.05.

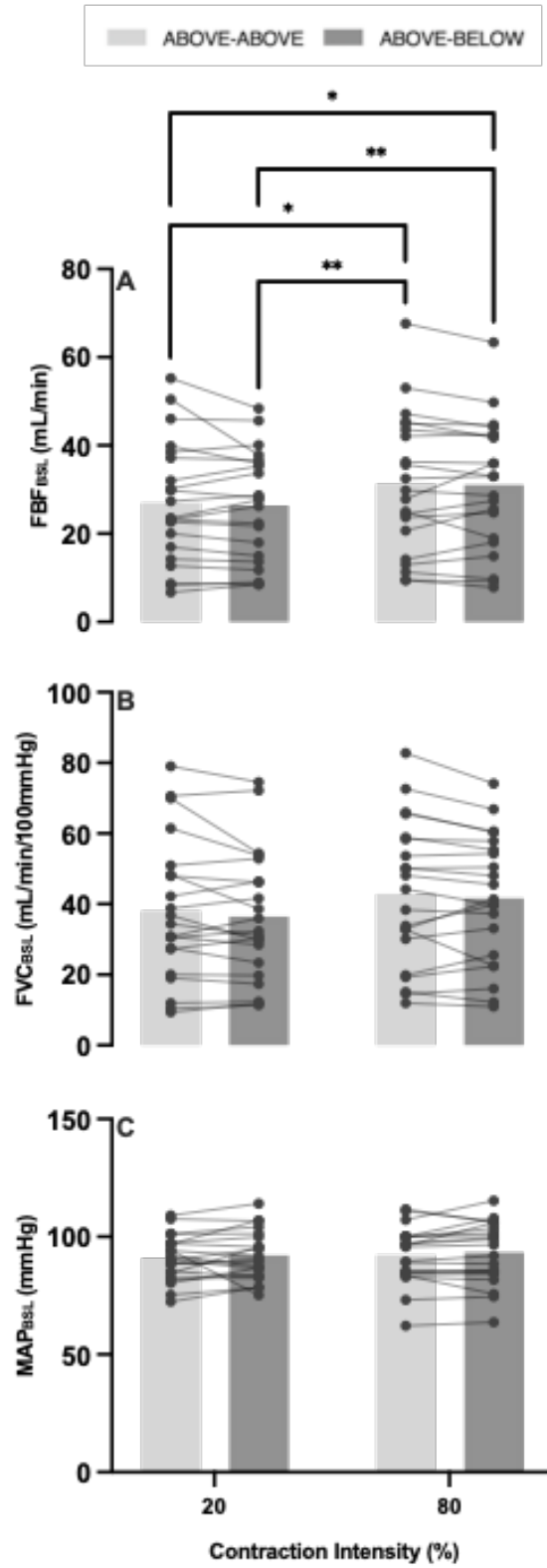
### ***Was MAP different between conditions?***

There was no difference between experimental conditions in baseline MAP prior to single contraction (F(1, 20) = 0.0002, P = 0.9871), or during the first cardiac cycle following a contraction (F(1, 20) = 0.3504, P = 0.5605).

### ***Were baseline forearm hemodynamics different between conditions?***

See Figure 5. By design, all baseline measurements prior to single contractions were performed with the forearm above heart level, as single contractions were initiated in this position. There was no main effect of arm position condition on either FBF (F(1,20) = 0.5664, P = 0.4605) or FVC (F(1,20) = 2.380, P = 0.1386). There was no interaction effect between arm

position and contraction intensity ( $F(1,20) = 0.2214$ ,  $P = 0.6431$ ). However, baseline FBF prior to single contractions was greater in 80% MVC ( $31.16 \pm 15.1$  mL/min) vs. 20% MVC ( $26.65 \pm 13.0$  mL/min) contractions (main effect  $F(1,20) = 16.37$ ,  $P = 0.0006$ ). This was also the case for baseline FVC prior to single contractions (main effect arm position:  $F(1,20) = 2.380$ ,  $P = 0.1386$ ; interaction effect:  $F(1,20) = 0.1136$ ,  $P = 0.7396$ ; main effect of contraction intensity  $F(1,20) = 5.28$ ,  $P = 0.0325$ , 80% MVC  $42.23 \pm 19.2$  mL/min/100mmHg vs. 20% MVC  $37.21 \pm 19.0$  mL/min/100mmHg).

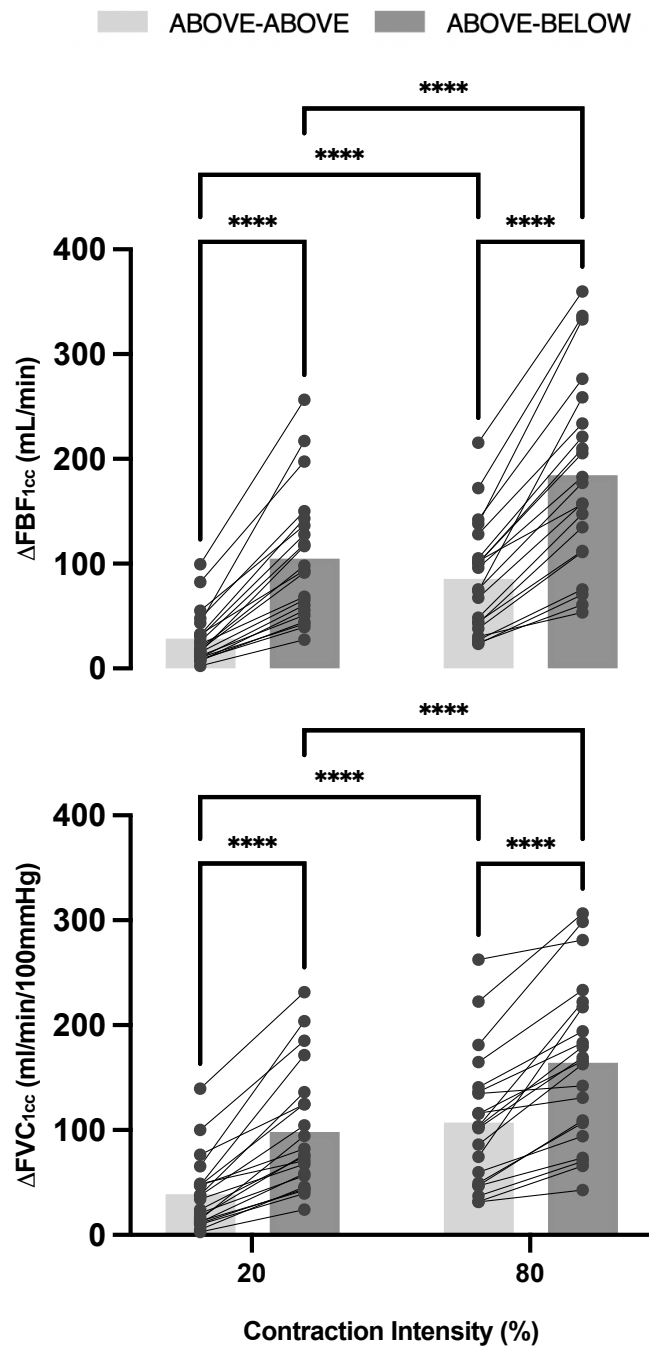


**Figure 5.** A) Baseline forearm blood flow; B) baseline forearm vascular conductance, calculated based MAP that is adjusted for hydrostatic column pressure; C) baseline MAP.

***What was the effect of contraction intensity and arm position on forearm hemodynamics following a single contraction?***

See Figure 6.  $\Delta\text{FBF}_{1\text{cc}}$  was greater following 80% MVC vs. 20% MVC contractions in both AA ( $85.63 \pm 52.5$  mL/min vs.  $28.57 \pm 25.2$  mL/min) and AB ( $184.6 \pm 91.7$  mL/min vs.  $104.9 \pm 62.4$  mL/min) (main effect of contraction intensity:  $F(1,20) = 68.73$ ,  $P < 0.0001$ ). Furthermore,  $\Delta\text{FBF}_{1\text{cc}}$  was greater in AB vs. AA in both 20% ( $104.9 \pm 62.4$  mL/min vs.  $28.57 \pm 25.2$  mL/min) and 80% MVC ( $184.6 \pm 91.7$  mL/min vs.  $85.63 \pm 52.5$  mL/min) (main effect of arm position:  $F(1,20) = 91.13$ ,  $P < 0.0001$ ). There was a significant interaction effect between contraction intensity and arm position ( $F(1,20) = 16.35$ ,  $P = 0.0006$ ). Post-hoc analysis indicated a significant difference between all comparisons ( $P < 0.0006$ ).

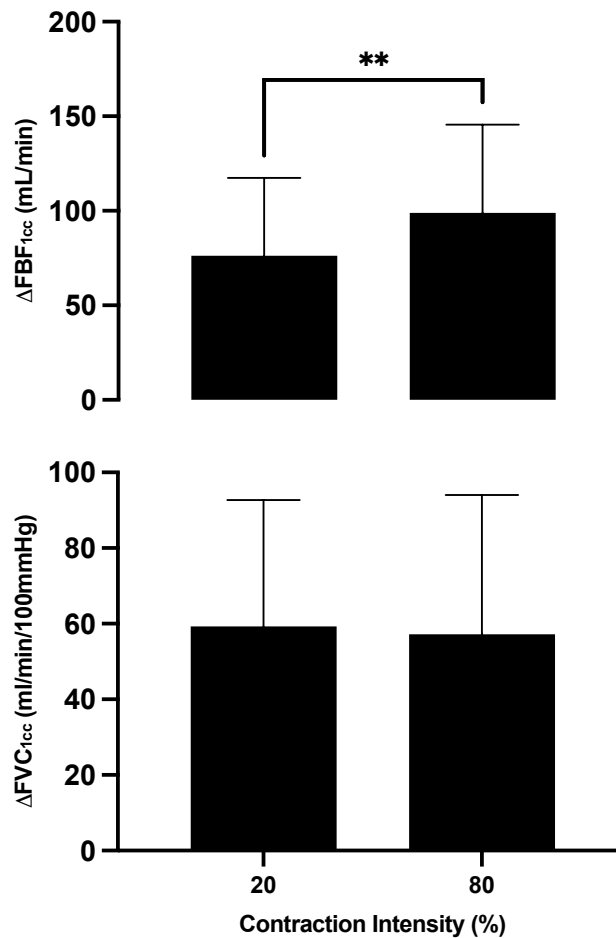
$\Delta\text{FVC}_{1\text{cc}}$  was greater following 80% MVC vs. 20% MVC contractions in both AA ( $107.0 \pm 63.2$  mL/min/mmHg vs.  $38.76 \pm 34.2$  mL/min/mmHg) and AB ( $164.2 \pm 77.1$  mL/min/mmHg vs.  $98.04 \pm 58.7$  mL/min/mmHg) (main effect of contraction intensity:  $F(1,20) = 79.33$ ,  $P < 0.0001$ ). Furthermore,  $\Delta\text{FVC}_{1\text{cc}}$  was greater in AB vs. AA in both 20% ( $98.04 \pm 58.7$  mL/min/mmHg vs.  $38.76 \pm 34.2$  mL/min/mmHg) and 80% MVC ( $164.2 \pm 77.1$  mL/min/mmHg vs.  $107.0 \pm 63.2$  mL/min/mmHg) (main effect of arm position:  $F(1,20) = 68.61$ ,  $P < 0.0001$ ). There was a no significant interaction effect between contraction intensity and arm position ( $F(1,20) = 0.1137$ ,  $P = 0.7295$ ).



