THE EFFECT OF UPRIGHT POSTURE ON ENDOTHELIAL FUNCTION IN WOMEN AND MEN

KARIM HABIB

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Abstract

We investigated flow-mediated dilation (FMD) and reactive hyperemia peripheral arterial tonometry (RH-PAT) responses during the supine and 70° head-up tilt postures to assess vascular function in young healthy women and men. During the FMD protocol: 1) FMD increased in both sexes during tilt (P=0.005), 2) women had higher shear stress responses in both postures vs. men (P=0.03), 3) at the time of maximal vasodilation during tilt, both sexes had lower mean arterial pressure responses (MAP; P=0.02) compared to supine. During the RH-PAT protocol: 1) at all timepoints men had greater RH-PAT in comparison to women (P=0.035), 2) both sexes had similar arterial stiffness in both postures (P>0.05), and 3) at the time of maximal vasodilation, both sexes showed similar MAP responses across both postures (P>0.05). We suggest that the increased vasodilatory response as measured by FMD in the tilt posture could be attributed to increasing metabolite production from postural muscles.

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List of Abbreviations

AI: Augmentation index

AI @ 75bpm: Augmentation index at heart rate 75 beats per minute

BP: Blood pressure

cGMP: Cyclic Guanosine Monophosphate

CFR: Coronary flow reserve

DBP: Diastolic blood pressure

EC: Endothelial cell

EDRF: Endothelium-derived relaxing factor

eNOS: Endothelial nitric oxide synthase

FMD: Flow mediated dilation

HR: Heart rate

IMR: Index of microvascular resistance

iNOS: Immunological Nitric oxide synthase

Ln-RHI: Natural logarithm of the reactive hyperemic index

MAP: Mean arterial pressure

MI: Myocardial infarction

MSNA: Muscle sympathetic nerve activity

NE: Norepinephrine

NO: Nitric Oxide

NOS: Nitric oxide synthase

nNOS: Neuronal Nitric oxide synthase

OH: Orthostatic hypotension

PNS: Parasympathetic nervous system

Q: Cardiac output

Qi: Cardiac output index

RHI: Reactive hyperemia index

RH-PAT: Reactive hyperemia peripheral arterial tonometry

SBP: Systolic blood pressure

SNS: Sympathetic nervous system

SR: Shear rate

SR_{AUC}: Shear rate area under the curve

SV: Stroke volume

SVi: Stroke volume index

TPR: Total peripheral resistance

TPRi: Total peripheral resistance index

VSMC: Vascular smooth muscle cells

Chapter 1: Literature Review

1.1 Introduction

Orthostatic hypotension (OH) is a form of autonomic dysfunction where the cardiovascular system is unable to maintain blood pressure during upright posture. Symptoms include; fatigue, dizziness, and in some cases syncope (i.e. fainting). OH affects 6% of the general population and is the second most common cause of syncope, approximately 15% of syncope cases are attributed to OH (reviewed in Sutton, 2013). The incidence of OH in women is two-fold higher than in men, although the sex-related mechanisms are still unclear and require further investigation (reviewed in Ricci, De Caterina, et al., 2015).

1.2 Blood vessel anatomy & Circulation

The blood vessels that are responsible for carrying oxygenated blood to peripheral tissues can be divided into two general categories: large/conduit arteries and arterioles. The first category is usually associated with macrocirculation, while the latter is associated with microcirculation. Arteries consist of 3 distinct layers: tunica intima, tunica media, and tunica adventitia (reviewed in Milutinović et al., 2020). The tunica adventitia is the third and outermost layer of the artery. It is composed of primarily connective tissue (collagen fibers, along with a sheath of elastic fibers) (reviewed in Sangiorgi et al., 2006). Its primary purpose is to provide shape and support to the artery. It also plays a crucial role in connecting the artery to other parts of the body, such as the organs and vascular nerves (Lohman et al., 1995).

The tunica media is the middle layer of the arteries. It is the thickest layer, composed of mainly vascular smooth muscle cells (VSMC), along with collagen and elastic fibers (reviewed in Tellides & Pober, 2015; Waller et al., 1992; reviewed in Zorc-Pleskovič et al., 2018). This layer is responsible for controlling the vascular tone via dilation and constriction. One of the primary sources of vasoconstriction is the sympathetic nervous system (SNS), which innervates the tunica media directly via small vascular nerves referred to as *nervi vasorum* (reviewed in Loesch & Dashwood, 2009).