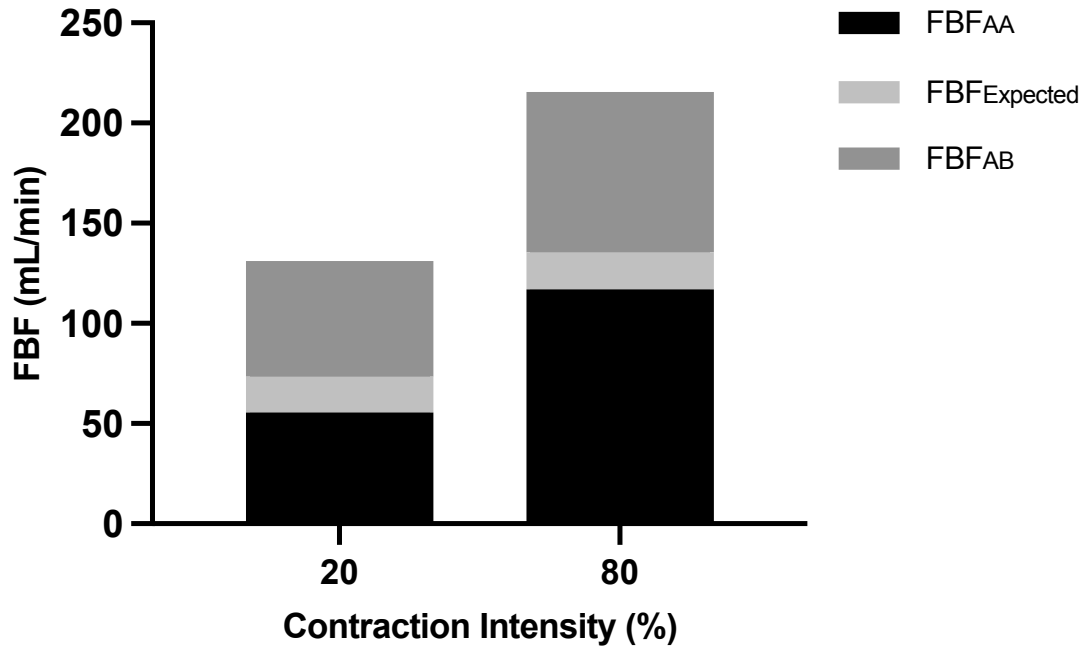
**Figure 6.** A) Change in forearm blood flow during the first cardiac cycle ( $\Delta\text{FBF}_{1\text{cc}}$ ) following a single contraction in the above-above (AA, light grey) condition and the above-below (AB, dark grey) condition; B) Change in forearm vascular conductance during the first cardiac cycle ( $\Delta\text{FVC}_{1\text{cc}}$ ) following a single contraction in the AA and AB condition; \*\*\*\*P < 0.0001.

**Was there an effect of active vasodilation magnitude on  $\Delta FVC_D$ ?**

Distension-mediated increase in FVC following a single contraction was not different between the 20% MVC condition ( $59.28 \pm 33.4$  mL/min/100mmHg/100mL) and the 80% MVC condition ( $57.20 \pm 36.84$  mL/min/100mmHg/100mL,  $P = 0.7395$ ) (see Figure 7, panel B).



**Figure 7.** A) Change in forearm Blood flow attributed to distension; B) change in forearm vascular conductance attributed to distension. \*\* $P < 0.01$ .



**Figure 8.** Proportion of the observed forearm blood flow response (FBF<sub>AB</sub>) that is attributed to active vasodilation mechanisms (FBF<sub>AA</sub>), and the proportion that is attributed to the increase in perfusion pressure when the arm is in the below-heart position (FBF<sub>Expected</sub>).

## DISCUSSION

The purpose of the current study was to test the hypothesis that distension mediated dilation with increasing arteriolar transmural pressure is increased with increased active vasodilation. The key findings from the current study are: 1) as intended, a greater forearm contraction intensity resulted in a greater increase in forearm blood flow (FBF) and active vasodilation (forearm vascular conductance (FVC) observable in the first cardiac cycle following contraction, 2) FBF and FVC immediately following a single contraction increased more when contraction was released in the higher (arm below heart) vs. lower (arm above heart) transmural pressure condition, regardless of contraction intensity, and 3) the increase in transmural pressure mediated  $FVC_D$  was not different between low and high active vasodilation magnitudes.

The observation that distension mediated vasodilation was not greater when greater active vasodilation was created does not support our hypothesis that distension mediated vasodilation with increased transmural pressure increases with active vasodilation magnitude, at least within the range of active vasodilation created in this study.

### ***Isolating the contribution of arteriolar distension to the change in forearm vascular conductance***

In the current study, all contractions were initiated in the above-heart position. Thus, the contribution of active vasodilation mechanisms was the same regardless of whether contraction was released with the arm remaining above heart level (lower transmural pressure condition) or after the arm was repositioned below heart level (higher transmural pressure condition). The arm position change was used to induce an increase in local forearm transmural pressure via

gravity-induced hydrostatic pressure. Mean blood velocity of only the first cardiac cycle following a contraction was used to determine FBF and FVC. Using the first cardiac cycle allowed us to avoid the effects of venous filling which gradually reduces the pressure gradient when the arm remains in the below-heart position following a contraction. Moreover, it has been demonstrated that in subsequent cardiac cycles (i.e., cardiac cycles occurring after the first full cardiac cycle following an arm position change) FVC returns to pre-movement values during steady state handgrip exercise (Walker et al., 2007). With single contractions, peak FBF and FVC responses typically occur within 3-5 cardiac cycles following a contraction (Kirby et al., 2007) and therefore may be susceptible to this counteractive vasoconstriction. Thus, we used the first cardiac cycle following contraction to avoid potential active vasoconstriction mechanisms that work to oppose the sudden change in transmural pressure.

### ***Contribution of driving pressure to limb position differences in exercising muscle blood flow***

The blood flow response to exercise is strongly dependent on limb position, with greater responses occurring when the limb is in a dependent position, below heart level (Groot et al., 2013; Hartling et al., 1976; Jasperse et al., 2015; Krishnan et al., 2011; Trinity et al., 2012). A prominent contributing factor to this observation is an increase in perfusion pressure to the exercising muscle (Groot et al., 2013; Lynn et al., 2020; Trinity et al., 2011). Local forearm arterial pressure is greater when the limb is in a below heart position due to hydrostatic column pressure; meanwhile, venous pressure is reduced to 0 mmHg when the limb is in the above heart position as gravity empties the veins (Tschakovsky et al., 1996). Together, these data indicate that

perfusion pressure is greater following contraction release when the limb is in the dependent position compared to the above heart position.

While a greater perfusion pressure may account for a portion of the increase in blood flow when the exercising limb is in a dependent position, it has been demonstrated previously (Lynn et al., 2020; Walker et al., 2007) and again in the current study, that perfusion pressure alone cannot account for all of the greater blood flow response following contraction release below vs. above heart level (see figure 8). The greater increase in calculated FVC following contraction when contraction is released in the HI vs. LO transmural pressure condition is necessary to explain the observed blood flow response. This increase in conductance has been attributed to the mechanical distension of arterioles in response to an increase in transmural pressure occurring when the limb is in the dependent position (Lynn et al., 2020; Walker et al., 2007).

***Distension mediated increase in forearm vascular conductance is not dependent on active vasodilation magnitude***

We had hypothesized that distension mediated forearm vascular conductance ( $FVC_D$ ) was dependent on active vasodilation magnitude: as active vasodilation increased, distension mediated vasodilation would also increase. However, results from the current study demonstrate that the increase in  $FVC_D$  was consistent between the low and high active vasodilation magnitude conditions (see Figure 7, panel B). There are a number of potential explanations for our observation.

First, these findings may be in part explained by transmural pressure mediated vascular smooth muscle cell depolarization. In animal models, it has been demonstrated that graded increases in transmural pressure resulted in graded depolarization of the vascular smooth muscle (Harder, 1984; Harder et al., 1987), increased intracellular calcium concentration ( $[Ca_{2+}]_i$ ) (Nystoriak et al., 2011), and activation of voltage-gated calcium channels (VGCC), which causes smooth muscle vasoconstriction. Despite this, Harder et al. (1987) demonstrated that changes in canine interlobular vessel diameter was biphasic: increases in transmural pressure from 20 mmHg to 60 mmHg resulted in a proportional increase in vessel diameter; however, transmural pressure increases beyond 60 mmHg up to 120 mmHg resulted in a proportional decrease in vessel diameter. When the vessels were treated with a calcium channel blocker, vessel diameter increased throughout the entire range of transmural pressures. These results indicated that beyond a threshold occurring at 60 mmHg, concomitant depolarization and vasoconstriction occurs. Thus, preventing further increases in vessel diameter despite an increase in transmural pressure. However, the time course of this vasoconstriction response is not reported in a timeframe that is comparable to the current study. Therefore, whether this counter vasoconstriction response contributed to the lack of difference in distension-mediated dilation when transmural pressure was increased under different active vasodilation conditions is unclear.

In a study conducted by Nystoriak et al. (2011), these researchers demonstrated graded pressure-mediated increases from 5 mmHg to 60 mmHg in rat parenchymal arterioles induced graded increases smooth muscle cell  $[Ca_{2+}]_i$  and decreases in vessel diameter. Both these responses to an increase in transmural pressure were abolished and returned to baseline levels

when a VGCC blocker was introduced. Additionally, when the VGCC blocker was introduced prior to a graded increase in transmural pressure, arteriolar tone remained unchanged at all pressures. These data not only demonstrate the potential for transmural pressure mediated vasoconstriction, but further suggest that vascular smooth muscle cell depolarization in response to an increase in pressure is dependent on L-type VGCCs.

It is important to note that as we move down the branches of the vasculature, from arteries to arterioles, arterial pressure and thus transmural pressure diminishes. While we can be confident that arterial pressure is increased below heart compared to above heart due to hydrostatic column pressure, the actual pressure in the arterioles is not measured. However, we might infer that if the increase in transmural pressure in the above-below heart conditions in the current study occurs above the threshold demonstrated by Harder et al. (1987), that perhaps transmural pressure mediated vascular smooth muscle depolarization may be occurring. This would likely cause deconstructive interference with the relationship between transmural pressure and FVC as depicted by Harder et al. (1987) via calcium channel blockade. However, evidence for transmural pressure mediated smooth muscle cell depolarization has only been demonstrated in animal kidney and cerebral vessels (Harder, 1984; Harder et al., 1987; Nystoriak et al., 2011). Although it remains unknown whether this relationship and its characteristics are mimicked in humans, it may explain the results demonstrated by Walker et al. (2007) in which FBF and FVC returned to pre-arm-repositioning values by the second cardiac cycle following arm repositioning.

The time course of the onset of transmural pressure mediated smooth muscle cell depolarization should also be taken into consideration. While the myogenic response to changes

in transmural pressure occur quickly, there is a small time delay at the onset and offset of an increase in transmural pressure (Borgstrom & Grande, 1979). Borgstrom and Grande (1979) developed a mathematical model of the myogenic microvascular responses to changes and rates of changes in transmural pressure based on biological animal data in which the skeletal muscle was sympathectomized. While these researchers developed a mathematical model that predicted myogenic responses to changes in transmural pressure that very closely resembles that of the in vivo models they used as a reference, they recognized their model could not account for the short time delays at the onset and offset of the increase in transmural pressure. They hypothesized that the mathematical model did not account for the general inertia of the receptor-effector system and overall dynamic system that exists with in vivo models. Thus, whether transmural pressure mediated smooth muscle cell depolarization and subsequent vasoconstriction is occurring within the first cardiac cycle in the current study remains unknown.

It is possible that this counteractive vasoconstriction effect was greater under conditions of greater active vasodilation, therefore offsetting any potentially greater distension mediated dilation. However, the 20% MVC and 80% MVC conditions in the current study provide varying levels of active vasodilation magnitude while the increase in transmural pressure remains the same. Furthermore, this effect would have to be immediate, given we examined only the first cardiac cycle following contraction.

Another explanation for a lack of difference between active vasodilation conditions in distension mediated dilation with increased transmural pressure may be that our single contraction protocol was insufficient in creating a range of active vasodilation magnitude large enough to detect an effect of active vasodilation on distension-mediated vasodilation. FVC

increases due to active vasodilation (AA condition) in the current study increased twofold in the 20% MVC condition and threefold in the 80% MVC condition. This is much lower than the approximate eleven-fold increase demonstrated in rhythmic handgrip exercise studies at 30% MVC (Walker et al., 2007). Thus, future studies may consider a steady state exercise model.

The interaction between active vasodilation and the distensibility of resistance vessels must also be considered. As active vasodilation increases, the resistance vessels dilate which may distend the structure prior to an increase in transmural pressure. This distension could increase the inward acting elastic recoil forces from the resistance vessel structure itself. When transmural pressure is then increased, it may offset the reduced inward smooth muscle force, resulting in the same  $FVC_D$  observed in both the 20% and 80% MVC conditions.

***Payoff between transmural pressure outward forces, vascular smooth muscle inward forces, and whole limb inward forces***

The current hypothesis was developed based on our current understanding of the forces at play regarding the structure of the arteriole wall. Naturally, the volume of blood inside a vessel exerts an outward force onto the walls of the vessels, representing arterial blood pressure. The structural components that make up the various layers, specifically the collagen and elastin components found in the tunica media, exert an inward recoil force that opposes mean arterial pressure. Vascular smooth muscle provides another inward acting force, and this force is dependent on smooth muscle tone. When vascular smooth muscle is more vasoconstricted, this inward acting force is greater in magnitude. The opposite is also true in which there is less inward acting force provided by smooth muscle tone when the smooth muscle is more relaxed. Thus, we

hypothesized that distension mediated forearm vascular conductance was dependent on active vasodilation magnitude. However, the current results demonstrate that distension mediated forearm vascular conductance was not different between HI and LO conditions ( $P > 0.05$ ). The current hypothesis fails to consider additional limiting inward facing forces via the adventitia, adjacent vessels, other surrounding tissues, interstitial fluid, and the epimysium sheath, all which remain unchanged despite active vasodilation magnitude. These confounding inward facing forces are inevitable with in vivo models and may be contributing to the results found in the current study.

#### ***Discrepancy in active vasodilation magnitude between arm positions***

The purpose of the current study was to determine whether distension mediated increases in forearm vascular conductance were dependent on active vasodilation magnitude. To address this inquiry, the same magnitude of active vasodilation needed to be initiated in both the AA and AB arm positions. However, results demonstrate that different force integrals were achieved between the AA and AB arm positions in the HI condition (see Table 2). Upon further analysis, it appears that when the force integral data was analyzed for sex differences, the male participants demonstrated a significant difference in force production while the female participants did not. Despite this discrepancy in force integral production in the HI condition, it appears that both female and male  $\Delta FVC_D$  are not different between LO and HI conditions (see Appendix B for sex differences). Therefore, while a discrepancy in force integral production and subsequent active vasodilation magnitude exists in the HI condition, we believe that the

inconsistency was not large enough to impact the distension mediated response to increased transmural pressure.

## **CONCLUSION**

This is the first study to investigate whether distension mediated dilation of resistance vessels with increased transmural pressure is greater when active vasodilation is greater. We have demonstrated that increased transmural pressure resulted in an increase in  $FVC_D$ . However, the contribution of distension-mediated dilation was not different between lower and higher active vasodilation magnitudes created in this study. In conclusion, distension-mediated forearm vascular conductance is not dependent on active vasodilation magnitude within the range created in this study.

### ***Grants***

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### ***Disclosures***

No conflicts of interest, financial or otherwise, are declared by the authors.

### ***Author Contributions***

T.L and M.E.T conceived and developed the research protocol; T.L, E.T.Z, A.K.Z, and S.P.S.M performed data collections; T.L analyzed the data; T.L and M.E.T interpreted the results; T.L prepared figures and drafted the manuscript; T.L, E.T.Z, A.K.Z, and S.P.S.M edited and revised the manuscript; M.E.T approved final version of the manuscript.

## Chapter 4 – General Discussion

### Integrated Summary

An increase in oxidative phosphorylation and thus concomitant increase in oxygen demand occurs in the exercising muscle at the onset of exercise. This increase in oxygen demand is met with an increase in oxygen delivery via increased exercising muscle blood flow (Clifford & Hellsten, 2004; Hughson et al., 1996; Musch et al., 1987; Rogers et al., 2006). As muscle blood flow is a production of vascular conductance and the pressure gradient across a capillary bed, an increase in flow must be achieved through an increase in vascular conductance, an increase in the pressure gradient, or both. An increase in vascular conductance is the predominant contributor to increases in flow, with small contributions of increased arterial pressure at higher exercise intensities (Joyner & Casey, 2015).

Traditionally, increases in vascular conductance have been attributed to mechanisms that act to reduce vascular smooth muscle tone. These mechanisms have been linked to muscle activation via skeletal muscle fibre  $K^+$  release (Armstrong et al., 2007; Mohrman & Sparks, 1974b) or through mechanical compression of arterioles by the extravascular force created by the adjacent contracting muscle (Clifford et al., 2006; Kirby et al., 2007; Koller, 2002; Turturici & Roatta, 2013). Concomitant sympathetic vasoconstrictor activity is also present and opposes the relaxation of vascular smooth muscle (Buckwalter & Clifford, 2001; Hamann et al., 2002). However, the vasodilating mechanisms, along with the exercising muscle substances that work to blunt sympathetic vasoconstriction, termed functional sympatholysis (Remensnyder et al., 1962; Saltin et al., 1998), prevail neural sympathetic vasoconstriction.

While mechanisms that reduce vascular smooth muscle tone are the principal determinants of increased vascular conductance at the onset of exercise, resistance vessels are elastic and therefore distensible in response to increases in transmural pressure (Jasperse et al., 2015; Lynn et al., 2020; Walker et al., 2007). Previous findings from our laboratory have demonstrated an increase in FBF when the arm was moved from an above to below heart position during a contraction, where gravity works to increase the local transmural pressure (Lynn et al., 2020; Walker et al., 2007). This increase in FBF was greater than what would be explained by the increase in perfusion pressure in the below-heart position; thus, calculated FVC was increased. This increase in FVC was attributed to the distensibility of the arterioles as active vasodilating mechanisms remained stable when the arm was moved during a contraction. Therefore, the purpose of the current study was to explore the relationship between  $FVC_D$  and active vasodilation magnitude. We predicted that this relationship would be linear: as active vasodilation magnitude increased, so would  $FVC_D$ . Although both FBF and FVC increased with increasing active vasodilation magnitude, when transmural pressure was increased via arm repositioning,  $FVC_D$  was not different between LO and HI conditions. We interpret these findings to support the idea that  $FVC_D$  is not dependent on the active vasodilation magnitudes created in the current study.

### **Strengths of the thesis work**

In the current model, we assessed the FBF and FVC responses that occurred within the first cardiac cycle following a single contraction. This allowed us to avoid any effects of venous filling when the arm was moved into a below heart position. Further, we can be confident in our ability to isolate active vasodilation magnitude as a single handgrip contraction was initiated prior to repositioning the arm. Additionally, as all contractions despite arm position condition were initiated in the above heart position, we can be confident that initial perfusion pressure also remains consistent throughout the protocol.

### **Limitations of the thesis work**

There are several aspects to the current methodology that were limiting and require consideration. First, while an increase in transmural pressure was demonstrated when the arm was moved to a below heart position regardless of contraction intensity, transmural pressure tapers along the microvasculature. Thus, the transmural pressure in the arterioles is unknown. Second, the current methodology consists of a single contraction protocol; thus, limiting our ability to create a robust active vasodilation range. Therefore, not only can the current results only speak to the small active vasodilation range created, but perhaps the created range was not different or great enough to detect an influence of active vasodilation magnitude on  $FVC_D$ . Lastly, the current hypothesis was developed based on our current understanding of the forces at play regarding the structure of the arteriole wall. Transmural pressure is the difference between outward acting pressure of blood volume against resistance vessel walls and forces external to the vessel acting inward. The resulting distension of the vessel is therefore determined not only

by the vessel wall inward acting forces, but also all forces external to the vessel walls. The magnitude of inward force exhibited by the vascular smooth muscle depends on vascular smooth muscle tone. When the smooth muscle is more constricted, the magnitude of inward force is larger; conversely, when the smooth muscle is in a more relaxed state, the magnitude of inward force is less. However, this schema fails to consider that the arterioles in question are not isolated from their surrounding environment. The current model does not consider extravascular inward facing forces that stem from the surrounding adventitia, adjacent vasculature, surrounding muscle tissue and other tissues, and interstitial fluid, and their role in determining the total forearm vascular conductance. Ultimately, these extravascular forces are inevitable and difficult to account for in human in vivo models.