The tunica intima is the innermost layer. It primarily consists of endothelial cells (EC) known to be critical in regulating blood flow and controlling the exchange of substances at the level of the capillaries (reviewed in Nakashima et al., 2007, 2008). Damage to the endothelium

could result in hypertension and other forms of cardiovascular disease (Rizzoni Damiano et al., 2003). The endothelium is connected to the smooth muscle via the basal lamina. The basal lamina is an essential component of the intima layer as it provides strength to the intima while maintaining its flexibility (reviewed in Milutinović et al., 2020).

1.2.1 Macrocirculation

Large arteries and conduit arteries are usually composed of more elastic fibers in comparison to arterioles. Both types of arteries typically have a thicker intima-media layer (Boutouyrie et al., 1999), which dampens the pressure generated by left ventricular contraction so that flow can be delivered efficiently and safely to the microvasculature (reviewed in Climie et al., 2019). The aorta is the largest artery in the human body, and it temporarily stores half of the stroke volume (SV) ejected during systole before propelling it to the rest of the body during diastole, allowing for continuous blood flow to the large arteries; this is known as the Windkessel effect (reviewed in Laurent & Boutouyrie, 2015). The Windkessel effect is primarily dependent on 1) the level of arterial stiffness or compliance (Westerhof et al., 1972) and 2) the diameter of the artery (Vlachopoulos et al., 2011). Arterial stiffness usually occurs due to the loss of elastic fibers. In the aorta, greater stiffness impairs the ability to temporarily store blood during systole, leading to the potential for damage to the arteries (reviewed in Climie et al., 2019).

1.2.2 Microcirculation

Arterioles have the same three layers as large arteries; however, each layer is thinner. For example, the tunica media in the arterioles is composed of only 1 or 2 layers of VSMC compared to the multiple layers found in larger arteries (reviewed in Martinez-Lemus, 2012). This arteriolar medial layer, composed primarily of VSMC and an internal elastic lamina, provides further compensation for the pulsatile property of blood pressure (reviewed in Martinez-Lemus, 2012). On the internal elastic lamina, fenestrae (small holes) are found, allowing for communication between VSMC and EC to control vascular tone (Arribas et al., 2008).

Arterioles are a key component in regulating blood pressure and blood flow distribution to the various organs and tissues of the body (Meininger G A et al., 1984). Blood flow in the microcirculation is mainly regulated via the interaction of arteriolar, capillary, and venular

segments according to local and regional metabolic demand (Segal, 2005). Blood flow in arterioles can be impacted by multiple factors, including exercise and sympathetic nerve activation. Local blood flow can change by as much as 100 fold in response to exercise (reviewed in Thomas & Segal, 2004). Therefore, arterioles are the main contributors to total peripheral resistance (TPR), which allows a significant role in regulating mean arterial pressure (MAP) and perfusion to organs and tissues (reviewed in Martinez-Lemus, 2012).

1.3 Endothelial dependent vasodilation

1.3.1 Nitric oxide (NO)

Endothelial-dependent vasodilation is a process that occurs primarily through the release of nitric oxide (NO) from the endothelial layer (reviewed in Tousoulis et al., 2011). Furchgott and Zawadzki were the first to discover that the endothelial layer is capable of releasing substances that can relax vascular smooth muscle. Furchgott and Zawadzki came to this discovery after noticing that the substance released by the endothelium offsets the constrictive effect that acetylcholine exhibits on the aorta of a rabbit. Further, they observed no relaxation effect when the EC were removed (Furchgott & Zawadzki, 1980). Furchgott and Zawadzki labeled this substance as an endothelium-derived relaxing factor (EDRF), and Palmer et al. (1988) were able to identify EDRF as NO. A decrease in NO bioavailability can decrease dilation of coronary arteries, contributing to the increased risk of an individual suffering a myocardial infarction (MI), vascular inflammation, and cardiovascular disease (reviewed in Tousoulis et al., 2011).

1.3.2 Nitric oxide production and synthesis

Palmer et al. found that L-arginine is converted to NO via an enzyme called nitric oxide synthase (NOS). They also found that NOS function depends on the presence of calmodulin, a calcium-modulated protein (Palmer et al., 1988). NOS has 3 isoforms: endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS). The first two isoforms (eNOS and nNOS) are calcium-dependent enzymes, unlike iNOS, which is a calcium-independent enzyme (Stuehr, 1997). Shear stress has been identified as a crucial stimulus for the activation of vascular NO production (reviewed in Lu & Kassab, 2011).