### **Future Directions**

This study is the first to explore the mechanical distensibility of arterioles and its relationship to active vasodilation magnitude in response to an increase in transmural pressure. This study has demonstrated that FBF and FVC increase with increased active vasodilation and increase further with increased transmural pressure. Interestingly,  $FVC_D$  in response to an increase in transmural pressure was not dependent on the range of active vasodilation magnitude created as we had hypothesized. However, the current study is restricted to the limited range of active vasodilation magnitude created by the single contraction protocol. Further research should consider utilizing a steady state model to create a greater range of active vasodilation magnitudes. Further, individual response data (not shown) demonstrated a large variability in interindividual responses to increased transmural pressure. Thus, future research

may consider exploring the interindividual response is arteriolar distensibility as it pertains to exercising muscle blood flow and vascular conductance.

## Appendix A: Subject Information Forms



Dear Dr. Tschakovsky:

**RE: HSREB Annual Renewal Clearance TRAQ #: 6005158" PHE-036-02 Non-Invasive Investigation into Peripheral Vascular Control in Humans"**

**Date Ethics Clearance Effective: July 06, 2022**

**Ethics Clearance Expiry Date: July 06, 2023**

This email serves as your notification that the Queen's University Health Sciences & Affiliated Teaching Hospitals Research Ethics Board (HSREB) has reviewed your annual renewal event form submission and ethics clearance is extended for one year as indicated above.

Prior to the expiration of your ethics clearance, you will be reminded to submit your renewal report through TRAQ. Any lapses in ethical clearance will be documented above. Ethics Clearance from the HSREB must be renewed at least once per year ([TCPS 2 Article 6.14](#)).

Regards,

A handwritten signature in cursive script that reads "Albert F. Clark".

Albert F. Clark, PhD  
Chair, Queen's University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board

The HSREB operates in compliance with, and is constituted in accordance with, the requirements of the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the international Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Product Regulations; Part 3 of the Medical Devices Regulations, and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The HSREB is qualified through the CTO REB Qualification Program and is registered with the U.S. Department of Health and Human Services (DHHS) Office for Human Research Protection (OHRP). Federalwide Assurance Number: FWA#: 00004184, IRB#: 00001173. HSREB members involved in the research project do not participate in the review, discussion or decision.

**School of Kinesiology and Health Studies**



**MEDICAL QUESTIONNAIRE FOR RESEARCH STUDY**

**Understanding the contribution of arteriolar  
distension to rapid onset vasodilation during exercise**

**Faculty Investigator:**

Michael Tschakovsky, PhD, School of Kinesiology and Health Studies

**Graduate Student Investigator: Taylor Liu-Kuo**

*To the study participant:* Please answer all questions in sections 1 and 2 of this form.

**Identification code (To be filled out by investigator)** \_\_\_\_\_

**SECTION 1: PERSONAL DATA (please print)**

Year of Birth: \_\_\_\_\_

Date filling out form: \_\_\_\_\_

**SECTION 2: MEDICAL HISTORY**

1. Do you have, or have you ever had, problems with any of the following?

	Yes	No
i. Heart or blood vessels	___	___

**(these might include but are not limited to: heart attack, stroke, heart murmur, angina, coronary artery disease, high blood pressure, high cholesterol, congenital heart disease, any heart operation, anemia, bleeding or clotting disorders)**

ii. Kidney	___	___
iii. Nerves or brain	___	___
iv. Breathing or lungs	___	___
v. Hormones, thyroid, or diabetes	___	___
vi. Muscles, joints, or bones*	___	___

vi. Other (please list) \_\_\_\_\_

\*related to the neck or arms

2. Please list the diagnosis and/or briefly describe any problems identified above

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3. Are you presently taking any medications? If yes, please list.

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4. Do you have any allergies/sensitivities to adhesive tape or latex?

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5. Do you currently smoke (cigarettes or vapes)? \_\_\_\_\_ **yes/no**

If previous smoker date of last cigarette/vape \_\_\_\_\_ (year, month)

**6. To be completed on the day of GTN administration visits only.**

Have you consumed any alcoholic beverages within the last 12h? yes/no \_\_\_\_\_  
(initials) \_\_\_\_\_ (date).

Have you consumed any food within the last 4h? yes/no  
\_\_\_\_\_ (initials) \_\_\_\_\_ (date).

**For women only:**

Are you pregnant or is there a chance that you could be pregnant? **Yes/no**

Hormonal fluctuations during the menstrual cycle can impact vascular function. We need to schedule your two visits so that they are in the same phase of your menstrual cycle, specifically, days 1-7 of the menstrual phase.

Please answer the following questions (*i - vi*) regarding your menstrual cycle history:

i. Are you currently having menstrual periods?

\_\_\_\_ *No (skip rest of form).*

\_\_\_\_ *Yes*

Date of the start of last menstrual period \_\_\_\_\_

ii. Have you given birth in the last 12 months?

\_\_\_\_ *No*

\_\_\_\_ *Yes (skip rest of form).*

iii. Are you currently taking oral or any other form of hormonal contraceptives (e.g. intrauterine devices)?

\_\_\_\_ *Yes Brand* \_\_\_\_\_

\_\_\_\_ *No*

iv. Currently, what is the average duration of your menstrual cycle (A full cycle goes from the start of menstrual flow [menses] to the start of the next menstrual flow [menses])? The average cycle length is 28 days.

\_\_\_\_ *days.*

v. How many days do you typically experience menstrual flow each cycle?

Please check the correct response below:

*0 days*

*1 day*

*2 days*

*3 days*

*4 days*

*5+ days*

vi. Please estimate the number of menstrual cycles you have had in the past 12 months:

\_\_\_\_ *(number) menstrual cycles.*

**SECTION 3: DEMOGRAPHIC QUESTIONS**

1. What was your assigned sex at birth? (Check ONE only):

- Male
- Female
- Undetermined

2. Which best describes your current gender identity? (Check ONE only):

- Male or primarily masculine
- Female or primarily feminine
- Indigenous or other cultural gender minority identity (e.g. Two-Spirit)
- Non-binary
- Not listed (e.g. gender fluid)

3. Which of the following BEST describes your racial or ethnic group? (Check ONE only):

- White (European decent)
- Black (African, Afro-Caribbean, African-American decent)
- East/Southeast Asian ( e.g. Chinese, Korean, Japanese, Cambodian, Vietnamese)
- Latino (Latin American, Hispanic decent)
- Middle Eastern (Arab, Persian, West Asian decent, e.g. Afghan, Egyptian, Iranian)
- South Asian (South Asian decent e.g. East Indian, Pakistani, Bangladeshi, Sri Lankan)
- Other (please briefly describe) \_\_\_\_\_

**I acknowledge that I or the study investigators completed this form according to my specifications; this information is true to the best of my knowledge.**

\_\_\_\_\_

Participant Name

\_\_\_\_\_

Participant Signature

Date (dd/mm/yyyy) : \_\_\_\_\_



**“Peripheral Vascular Control in Humans”** Research Program  
 Human Vascular Control Laboratory  
 Room 400B, Kinesiology and Health Studies Building  
 Michael E. Tschakovsky, Ph.D., PRINCIPAL INVESTIGATOR  
 Funded by Natural Sciences and Engineering Research Council of Canada Discovery Grant  
 RGPIN-2022-05309

**LETTER OF INFORMATION AND CONSENT FORM  
 FOR RESEARCH PROJECTS ENTITLED:  
*Investigation into Peripheral Vascular Control in Humans***

You are invited to participate in a research study conducted under the “Peripheral Vascular Control in Humans” Research Program.

This is an important form. Please read it carefully. It tells you what you need to know about this study. If you agree to take part in this research study, you need to sign this form. Your signature means that you have been told about the study and what the risks are. Your signature on this form also means that you want to take part in this study.

This study has been reviewed for ethical compliance by the Queen’s University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board.

**Purpose of the Research:**

*The purpose of this study is to improve our understanding of how the flow of blood through the cardiovascular system is controlled in exercise.*

**Benefits of Research in General, and Benefits For You:**

This research advances basic understanding of cardiovascular physiology in exercise. There are no direct benefits to you by participating in this study.

**Conflicts of Interest, Personal Benefit to Researchers AND/OR Possibility of Commercialization of Research Findings:**

There are no real, potential or perceived conflicts of interest/personal benefits to the researchers. There is no possibility of commercialization of research findings.

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**Description of Experiment and Risks:**

What will happen? During this study, you will take part in the specific experimental procedures below that have a check mark. **They may be performed at one or more of three sites: The Human Vascular Control Laboratory (HVCL) on the Queen’s University Campus, the Laboratory for Clinical Exercise Physiology (LACEP) of Dr. Alberto Neder in Kingston General Hospital, or at Hotel Dieu Hospital. Depending on the specific experimental protocol, the combination of these procedures will be different. The investigator will explain to you in detail how each of these procedures will be combined in the study involving your participation. Please initial by each bullet point that is marked.**

The investigator will also identify the time commitment for participation in each stage of the research.

The data collected will quantify one or more of: cardiovascular responses including blood pressure, blood flow, the output of the heart; muscle responses such as muscle electrical activity and muscle force; blood characteristics such as oxygen content or products of muscle metabolism in exercise; perception of effort and discomfort; breathing and consumption of oxygen. These measurements allow us to understand the responses to exercise.

**There is a remote possibility that during your research activities you could come into contact with someone with COVID-19. If this highly unlikely event were to occur, we are required by the Public Health Unit to retain on file your email address or phone number to share with them for contact tracing purposes.**

- **HEART RATE MEASUREMENTS:** Heart rate is continuously monitored by an electrocardiogram (EKG) through 3 spot electrodes on the skin surface. The electrodes are normally placed in the lower portion of the chest and they can detect the electrical activity that makes your heart beat.

**RISKS:** This procedure is entirely safe. In a very small group of individuals, a skin rash might occur from the adhesive on the electrodes. There is no way of knowing this ahead of time. The rash, if it develops, will resolve itself within a day or so. Avoid scratching the rash and keep clean.

- **BLOOD PRESSURE MEASUREMENTS:**
  1. A cuff that can be inflated with air is wrapped around your upper arm, just as would occur if you had your blood pressure measured at the doctor’s office. This cuff is inflated to a pressure higher than your systolic blood pressure (the pressure in your blood vessels when the heart beats), and gradually deflated over a number of seconds to measure systolic blood pressure and diastolic (the pressure in your blood vessels when the heart is relaxed) blood pressure. Meanwhile, your wrist is secured in a wrist brace and a small pressure sensor is placed over your radial artery at the wrist. This pressure sensor is able to detect the increases and decreases in size of your radial artery that occur with each heart beat, and what the pressure sensor

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measures is compared to the pressure that the upper arm cuff measures (this calibrates the sensor). From then on, the pressure sensor at the wrist measures blood pressure continuously, while the upper arm cuff may be inflated intermittently.

OR

2. A small cuff is fit around your finger. This cuff inflates to pressures that match the blood pressure in your finger, so you feel the cuff pulsing with your heart beat. It shines infrared light through your finger to measure changes in the size of your finger with each heartbeat.

**RISKS:** These techniques are non-invasive and pose no risk.

- **LIMB BLOOD FLOW AND BLOOD VESSEL DIAMETER MEASUREMENTS:** The blood flowing through your brachial (above the elbow), radial (above the wrist), or femoral (above the groin) artery can be detected and your artery diameter measured using Doppler and imaging ultrasound. A probe will be placed on the skin over your artery and adjustments in its position controlled by hand by the investigator. Measurement of femoral artery flow takes place on the lower abdomen just above the groin. Shorts will be tied up at the site of measurement to expose the skin in this region. High frequency sound (ultrasound) will penetrate your skin. The returning sound provides information on blood vessel size and blood flow.

**RISKS:** This technique is non-invasive and poses no risk.

- **ELECTROENCEPHALOGRAPHY (EEG):** This measures the electrical activity of your brain. Depending on the type of electrical activity of interest, electrodes will be placed on your scalp in different arrangements.

**RISKS:** This procedure is entirely safe and painless. In a very small group of individuals, a skin rash might occur from the adhesive on the electrodes. There is no way of knowing this ahead of time. The rash, if it develops, will resolve itself within a day or so. Avoid scratching the rash and keep clean.

- **ELECTROMYOGRAPHY (EMG):** This measures the electrical activity of your muscles. Electrodes will be placed on muscles of interest for a given study.

**RISKS:** This procedure is entirely safe. In a very small group of individuals, a skin rash might occur from the adhesive on the electrodes. There is no way of knowing this ahead of time. The rash, if it develops, will resolve itself within a day or so. Avoid scratching the rash and keep clean.

- **GAS EXCHANGE:** This measures your breathing and the changes in oxygen and carbon dioxide as a result of your body utilizing oxygen and producing carbon dioxide. It involves breathing through a mouthpiece attached to a one way valve system, and wearing nose clips.

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**RISKS: This procedure is entirely safe. There are no known risks.**

- **MULTIPLE INERT GAS REBREATHE:** This technique measures the amount of blood pumped by your heart. You will breathe through a mouthpiece connected to an inflatable bag. This bag will contain two gases (nitrous oxide, sulfur hexafluoride) that are completely inert, which means that they do not react with anything in your body and are completely safe to breathe. When measurements are taken, you will be asked to breathe at a certain rhythm for up to 10 seconds and to inhale enough to empty the bag with each breath in.

**RISKS:** The inert gases are completely safe to breathe. There are no known risks to this procedure.

- **NEAR INFRARED SPECTROSCOPY:** This technique is used to measure the oxygen in your muscle. It consists of an infrared light emitter and sensor block that is positioned on the skin surface of your leg or arm and secured by wrapping with bands that prevent penetration of outside light. The infrared light that shines into your tissue is partially absorbed and partially reflected back to the sensor and this provides information on how much oxygen there is in the blood in your muscle.

**RISKS:** There are no risks to this non-invasive procedure.

- **FEMORAL NERVE MAGNETIC STIMULATION:** This technique involves placing a magnetic coil over your femoral nerve just above your thigh. This magnetic coil is activated in order to cause your femoral nerve to fire. This will result in the thigh muscles on the front of your thigh to contract.

**RISKS:** The sensation of nerve stimulated muscle contraction can be strange and even uncomfortable. However, this technique is well established and has minimal risk, other than the typical risk of muscle strain that occurs with any everyday activity involving voluntary muscle contraction.

- **VENOUS BLOOD SAMPLING:** Blood samples from veins are used to measure the amount of lactic acid and oxygen in your blood. We need to take a blood sample from a vein on the back of your hand, after we have increased blood flow to that hand by having you hold it in tolerably hot water until blood flow is maximized. For this, a researcher trained and certified in venipuncture (needle or catheter placement into a vein) will use sterile technique to draw a blood sample of ~1 ml into a syringe. We also need to take multiple 1 ml samples of blood from a vein at the elbow. In this instance, the researcher will place a teflon catheter into your vein using sterile technique. The catheter will be secured to your skin with tape and a self-sealing access attached to allow for drawing blood from the vein. We will take a volume of blood that is in total no more than ~120 ml. This represents approximately 1/3 of the volume of blood taken when you donate blood (370-400 ml). Periodically, the researcher may, after drawing some blood, inject (flush) sterile saline through the catheter into your vein. When the study is over, we will

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remove the catheter and secure sterile gauze over the puncture site. Blood samples for blood gas analysis are analyzed within hours of collection and then discarded using appropriate biohazard protocol. Blood samples for analysis of hormones are prepared according to analysis requirements and frozen for later analysis.

**RISKS:** The most common complications of inserting a small catheter in the arm is a small bruise and pain at the site of catheter insertion. This might last several days after removal of the catheter. It is also possible that this pain may refer down the arm (a “shooting” pain sensation), if there has been nerve irritation in the catheterization process. When the catheter is removed pressure must be applied to the vein to prevent internal bleeding. If adequate pressure is not applied a bruise and some discomfort might result for a short period of time. The puncture site should be kept clean and covered with a sterile gauze pad while stopping the bleeding after catheter removal to prevent infection. There is very little risk of infection or injury to the vein. The amount of blood taken can result in at most a 2% reduction in the hemoglobin content in your blood (hemoglobin carries oxygen in your blood), in comparison to ~7.5% reductions experienced when you donate blood. Nevertheless, this 2% does constitute a very mild anemia, and in the case of a person with chronic hemoglobin disorders it could increase the risk of adverse health consequences.

- **FOREARM AND LEG VOLUME MEASUREMENTS:** The volume of your forearm or calf can be measured by a thin, stretchable rubber band placed around your respective limb that is filled with mercury. A very small electrical current runs through this gauge and changes in the length of this mercury-filled rubber band are detected by changes in this current that occur in proportion to changes in the length of the rubber band.

**RISKS:** This technique is non-invasive and poses no risk.

- **BLOOD OXYGEN CONTENT:** A plastic clip is placed over your left index finger. This clip aims light through your finger, and the absorption of that light by the blood provides information on how much oxygen the blood contains.

**RISKS:** This technique poses no risks.

- **MUSCLE MASS:** Circumference and length measurements of segments of your arm or leg will be taken via manual placement of a tape measure on your limbs by the investigator.

OR

At Kingston General Hospital, you will lay on a table and a scan of your body will be performed using a technique called “dual-energy x-ray absorptiometry” (DXA). This technique uses a small amount of x-ray energy to scan a “picture” of your body and identify how much muscle there is on your arms and legs.

**RISKS:** Radiation levels with DXA are considered trivial by radiation regulatory agencies. The technique uses less radiation than a dental X-ray, roughly equivalent to

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the background amount a person would be exposed to when flying from Cincinnati to the West Coast. This is a mere fraction of the radiation dose we are all exposed to every week, from just being alive.

- **FOREARM OR LEG OCCLUSION:** In order to completely block the blood flow through your forearm or leg, a pressure cuff will be inflated around your arm or around your upper or lower leg for 1-10 min or inflated and deflated rhythmically depending on the protocol. You may feel a strong pressure and some mild tingling with cuff inflation but it should not be uncomfortable. If there is pain, immediately notify the investigator and the cuff will be deflated and repositioned. Upon cuff release there will be a large rush of blood into your forearm or leg. This may feel warm and you may experience mild tingling but no discomfort.

**RISKS:** This technique is non-invasive and poses no risk.

- **FOREARM COMPRESSION:** A stylus will be positioned over your artery pulse to control the amount of flow through the artery. The arterial compression provided by the stylus will be varied to create different blood flow profiles. Increases in stylus downward pressure will result in decreases in blood flow, while controlled release of stylus downward pressure will result in increases in blood flow. The blood flow to your limb will never be completely occluded by the arterial compression. In some cases, manual finger pressure will be used instead of the stylus.

OR

A cuff will be positioned around your forearm or leg, and can be inflated and deflated at will to increase and decrease blood flow to your limb.

**RISKS:** The brachial artery and nerve run close together, thus the compression of this particular artery may result in a tingling sensation and some temporary numbness in the forearm. The compression of the artery can also become somewhat uncomfortable over time. These symptoms will subside within 5 minutes of compression release. There are no risks to your forearm from temporarily stopping blood flow to the forearm.

- **FOREARM OR HAND HEATING:** In order to increase the blood flow through your brachial artery and/or radial artery, your forearm or hand will be enclosed in a water bath that is circulated with warm water. The warm water will result in the dilation of your skin blood vessels. The water bath consists of a cylinder that is circulated with heated water. Your arm will rest inside the tube enclosed in a plastic glove that prevents your skin from being in direct contact with the water. A temperature sensor will be fixed to your skin and your skin temperature will be maintained between 41 and 42° Celsius. The water for the bath is heated remotely to a temperature not exceeding 45° Celsius and is circulated into the bath via a water pump. The water in the bath will feel quite warm, but not too hot. If at any time you feel discomfort the warm water inflow will be stopped and replaced with cooler water to allow the bath temperature to drop to a more comfortable level. Your forearm may be heated for a total of one to two hours.

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**RISKS:** When the skin blood vessels fill with blood for an extended period while undergoing arterial compression it causes a temporary swelling as some fluid escapes from the blood vessels into the surrounding tissue. This minor swelling should resolve itself within 24 hours. Elevation of the arm will help to speed up the process. Your skin may appear red after removal from the bath. This is due to the increased skin circulation. The redness should resolve within 24-36 hours.

- **CONGESTION OF YOUR FOREARM OR LEG VEINS:** One inflatable cuff will be placed around your upper arm or above the knee and another may be placed around your wrist or ankle. The wrist cuff will be inflated to a pressure that prevents blood flow to your hand for a period of 10-15 minutes at a time. This should not be uncomfortable. If it is, notify the investigator and the position of the cuff will be adjusted until inflation without discomfort is achieved. These cuffs will be inflated to pressures that feel like a mild to moderate squeeze. This will prevent blood from flowing out of your limb back to the heart, but allow blood to flow in to your arm. Your limb will fill with blood and if the cuff inflation is maintained for a number of minutes, you may feel a sensation of swelling. This is because some of the plasma (water portion of your blood) will leak out of the small blood vessels and into the space between other cells in your limb. This is similar to when you stand up in the morning and stay upright during the day. In that case, gravity makes it difficult for blood to flow back to the heart from the legs, and they slowly swell over the course of the day as plasma leaves the blood vessels. When the cuff is released, the limb will slowly return to normal as the plasma moves back into the blood vessels.