Palmer et al. (found that NO is a soluble gas with a half-life of 6-30 seconds (this duration could vary depending on pH, oxygen levels, reactive oxygen species levels and activity of the tissue) and is capable of moving through the endothelium to the VSMCs. Once NO enters the VSMCs, it stimulates the release of guanylate cyclase, an enzyme responsible for converting cyclic guanosine triphosphate to cyclic guanosine monophosphate (cGMP). An increased concentration of cGMP will activate GMP-dependent kinases which decreases the concentration of intracellular calcium (Ca^{2+}) causing vasodilation (reviewed in Moncada et al., 1991).

1.3.3 Impact of shear stress on NO production and vessel vasodilation

Along straight branches of the arterial tree, flow is unidirectional and laminar, which results in increased shear stress. Blood cells make direct contact with the wall of the artery while moving in a forward direction causing shear stress. The resultant shear stress leads to the initiation of the process of NO synthesis via a non-surface receptor mechanism (Rubanyi et al., 1986) (Joannides et al., 1995) referred to as mechano-transduction (Baeyens et al., 2016)(Kutys & Chen, 2016)(Baratchi et al., 2017). Integrins have been reported to have a role in mechano-transduction (Tzima et al., 2001) by helping to anchor the EC to the extracellular matrix in order to sense and transduce signals in response to shear stress (Takahashi & Berk, 1996). Integrins are glycoproteins located at the cell membrane and are activated via a conformation change in response to shear stress (Shyy John Y.J. & Chien Shu, 2002). Increased shear stress causes EC elongation, enhanced EC cellular function, helps EC maintain their shape and orientation while also providing anti-inflammatory properties to the EC (reviewed in Cahill & Redmond, 2016)(reviewed in Jensen & Mehta, 2016). On the other hand, when shear stress is reduced chronically, it stimulates apoptosis (cell death) of ECs which can lead to vascular inflammation (Gimbrone & García-Cardeña, 2016)(Yurdagul et al., 2016).

1.4 Endothelial independent vasodilation

Nitroglycerine is a pharmacological agent that induces vasodilation of the arteries and arterioles via an eendothelial-independent pathway. It is most often used to treat those who suffer from ischemic heart disease and/or myocardial infarction (reviewed in Boden et al., 2015). Nitroglycerine-induced vasodilation works via the biotransformation of nitroglycerine to a NO molecule with glycerol dinitrate as an intermediate product (reviewed in Kawamoto et al., 1990).

Brien et al. found that vascular tissue relaxes concurrently with the biotransformation of nitroglycerine in isolated rabbit aortic strips (Brien et al., 1986).

Endothelial independent vasodilation can involve stimulation of β_2 adrenergic receptors (Dawes Matthew et al., 1997) which are primarily bound by epinephrine with some affinity for norepinephrine (NE) (reviewed in Alhayek & Preuss, 2020). Stimulation of β_2 receptors increases the concentration of cyclic adenosine monophosphate which allows for the relaxation of VSMCs and vasodilation of the artery (Sutherland & Rall, 1960). Sutherland and Rall's finding provides evidence that the activation of β_2 receptors provides an endothelium-independent pathway for arterial vasodilation. Since β_2 receptors are very prevalent in small vessels such as arterioles (Parent R et al., 1993) they have a crucial role in blood flow regulation by contributing to the control of vascular tone in the arterial tree. Furthermore, using autoradiography, Molenaar et al. were able to determine that β_2 receptors are also located on the endothelial layer of human arteries (Molenaar et al., 1988) and can activate eNOS to increase NO (Dawes Matthew et al., 1997). This signifies that β-adrenergic receptors have both endothelial independent and dependent vasodilation properties while acting as the mechanism for sympathetically mediated vasodilation.