**RISKS:** The movement of fluid out of the blood vessels into your limb may in extreme cases cause discomfort. This discomfort should resolve itself within minutes of deflating the cuff, and the swelling should subside within 24 hrs. Elevating the arm above the heart for 15 minutes should speed this process.

- **INTERMITTENT COMPRESSION OF THE FOREARM OR LEG:** You will have an inflatable cuff placed around your forearm or leg. We can rapidly inflate and deflate this cuff to different pressures that are able to squeeze the blood out of the veins in your limb. Inflation is maintained for only a brief period of time (a few seconds). The sensation of limb compression will feel like a strong grip, but should not be painful. If it is uncomfortable, notify the investigator and the position of the cuff can be adjusted.

**RISKS:** There are no risks associated with this procedure.

- **ALTERNATING FOREARM SUCTION AND COMPRESSION:** Your forearm will be enclosed in a plexiglass box and sealed with a neoprene sleeve around the upper arm. Suction or compression of your forearm can be created by rapidly adding or removing air in the box via a connected automated air compressor. The sensation of suction and compression should not be painful. Notify the investigator if there are any feelings of discomfort.

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**RISKS:** There are no risks associated with this procedure.

- **EXERCISE MANEUVERS THAT ALTER BLOOD PRESSURE:** You may be asked to perform one of the following MANEUVERS to temporarily increase your blood pressure: 1) squeezing a handgripper with your forearm for a few minutes with or without blood flow to your forearm being prevented 2) contracting your leg muscles with or without blood flow to your leg being prevented.

**RISKS:** When muscle contractions are performed while the blood flow to the limb is prevented, you may experience considerable discomfort similar to that when doing maximal weightlifting repetitions. However, there is no risk to your muscles in performing this exercise.

- **STROOP TEST:** In order to create a mental stress a “STROOP” test will be performed. A series of words for colours will be displayed such as “RED”. However, the word will be displayed in a different colour, perhaps the colour green. You must read out the colour in which the word is written in, not the word itself. Therefore, upon seeing “RED” (written in green text) you will respond by saying “green”. You will be asked to perform the task as fast as you can. Part of the study evaluates the score you achieve on the test and it is very important that your score achieves the normal range for persons of your age and education. Your performance will be measured by how much of the list you read through in two minutes time, as well as how many mistakes you make.

**RISKS:** There are no risks posed by this procedure.

- **ANGER TEST:** In order to create emotional stress an anger test will be performed. Prior to the testing day, you will have been asked to fill out an anger questionnaire in order to recall a past event that made you very angry. We will use the questionnaire to elicit momentary anger. You will be asked to describe the event while re-experiencing the event in your imagination, as well as report on thoughts, feelings, and physical aspirations about the situation. The test will last two minutes.

**RISKS:** You will feel momentary anger that will subside following the interview. It is possible that this anger interview might contribute to renewing problems between yourself and this individual. If you believe that this might in any way be problematic, you are encouraged to withdraw from participation in this study.

- **CONTROL TEST:** A control test will be performed in order to understand if verbalization is contributing to the blood vessel response. You will simply count from ‘one’ in Mississippi’s. Your verbalization will start as “one Mississippi, two Mississippi, three Mississippi” and will continue for two minutes.

**RISKS:** There are no risks posed by this procedure.

- **LOWER BODY NEGATIVE PRESSURE:** You will lay on your back and your lower body will be enclosed in an air-tight box. Various levels of suction will then be applied to the

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box to simulate how the blood normally shifts in the body during activities like standing up. This will cause your heart rate to increase and your blood vessels to constrict to maintain blood pressure. This is a normal response that you experience every morning when you get up out of bed.

**RISKS:** There is a small chance that you may begin to faint with this procedure. We will be monitoring your blood pressure continuously. If you experience any of the following symptoms, notify the investigator immediately: nausea, narrowing field of vision, sweating. Changes in your blood pressure that we detect will most likely indicate that fainting is imminent well before you experience any of these symptoms. By shutting off the suction, blood will rapidly return to your heart and symptoms of fainting will be reversed. You may feel nauseous for a few hours after this procedure if you came close to fainting. This should resolve itself without any complications.

- **COLD PRESSOR TEST:** In this test, you will place your hand or foot in an ice water bath for a few (1-3) minutes. This will cause your heart rate to increase and your blood vessels to constrict as the cold will activate your sympathetic nervous system (the part of your nervous system involved in the “fight or flight” response).

**RISKS:** There are no risks posed by this procedure. However, it can be quite painful. You have the right at any time to withdraw your hand or foot from the ice water bath if you feel unable to continue.

- **CHEST WALL STRAPPING:** You will have either a tensor bandage or a custom strapping device applied to your chest and abdomen. You are asked to breathe out as much air from your lungs as you can, and then hold that as the strapping is tightened around your chest and abdomen. You can indicate the need to breathe during this procedure at any time. After catching your breath you will again empty your lungs and strapping will continue. This will be repeated until the strapping is complete. The purpose of this is to restrict how much you can expand your chest and abdomen in the effort of breathing in and to reduce the amount of air left in your lungs at the end of a normal expiration. This mimics “restrictive” lung disease.

**RISKS:** The strapping can feel uncomfortable but should not be painful. If it is painful notify the investigator immediately and strapping will be adjusted. There is a small chance that you may begin to faint with this procedure. We will be monitoring your blood pressure continuously. If you experience any of the following symptoms, notify the investigator immediately: nausea, narrowing field of vision, sweating. Changes in your blood pressure that we detect will most likely indicate that fainting is imminent well before you experience any of these symptoms. These are reversed by rapidly removing the strapping and having you rest laying on your back with your legs raised.

- **HANDGRIP EXERCISE:** You will be asked to perform handgrip squeezing exercise. The duration of this exercise can vary from a few seconds to 10-20 minutes, and at an intensity that can vary from very mild to maximal contraction force. Exercise may take place in combination with any of the above-mentioned techniques which can control the

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blood flow to your limbs, congest the limbs, and which can alter your blood pressure.

**RISKS:** When forearm muscle contractions are performed while the blood flow to the forearm is prevented, you may experience considerable discomfort similar to that when doing maximal weightlifting repetitions. However, there is no risk to your muscles in performing this exercise. You may experience muscle soreness in the muscles of your forearm for 24-72 hours after performing the handgrip exercise, much as you would if you had been lifting weights.

- **LEG EXERCISE:** You will be asked to contract your leg muscles, either continuously or intermittently. The duration of this exercise can vary from a few seconds to 10-20 minutes, and at an intensity that can range from very mild to maximal contraction force. Exercise may take place in combination with any of the above-mentioned techniques which can control the blood flow to your limbs, congest the limbs, and which can alter your blood pressure.

**RISKS:** When leg muscle contractions are performed while the blood flow to the forearm is prevented, you may experience considerable discomfort similar to that when doing maximal weightlifting repetitions. However, there is no damage or risk to your leg from this. You may experience muscle soreness in the muscles of your leg for 24-72 hours after performing the leg exercise, much as you would if you had been lifting weights.

- **DIETARY NITRATE CONSUMPTION:** Nitrate is a compound that is present in several foods, including plant foods (vegetables and a few fruits), processed meats, baked goods, cereals, and drinking water. You will be randomly assigned to conditions in which you consume different beverages acting as sources of nitrate. This will be done in a double-blind fashion (meaning that neither you nor the experimenters involved in data collection and analysis will know which condition you are in until the completion of the study). You will be asked to either: 1. consume a specified amount each day for a few days prior to your testing day, and to consume your final amount a few hours before coming to the lab for your testing session, 2. Consume a specified amount a few hours before coming for your testing. The highest quantity of nitrate in these beverage sources is an amount achievable through normal dietary intake by making appropriate selections of high-nitrate-containing foods as part of the Dietary approaches to Stop Hypertension (DASH) diet, and is similar to the dose of dietary nitrate provided in several recent studies. You may experience red urine and red stools during this time period (due to the beverage colour), and this is a normal response. There are no known risks of acute dietary nitrate supplementation. Dietary nitrate can interact with certain medications (proton pump inhibitors, phosphodiesterase type 5 inhibitors, nitroglycerine or other “nitric oxide donor” drugs); if you are taking any of these medications, you will be excluded from the study.

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**RISKS:** There are no known risks of acute dietary nitrate supplementation. Dietary nitrate can interact with certain medications (proton pump inhibitors, phosphodiesterase type 5 inhibitors, nitroglycerine or other “nitric oxide donor” drugs); if you are taking any of these medications, you will be excluded from the study.

#### **How long will the study take?**

On an initial visit we will use ultrasound to get an image of the blood vessels in your limbs to determine whether you are eligible to participate in the main study.

For the main study: preparing all the techniques for measuring your response and creating the correct experiment conditions usually takes ~45 minutes. The actual experimental visit will take ~1-3 hours.

#### **Talking and Movements:**

Talking or moving during the times that we are taking measurements will cause variations in the measurements we are making. If you have any discomfort, please let us know immediately and we can temporarily break from data collection. However, if everything is comfortable, please maintain a very quiet posture. Even very slight movements interfere with our experiments.

#### **Special Instructions:**

Participants are asked to not drink alcohol or caffeine during the 12 hours prior to the study. Also, we ask that you do not consume any food during the 4 hours preceding the experiments. You should empty your bladder immediately prior to starting the test. When the study is finished, we will have you sit in the laboratory for a short time to allow you to readjust to the upright posture. These precautions should be enough to prevent any sensations of dizziness. Please be aware that sensations of dizziness are not normal and you should let us know if you experience any discomfort before you leave the laboratory.

#### **Attached Medical Screening Form:**

This questionnaire asks some simple questions about your health. This information is used to guide us with your entry into the study. Current health problems indicated on this form which are related to cardiovascular diseases (including high blood pressure) and liver or kidney problems will exclude you from the study **only** if the particular experiment in question requires healthy **participants**.

#### **Safety Precautions:**

Safety precautions for the study will include the following:

- **Participants** who enter the study will be identified as either healthy men and women,

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insulin resistant, or type II diabetic.

- Before entering the study, you will be screened using a medical screening form. You will not be able to enter the study if anything is found which indicates that it is dangerous for you to participate.
- We will continuously monitor your heart rate and blood pressure, and you will be laying on your back or seated upright. These precautions allow us to quickly identify if you are becoming faint and simply stopping the experimental manipulation will allow you to quickly recover.
- You will be provided with any new information/incidental findings/testing results that may be relevant to your decision to continue or withdraw from study participation (as applicable).

**Information on Stopping Study Participation:**

The investigator may end your participation in the study without your consent in cases where there are ongoing difficulties with obtaining valid measurements, or where there are medical reasons.