1.5 Vascular regulation

The autonomic nervous system plays an important role in regulating the various hemodynamic components of the human body (reviewed in Fisher, 2014). The interaction of the SNS and parasympathetic nervous system (PNS) is important for making sure that adequate blood flow is maintained throughout the body. The brainstem is considered to be the main regulator of the cardiovascular system and it has been referred to as "the command center of the cardiovascular system" (Krogh & Lindhard, 1917; Goodwin et al., 1972). The brainstem receives sensory information from many sensory receptors. For example, the mechanoreflex and metaboreflex within skeletal muscles can influence autonomic function via group III and IV afferent nerves, respectively (Coote et al., 1971; McCloskey & Mitchell, 1972), and the peripheral chemoreceptors relay sensory information such as arterial oxygen levels from the aortic arch and carotid artery to the brainstem (Fadel & Raven, 2012; Raven et al., 2006; Stickland et al., 2008).

Importantly, the cardiovascular system also contains arterial (carotid sinuses and aortic arch) and cardiopulmonary baroreceptors which are sensitive to blood pressure (BP) changes. Once a drop in BP is detected, the tonic baroreceptor signal to the medulla oblongata is inhibited due to a reduction in stretch. This inhibition of signals will stimulate sympathetic activation and parasympathetic withdrawal in order to increase BP and heart rate (HR) (reviewed in Brown, 2017).

During upright posture, gravity causes blood pooling in the lower extremities of the body leading to a decrease in venous return back to the heart (reviewed in Arnold & Raj, 2017). Lower venous return results in an immediate rise in HR to maintain cardiac output (Q) due to lower SV. This normally results in a compensatory increase in BP. Pathophysiologically, a person may develop OH where they are unable to maintain BP leading to pre-syncope or syncope (Streeten, 1995). This can be detected using head-up tilt (reviewed in Arnold & Raj, 2017) which is a non-invasive experimental method that allows for inducing hemodynamic changes in a controlled laboratory setting (reviewed in Macedo et al., 2011).

1.6 Orthostatic intolerance

OH is defined as a drop of 20 mmHg in systolic blood pressure (SBP) or 10 mmHg in diastolic blood pressure (DBP) within 3 minutes of standing up or head up tilt table testing (Grubb, 2008). OH, is associated with symptoms of dizziness, blurry vision, syncope and decreased vestibular balance. These symptoms can be caused by decreased cerebral perfusion (Figueroa et al., 2010) while impairment of sympathetic function and abnormal autonomic regulation can also cause OH (Jacob et al., 2000; Peltier et al., 2010). When going from a supine posture to an upright posture, gravity causes ~500-700 ml of blood to move from the upper torso to the lower extremities of the body (reviewed in Mathias, 2002). This increased hydrostatic pressure, will also cause a shift of 10% of plasma volume from the intravascular space to the interstitial space in the lower extremities (Jacob et al., 1998). This pooling of blood and plasma in the lower extremities will cause reduced venous return, SV and a reduced Q. The body normally compensates for venous pooling in order to maintain blood pressure, however, in those who suffer from OH, abnormal autonomic regulation hinders this compensatory mechanism.

Abnormal autonomic regulation can be due to baroreflex dysfunction where the baroreflex can suffer a loss of afferent baroreflex engagement which could reduce it's ability to

sense BP changes (Robertson et al., 1993) . This baroreflex dysfunction can cause decreased sympathetic activation and/or PNS (vagal) withdrawal in response to the low BP. Lower sympathetic activation could lead to less peripheral vasoconstriction in those suffering from OH exacerbating the venous pooling. Lower PNS withdrawal could prevent HR from increasing sufficiently in order to preserve Q (reviewed in Benarroch, 2008; reviewed in Thomas, 2011). OH is associated with increased morbidity and mortality. In a meta-analysis investigation, the presence of OH increased all cause- mortality by 1.5 times at the 5 year follow up mark (Ricci, Fedorowski, et al., 2015). OH also increases the risk of falling which is particularly relevant to older and elderly populations since falls can lead to head injuries and body trauma. Elderly persons who suffer from OH are 2.5 times more likely to experience falls in comparison to those who do not suffer from OH (Ooi et al., 2000).

Orthostatic tolerance is lower in women in comparison to men (Convertino, 1998). Women experience more OH symptoms in comparison to men, and women experience syncope more often and for prolonged periods of time in comparison to men (Park et al., 2010). At rest, women have a more active PNS in comparison to men (Fedorowski & Melander, 2013). However, men have a more active SNS which allows for a potentially different mechanism for BP control in comparison to women (Evans et al., 2015; Ramaekers et al., 1998). Further, after normalization for body size, Cheng et al. reported that women have smaller hearts and a less distensible left ventricle in comparison to men, often preventing women from producing a large enough SV in order to maintain Q and BP in the upright posture (Y.C. Cheng et al., 2011). Women also have a smaller blood volume in comparison to men (Fu et al., 2004).

Female sex hormones have been shown to have an effect on the regulation of the renin-angiotensin-aldosterone system (RAAS) (reviewed in O'Donnell et al., 2014). RAAS is designed for water and salt reabsorption in the kidney in response to a decrease of BP (Mathias, 2002). Chidambaram et al. reported that renin, angiotensin II, and aldosterone are all higher in the luteal phase (high estrogen and progesterone levels) in comparison to the early follicular phase (low estrogen and progesterone levels) and the responsiveness of RAAS was greater in the luteal phase during lower body negative pressure which is a simulation of orthostatic stress (Chidambaram et al., 2002). However, the increase of RAAS activity to simulated orthostatic stress during the luteal phase did not allow for the maintenance of MAP (Chidambaram et al., 2002). Cheng and Gruetter also reported that estrogen decreased angiotensin II-induced

vasoconstriction (D. Y. Cheng & Gruetter, 1992). Furthermore, Ferrer et al. reported that estrogen has increased β2-adrenergic mediated vasodilation (Ferrer et al., 1996), and under resting conditions, estrogen decreases vasoconstrictive responses to NE (Sudhir Krishnankutty et al., 1997) which is released in the synaptic cleft during sympathetic activation such as during upright posture. Estrogen's ability to reduce vasoconstrictive effects of NE will reduce the ability to compensate for any drops in BP endured during the transition from supine to upright posture which may partially explain why women are more prone to OH.

1.7 Sex differences in vascular regulation

Men and women have different levels of sex hormones in circulation such as higher testosterone for males and higher estrogen and progesterone for women. Women also exhibit more variability in sex hormone status in comparison to men. Abdel Rahman et al. found that young healthy men showed a greater baroreflex sensitivity to acute hypertension in comparison to young healthy women (Abdel-Rahman et al., 1994). This is an advantage for young healthy men as this greater sensitivity response will allow for a greater modulation of HR in response to the increased SBP. Minson et al. investigated the impact of fluctuating estrogen and progesterone levels during the menstrual cycle on cardiovagal baroreflex sensitivity, and they found that baroreflex sensitivity is the same during the early follicular phase (days 2-5 of the menstrual cycle, where estrogen and progesterone levels are low) compared to the mid-luteal phase (days 19-22 of the menstrual cycle, where estrogen and progesterone levels are high) (Minson et al., 2000). Together, this could signify that female sex hormones may not have an impact on cardiovagal baroreflex sensitivity, yet testosterone could have an influence.

Tank et al. found that healthy women tended to have lower SBP and muscle sympathetic nerve activity (MSNA) in comparison to healthy men (Tank et al., 2005), and Kneale et al. demonstrated that young healthy women have higher β2-adrenergic receptor sensitivity in comparison to young healthy men (Kneale et al., 2000). Kneale et al. noted that compared to men, young healthy women have a lower forearm vasoconstrictive response to NE, because they simultaneously experience α1-mediated vasoconstriction and enhanced β2-adrenergic induced vasodilation. Indeed, β2-adrenergic receptors have been found to counteract the α-adrenergic vasoconstriction effect of NE in young healthy women (Hart et al., 2011).

Importantly, estrogen has been reported to increase NO bioavailability in different ways including directly stimulating eNOS which then stimulates the production of NO leading to vasodilation (reviewed in Boese et al., 2017). Kharitonov et al. reported that NO production is greater in the preovulatory phase in comparison to the rest of the menstrual cycle because estrogen levels are highest at that timepoint (Kharitonov et al., 1994). In support of this, Hayashi et al. have reported that females release more NO from their aortas in comparison to men (Hayashi et al., 1992). This increase of NO bioavailability could be why estrogen is considered as a protective agent for premenopausal women against cardiovascular disease (reviewed in White, 2002), yet could be a contributing factor to greater orthostatic intolerance.