**Participant and Study Data Withdrawal:**

Participant AND study data, including biological materials (blood samples) can be withdrawn by e-mail request to the principal investigator, Dr. Michael E. Tschakovsky [mt29@queensu.ca](mailto:mt29@queensu.ca). For each study, a single identifiable master list of participants is created that contains each participant's identification code assigned to the stored study data.

**Access to Study Data During Collection, Use, Analysis, Dissemination, Retention, and/or Disposal:**

Study data will be accessible to the Principal Investigator, the graduate student conducting the study, other members of the study team and regulatory authorities mentioned below in the "confidentiality" section.

**Plans for Storage/Retention/Dissemination/Publication/Disposal of Data:**

Storage of data is indicated in the "confidentiality" section below. Data is retained for a minimum of 5 years as per Queen's University policy. Data is disseminated in published manuscripts, graduate student thesis or conference abstracts without subject identifiers such that individual subjects are not identifiable. After the 5-year period of storage the data files can be permanently deleted from the secure server site.

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Participant Initials    Witness Initials    Day/Month/Year **Ver. 4.0, 07/04/2022**

**Confidentiality:**

All information obtained during the study is strictly confidential and will not be released in a form traceable to you, except to you and your personal physician. A single master list identifying each participant and their non-identifiable study code will be retained in a separate password-protected file folder on a secure Queen's University server. The data labelled with the participant code will be kept in other file folders on the secure Queen's University server. Blood samples with these participant codes are stored prior to analysis in a -80 degree Celsius freezer in the Human Vascular Control Laboratory to prevent biological materials being linked to a participant. which is available only to the investigators and research assistants who will perform statistical analysis of the data. There is a possibility that your data file, including identifying information, may be inspected by officials from the Health Protection Branch in Canada in the course of carrying out regular government functions. The Queen's University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board (HSREB) may require access to study-related records to monitor the ethical conduct of the research. The study results will be used as anonymous data for scientific publications and presentations, or for the education of students in the School of Kinesiology and Health Studies at Queen's University.

**Study Compensation:**

A monetary compensation for your time will be identified by a research team member during the explanation of the study. This will include any expenses you incur and reflect imposition on your time by your participation in this study.

**Freedom to Withdraw from the Study:**

Your participation in this study is voluntary. You can decline to participate in any aspect of the research **without penalty AND/OR loss of benefits AND/OR impact on academic standing.**

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Participant Initials    Witness Initials    Day/Month/Year **Ver. 4.0, 07/04/2022**

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**Participant Statement and Signature Section**

I have read the Letter of Information/Consent Form. I have had all my questions answered. I have been provided a copy and have returned a copy to the Researcher. **By consenting, I have not waived any legal rights in the event of research-related harm.**

If at any time I have further questions, problems or adverse events, I will contact:

Michael E. Tschakovsky, Ph.D.  
(Principal Investigator)  
KHS 306, Kinesiology and Health Studies Building  
Queen's University, Kingston, ON, K7L 3N6  
Tel: (613) 533-6000, ext, 74697

Dr. Samantha King, Ph.D.  
Acting Director, School of Kinesiology and Health Studies  
KHS 206J, Kinesiology and Health Studies Building  
Queen's University, Kingston, ON, K7L 3N6  
Tel: 613-533-6601

If I have any ethics concerns, I will contact:

The Queen's University Health Sciences and Affiliated Teaching Hospitals  
Research Ethics Board (HSREB) at 1844-535-2988 (Toll free in North America) or e-mail  
HSREB@queensu.ca

By signing this consent form, I am indicating that I agree to participate in this study.

\_\_\_\_\_  
Signature of Participant or  
Substitute Decision Maker

\_\_\_\_\_  
Signature of Person Obtaining Consent

\_\_\_\_\_  
Participant or Substitute Decision  
Maker Name (please print)

\_\_\_\_\_  
Name of Person Obtaining Consent (please print)

\_\_\_\_\_  
Date (day/month/year)

\_\_\_\_\_  
Date (day/month/year)

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Participant Initials    \_\_\_\_\_  
Witness Initials    \_\_\_\_\_  
Day/Month/Year **Ver. 4.0, 07/04/2022**

### Seven-Day PAR Instructions / Script

The following is a sample script for the of the seven-day PAR Interview, as administered in this study.

- Now we are going to do a Physical Activity (PA) questionnaire, where I ask you about your PA over the last 7 days. This is simply a recall of actual activities for the past week, and isn't a history of what you "usually" do. It's not a test, and it will not affect the exercise that you do as part of this study, we're just interested in physical activity levels so that we can match our participants based on PA.
- I'm going to start off by asking you some questions about the past week.
- *Questions on page 1 of Seven-Day PAR.*
  
- Over the course of this interview, I'll be asking questions about yesterday, and then working backwards through the previous 7 days.
- So first, let's talk about the time you spent sleeping in the past week.
  - By "**sleeping**", I mean the time you went to bed one night and the time that you got out of bed the next morning. You may not necessarily have been asleep the entire time you were in bed. You may have been reading, watching TV, or doing paperwork. Time spent in sexual activity is not counted as "sleep".
  - Today is (i.e. Monday), so yesterday was (i.e. Sunday). What time did you go to bed (Sunday) night and get up (Monday) morning. Record to the nearest ¼ hour. Do this for each of the 7-d recall. Calculate total time spent sleeping after completing the interview. Did you have any **naps** on (Sunday)? Did you have any **disruptions** to your sleep – any times when you got out of bed for 15 minutes or more?
    - Repeat for all other days
  
- Now I'm going to ask you about physical activities done in the past 7 days. In talking about PA, we will classify activities into 3 categories:
  - The "moderate" category is similar to how you **feel** when you're **walking at a normal pace**, walking as if you were going somewhere
  - The "very hard" category" is similar to how you **feel** when you are **running**
  - The "hard" category just falls in between → in other words, if the activity seems harder than walking but not as strenuous as running, it should go in the hard category
- *These cards* give examples of some activities that fall into each of these categories (*sample activities were shown*).
- I'm going to ask you about the PAs you engaged in during three segments of the day, which includes morning, afternoon, and evening.
  - "Morning" is considered from the time you get up in the morning to the time you have lunch
  - "Afternoon" is from lunch to dinner
  - And "evening" is from dinner until the time you go to bed
  - NOTE: If a meal is skipped, "morning" is from the time a person wakes up to 12:00 pm, afternoon from 12:00-6:00pm, and evening from 6pm to bed.
- For this interview we are **not** considering light activities such as desk work, standing, light housework, strolling, and stop-and-go walking such as grocery shopping or window shopping.
- We **are** interested in occupational, household, and sports activities that make you **feel** similar to how you feel when you are walking at a normal pace.
- Remember that this is a recall of activities for the past week, not a history of what you usually do.
  - We'll start with yesterday. Today is (i.e. Monday), so yesterday was (i.e. Sunday). Think about what you did in general yesterday morning. Did you do any PA (Sunday morning)? How long did you do that activity? How much of that time was spent standing still or taking breaks? Did that activity feel similar

- to how you feel when you are walking or running or is it somewhere in between? Did you do any PA (Sunday afternoon)? (Duration, intensity). Did you do any PA (Sunday evening)? (Duration, intensity).
- If people are giving too much information, it is appropriate to ask “how much time in general?” – i.e. remind them they do not need to account for every minute of the day. **For an activity to be counted, it must add up to at least 10 min in one intensity category for one segment of the day (round to 15 min).**

1. **At the end of each day:** Are there any PAs you might have forgotten? Did you do any PA at work? Any other recreational or sport activities? Housework or gardening? Were there any other walks that you might have taken?
2. **On the last day of recall:** Take a moment to think back over the course of the week and think of any activities you may have forgotten.
3. **Last question:** The last question I’m going to ask you is, “Compared to your PA over the past 3 mo, was last week’s PA more, less, or about the same?”
4. Thank you.

Prompting questions (examples):

- What were you doing [day] morning?
- You said that you got up at 6am. Did you go anywhere after that?
- Did you watch any particular TV show?
- What did you make for dinner?
- What did you do that evening?
- Did you take any walks that you may have overlooked?
- Did you do any vigorous home repair or gardening?
- Are there any activities that you are unsure about?

Scoring

10 min and 22 min are rounded to 15 min = 0.25

23 min and 37 min are rounded to 30 min = 0.5

38 min and 52 min are rounded to 45 min = 0.75

53 min and 67 min are rounded to 60 min = 1.0

68 min and 1 hr 22 min are rounded to 1hr 15 min = 1.25

*Note:* This script was adapted from reference [1].



**Physical Activity Recall**

Day of the week form completed:  Sunday  Thursday  
 Monday  Friday  
 Tuesday  Saturday  
 Wednesday

Date: \_\_\_\_/\_\_\_\_/\_\_\_\_  
month / day / year

1. Were you employed in the last seven days (paid or volunteer)?  YES  NO →

2. How many days of the last seven did you work?  (round to nearest day)

3. How many total hours did you work in the last seven days?  hours

4. What days of the week do you consider to be your weekend or non-work days? For most people, this would be Saturday and Sunday, but it may be different for you.

- Sunday  Monday  Tuesday  Wednesday  Thursday  Friday  Saturday

\*\*\*\*\**Explain Moderate, Hard, and Very Hard Intensity levels*\*\*\*\*\*

*At the end of the interview:*

5. Compared to your physical activity over the past three months, was last week's physical activity more, less or about the same?