1.8 Vascular measures

1.8.1 Flow mediated dilation (FMD)

FMD refers to the process where EC react to an increase of shear stress via the production of NO ,causing the vascular smooth muscle to relax (reviewed in Tremblay & Pyke, 2017), and is used as a measure of endothelial health. FMD is also proportional to NO bioavailability (reviewed in Pyke & Tschakovsky, 2005). FMD is typically measured via a protocol where occlusion of the brachial artery using a blood pressure cuff occurs for a period of 5 minutes at a suprasystolic pressure (reviewed in Pyke & Tschakovsky, 2005; reviewed in Thijssen et al., 2011; described in detail in the Methods). During the period of occlusion, vasodilatory metabolite concentrations in the plasma increases surrounding ischemic yet active cells due to the ongoing cellular metabolism. The increased plasma concentration of metabolites allows for vasodilation at the level of the capillaries which then leads to the progressive vasodilation of the rest of the arterial branch network (Thomas & Segal, 2004; Welsh & Segal, 1997; Williams & Segal, 1993) and therefore increased shear stress at the time of cuff release/reperfusion allowing FMD to take place (reviewed in Tremblay & Pyke, 2017). Shear stress is proportional to the velocity and viscosity of the blood and is inversely proportional to the diameter of the artery. The increase of blood flow upon the release of the cuff (or after a period of occlusion/ischemia) is also called reactive hyperemia.

Many different factors could contribute to increased variability of FMD values including fatty foods and smoking (revieweed in Greyling et al., 2016). Padilla et al. found that ingesting a

high fat meal before a testing session led to reduced FMD and therefore reduced endothelial function (Padilla et al., 2006). Findlay et al. found that the FMD response to sustained shear stress was decreased in young healthy male smokers in comparison to young healthy male non-smokers which could potentially lead an individual to develop endothelial dysfunction (Findlay et al., 2013).

FMD measurements can help predict future cardiac events and cardiovascular disease since a small FMD value signifies low NO bioavailability which could be due to the emergence of vascular disease (reviewed in Cooke & Dzau, 1997; Gokce et al., 2002, 2003; Widlansky et al., 2003). Exercise training has been shown to improve endothelial dysfunction and FMD by increasing NO production (Hambrecht Rainer et al., 1998). Aerobic or resistance exercise, or a combination of both, improved NO-mediated vasodilation in patients suffering from heart failure (Belardinelli et al., 2005; Linke et al., 2001; Maiorana et al., 2000).

1.8.2 Reactive Hyperemia Peripheral Arterial Tonometry (RH-PAT)

RH-PAT is another non-invasive method that has been used to assess endothelial function in humans (Axtell et al., 2010). Kuvin et al. demonstrated that there was a significant linear relationship between RH-PAT (as measured by a reactive hyperemia index (RHI), described in the Methods) and brachial FMD (Kuvin et al., 2003). Their data highlighted the fact that endothelial function was similar in both conduit arteries and the microvasculature. A low RHI is indicative of vascular dysfunction (reviewed in Hedetoft & Olsen, 2014). Nohria et al. have also attributed RHI scores to NO bioavailability and endothelial health and function. (Nohria et al., 2006).

Our labratory investigated the relationship of RH-PAT and coronary microvascular function in patients with suspected microvascular dysfunction (Nardone et al., 2019). Nardone et al. infused patients with adenosine (causes endothelial independent hyperemia), acetylcholine (causes endothelial dependent hyperemia) and dobutamine (causes sympathetic mediated hyperemia). They measured the index of microvascular resistance (IMR) and coronary flow reserve (CFR) using invasive measurements in a hospital setting and correlated it with measurements of RH-PAT and FMD on the same patients. They found a correlation of RH-PAT with dobutamine IMR and CFR responses. Likewise, they also found correlations between FMD and adenosine and acetylcholine IMR and CFR. Hence, these findings suggests that RH-PAT is

related to sympathetic mediated dilation, while FMD is related to adenosine mediated endothelial dependent vasodilation (Nardone et al., 2019).

1.9 Purpose

FMD and RH-PAT have rarely been used to assess the physiological changes associated with upright posture. We will be using FMD in order to assess the endothelial dependent vasodilatory capacity of conduit arteries in upright and supine postures. Furthermore, since Nardone et al., showed a relationship between LnRHI and sympathetic dilatory control of the peripheral microvasculature, we will use RH-PAT as a measure of sympathetic control of the microvasculature in response to the different postures. Importantly, we will also investigate sex differences in vascular function during both postures in order to investigate potential mechanisms for greater orthostatic intolerance in women compared to men.

1.10 Hypotheses

We hypothesize that: 1) FMD will be lower in the upright position because of lower shear stress in the upright posture, 2) women will have a higher FMD and LnRHI in both postures because of their increased vasodilatory capacity, 3) LnRHI values will be lower in the upright posture because of the increased sympathetic activation associated with the upright posture (i.e. in the upright posture there will be a greater proportion of α-receptor mediated vasoconstriction counteracting the β2-receptor mediated dilation). In the upright posture, men and women will have similar decreases of LnRHI because of their similar increases of sympathetic activity in response to tilt (Fu et al., 2009).

Chapter 2: Materials and Methods

2.1 Study participants:

Young healthy adults were recruited from the graduate and undergraduate populations at York University. Each vascular measurement protocol included 2 groups (men and women), and different cohorts of participants were used for each measurement protocol. The FMD protocol consisted of 10 women and 9 men, while the EndoPAT protocol included 9 women and 7 men (for a total of 35 participants). It is worth noting, that the initial goal of this study was to recruit 12 women and 12 men for each testing (n=48 overall), however this was not possible because of the lab shutdown due to the COVID-19 pandemic. Male and female participants were excluded if they suffered from any previously diagnosed cardiovascular or pulmonary diseases such as hypertension, heart failure, or obstructive or restrictive pulmonary diseases. In order to be considered eligible, women must have never taken oral contraceptives or have stopped taking them for a period of at least three months prior to participating in the study. Women were also excluded if they have been using a hormonal intrauterine device, contraceptive patches, or any other form of hormonal contraceptives. Women were tested in the early follicular phase during days 2-5 of the menstrual cycle when estrogen and progesterone levels are low.

For 12 hours before testing all participants were asked to refrain from: smoking (e.g., cigarettes, vaping, marijuana), drinking alcohol, drinking caffeine (e.g., coffee, tea), heavy exercise (including sports, resistance training, and moderate to intense aerobic exercises), and eating fatty foods (e.g., fried foods, fatty breakfast meals such as bacon and sausages, fast food). Participants completed a screening survey (Appendix A) which asked about their self-identified ethnicity, the number of times they vigorously exercise per week (which will be used to estimate the VO_2max of the participants, as the number of times of exercise per week is correlated with VO_2 max measured) (Ainsworth et al., 1993), along with a self-reported medical background check (i.e., medical conditions and prescription medications). Anthropometric data and a supine blood pressure reading using a standard blood pressure monitor (BPTru BPM 200 monitor (Medaval, Coquitlam, Canada)) were measured and recorded. Variables such as age, sex, height, and weight will be used by the continuous blood pressure device NEXFIN® (BMeye, Amsterdam, The Netherlands) and the EndoPAT 2000^{TM} device (EndoPAT, Itamar Medical, Israel).

2.2 Hemodynamics measurements

Continuous BP was relayed to the NEXFIN® (BMeye, Amsterdam, The Netherlands) via a finger cuff. SV was calculated by the NEXFIN as it integrates the continuous BP measurement with a pulse contour method which calculates the systolic pressure area under the curve and uses the 3 element Windkessel model (Wesseling et al., 1993) (Ameloot et al., 2013). The three elements used are the assumed aortic characteristic impedance, TPR, and arterial compliance based on age, sex, height, and weight. The Nexfin then calculated Q as SV x HR.

Q and BP were relayed into PowerLab hardware and LabChart version 8 Pro software at 1000Hz (ADInstruments, Australia). In both protocols (FMD and EndoPAT; described below), LabChart was used to determine and record MAP, SBP, DBP, and Q. HR was calculated via a single-lead electrocardiogram. This occurs via a detection algorithm in LabChart that detects the R spike of each QRST complex calculating the R-R interval which is the inverse of HR. From these measurements, we were able to calculate total peripheral resistance (TPR) by dividing MAP with Q and SVwas calculated by dividing Q by HR and multiplying by 1000 to convert to mL. For each participant, Q, SV, and TPR was normalized to body surface area using the Du Bois formula (Du Bois & Du Bois,1916) in order to calculate cardiac index (Qi), stroke volume index (SVi), and total peripheral resistance index (TPRi).

2.3 Vascular measurement protocols

Each protocol (i.e. FMD and EndoPAT) consisted of 2 randomized trials (supine and upright postures). Participants were given a 30-minute break between trials in order to restore cardiovascular homeostasis before the start of the second trial. All upright trials involved 70° head-up tilt, a clinical standard. In the tilted position, both of the participant's arms were maintained at heart level in order to prevent a gravitational fluid shift from affecting results. The right arm used for vascular assessment was placed on a height-adjusted table with an arm rest and the left arm used for BP and Q (previously described) was maintained at heart level with either an arm board or sling.