- More  
 Less  
 About the same

Subject ID: \_\_\_\_\_ Interviewer Initials: \_\_\_\_\_

Naps [+] / Disruptions [-] > 30 min

	Yesterday						One Week Ago
Sleep	-	-	-	-	-	-	-
Moderate							
Hard							
Very Hard							
Moderate							
Hard							
Very Hard							
Moderate							
Hard							
Very Hard							

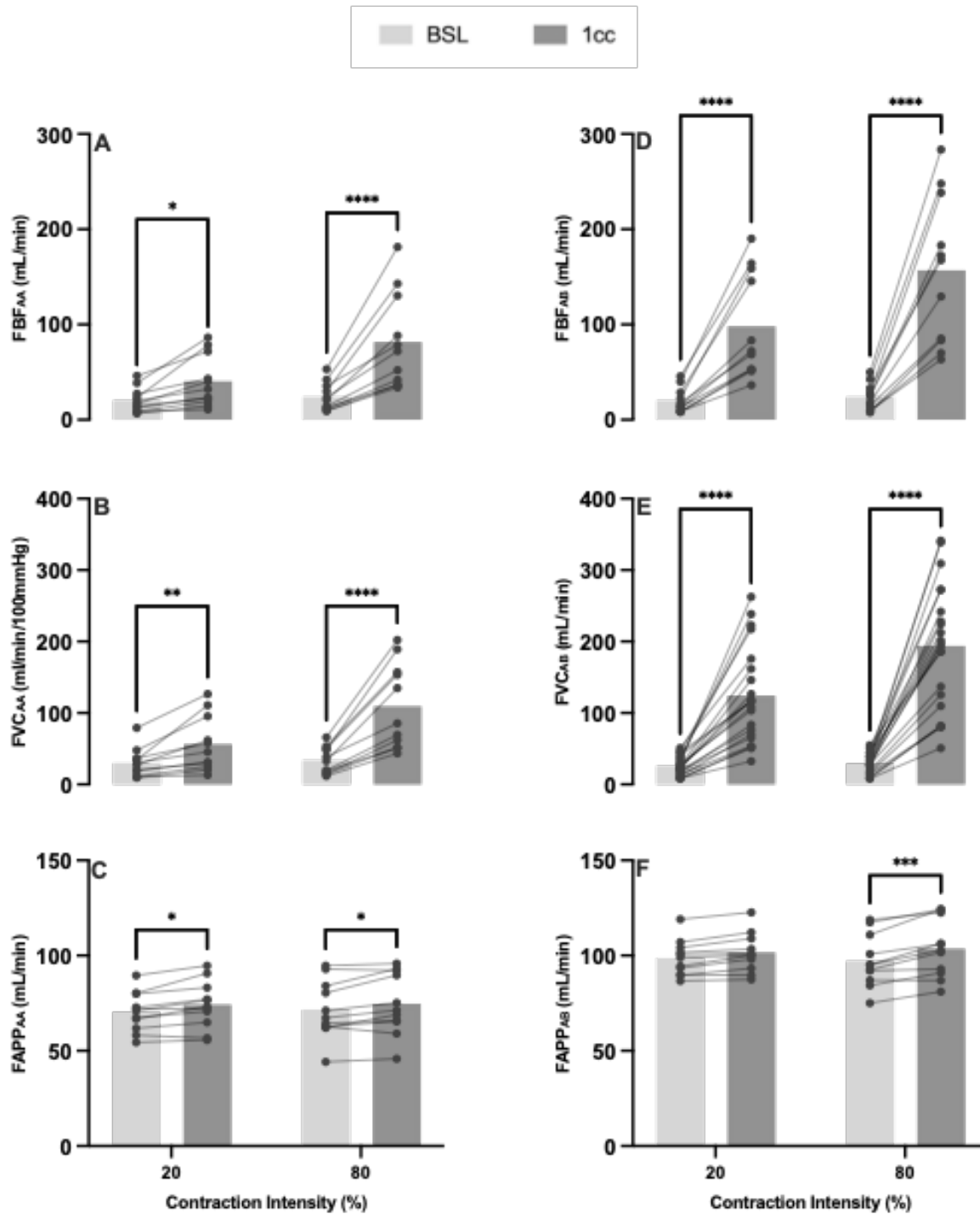
**Rounding:** 10-22mins = .25hrs    23-37mins = .50hrs    38-52mins= .75hrs    53-1:07mins=1.0hrs    1:08-1:22= 1.25hrs  
 Subject ID: \_\_\_\_\_ Interviewer Initials: \_\_\_\_\_



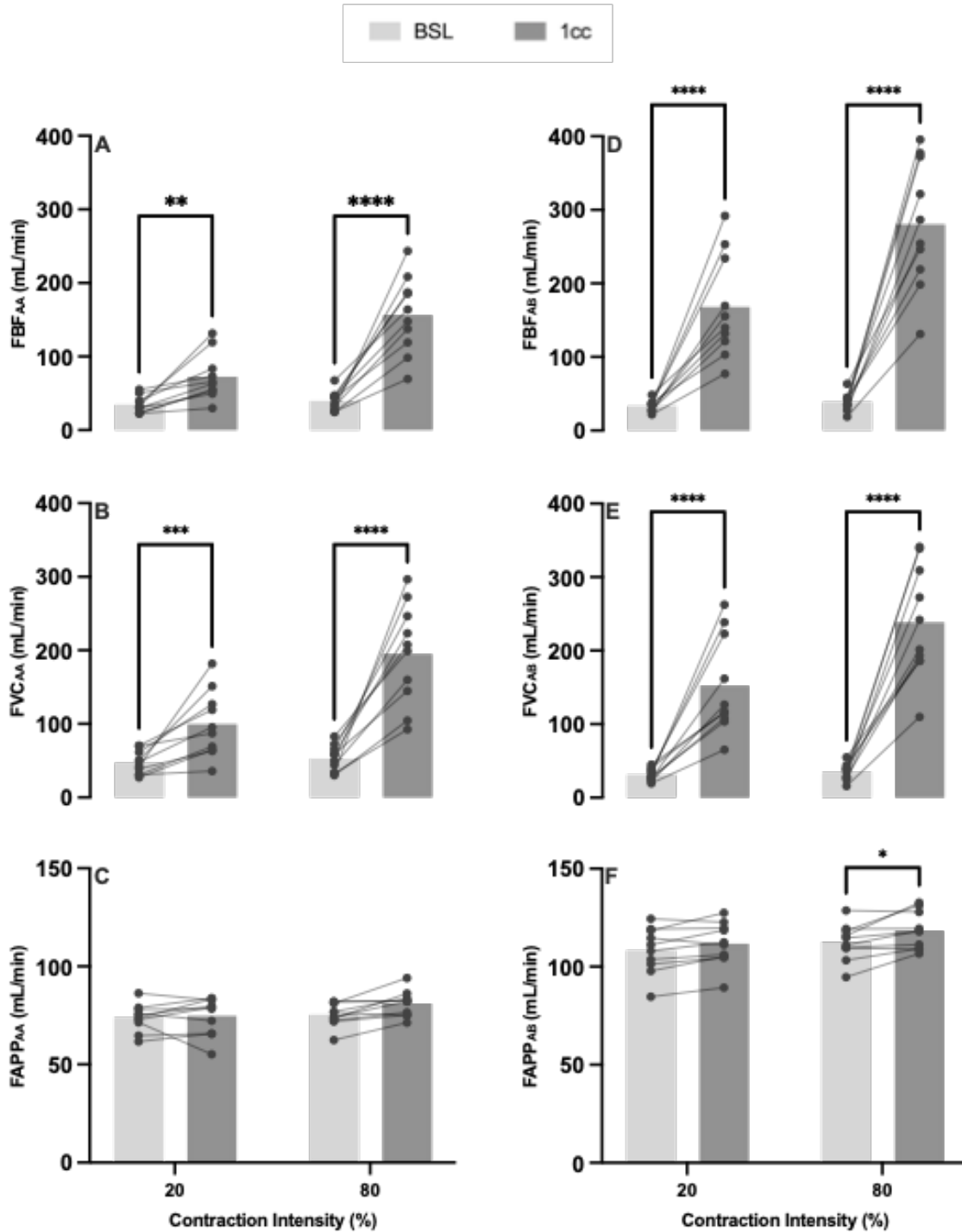
## References

- [1] , Seven-Day Physical Activity Recall, *Medicine & Science in Sports & Exercise*, **29** (1997).

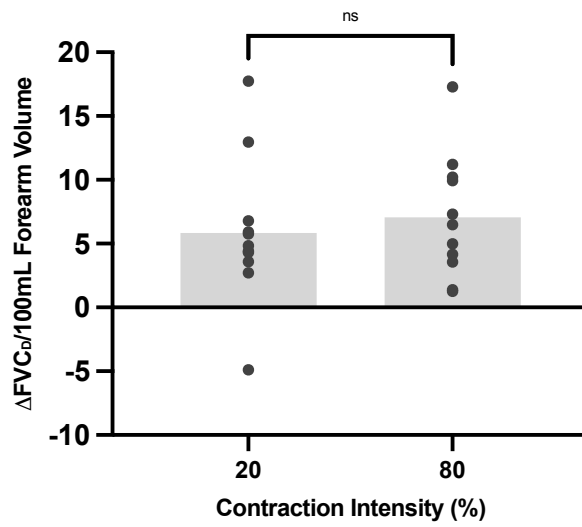
**Appendix B: Sex Differences**



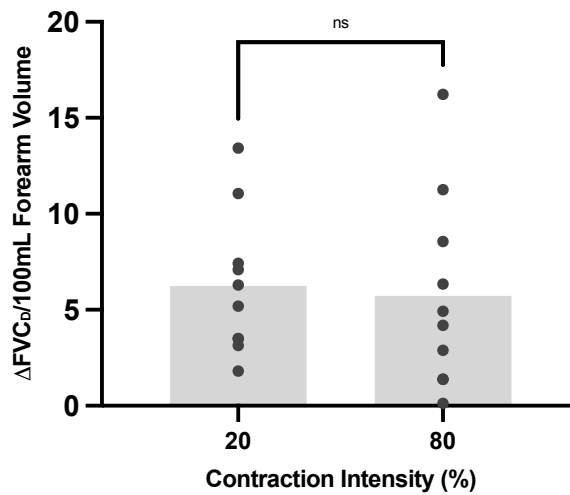
**Figure 9.** Female participant responses. Forearm blood flow in the above-above heart condition (FBF<sub>AA</sub>, panel A) and in the above-below heart condition (FBF<sub>AB</sub>, panel D) during a 10s average baseline (BSL) and during the first cardiac cycle following a contraction (1cc); Forearm vascular conductance in the above-above heart condition (FVC<sub>AA</sub>, panel B) and in the above-below heart condition (FVC<sub>AB</sub>, panel E) during BSL and 1cc; Forearm arterial perfusion pressure in the above-above heart condition (FAPP<sub>AA</sub>, panel C) and the above-below heart condition (FAPP<sub>AB</sub>, panel F) during BSL and 1cc. \* 0.05 > P ≥ 0.01. \*\* 0.01 > P ≥ 0.001. \*\*\* 0.001 > P ≥ 0.0001. \*\*\*\* P < 0.0001.



**Figure 10.** Male participant responses. Forearm blood flow in the above-above heart condition ( $FBF_{AA}$ , panel A) and in the above-below heart condition ( $FBF_{AB}$ , panel D) during a 10s average baseline (BSL) and during the first cardiac cycle following a contraction (1cc); Forearm vascular conductance in the above-above heart condition ( $FVC_{AA}$ , panel B) and in the above-below heart condition ( $FVC_{AB}$ , panel E) during BSL and 1cc; Forearm arterial perfusion pressure in the above-above heart condition ( $FAPP_{AA}$ , panel C) and the above-below heart condition ( $FAPP_{AB}$ , panel F) during BSL and 1cc. \*  $0.05 > P \geq 0.01$ . \*\*  $0.01 > P \geq 0.001$ . \*\*\*  $0.001 > P \geq 0.0001$ . \*\*\*\*  $P < 0.0001$ .



**Figure 11.** Change in forearm vascular conductance due to distension in female participants.



**Figure 12.** Change in forearm vascular conductance due to distension in male participants.

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