2.3.1 Flow-Mediated Dilation (FMD) protocol

Each FMD protocol (one supine and one upright) consisted of 2 minutes of baseline measurements, 5 minutes of forearm occlusion/ischemia, and 3 minutes or reperfusion and recovery for a total of 10 minutes per trial. This occlusion duration of 5 minutes is used because Mullen et al. found that if the duration exceeds 5 minutes, it will stimulate non NO-mediated vasodilation, as well as prolonging the duration of hyperemia in the recovery period (Mullen et al., 2001). Before each trial starts, a standard blood pressure cuff was placed on the forearm of the right arm. The participant's arm was then extended at heart level for the full duration of the trial. Using a linear array high resolution (10 MHz) ultrasound probe (9L, GE Healthcare Systems, Mississauga), duplex ultrasonography (Vivid i, GE Healthcare Systems, Canada) of the brachial artery in the right arm was conducted to measure continuous vessel diameter and blood velocity. Diameter and blood velocity baseline measurements were recorded for 2 minutes. At the 2-minute mark, the blood pressure cuff around the forearm was inflated to 180 mmHg (i.e. suprasystolic pressure) for the 5 minutes of ischemia. The cuff was then released allowing for a reperfusion period of 3 minutes.

Duplex ultrasound videos were recorded via a laptop as video files (.avi format) using a video grabber device (AV.io HD, Epiphan Video). These videos were then converted to an MP4 format via VLC media player (Videolan Organization, Paris, France). These MP4 files were analyzed via an automated software (Cardiovascular Suite, Quipu, Italy). The software uses edge detection technology and vessel wall tracking in order to obtain precise measurements of diameter and blood velocity (Bots et al., 1994) . The software continuously detected and output the diameter and blood velocity data. Maximal diameter and velocity are expected during the reperfusion period. After testing, the automated software measured changes in artery diameter, maximum shear rate (SR), and shear rate area under the curve (AUC) continuously via the following equations:

$$\text{FMD (\%)} = [(\text{maximal diameter} - \text{baseline diameter})/\text{baseline diameter}] \times 100. \quad \text{(Eq 1)}$$

$$SR = 4 * (\text{velocity}/\text{diameter}); \text{ where 4 indicates a constant blood viscosity} \quad \text{(Eq 2)}$$

$$SR_{AUC} = \sum \{yi\,(xi+1 - xi) + \tfrac{1}{2}\,(yi+1 - yi)(xi+1 - xi)\}. \quad \text{(Eq 3)}$$

In the SR_{AUC} equation xi is first time point, xi+1 is the second (s) following xi, yi is initial shear rate, and yi+1 is the shear rate following yi. This data was then exported to an Excel file providing second by second measurements of artery diameter and shear rate. AUC measurements are calculated during the three minutes of the recovery period only. AUC is also indicative of maximum shear rate.

2.3.2 Reactive hyperemia peripheral arterial tonometry (RH-PAT) Protocol

Each RH-PAT trial consisted of 15 minutes; 5 minutes baseline, 5 minutes of forearm occlusion/ ischemia, and 5 minutes of recovery/reperfusion. Before the start of the RH-PAT trial, two tonometry finger cuffs were placed on the index finger of each hand while the Nexfin finger blood pressure cuff was placed on the middle finger of the left hand. Each probe contained an air cushion that gets inflated applying pressure on the index finger. With each cardiac cycle, SBP increases blood flow leading to an increase of blood volume in the fingers which was sensed by the tonometry probes. Pulse waveforms throughout each cardiac cycle (Hamburg & Benjamin, 2009) were relayed to the EndoPAT 2000^{TM} device (EndoPAT, Itamar Medical, Israel) and recorded on proprietary software.

The EndoPAT software uses an automated algorithm to quantify the reactive hyperemia index (RHI) which represents microvasculature function (reviewed in Aozasa et al., 2020). RHI is the ratio of the mean amplitude of the pulse waves during baseline and the post occlusion period of the occluded arm, divided by the same ratio from the measurements of the non-occluded arm (reviewed in Hedetoft & Olsen, 2014)(Please refer to appendix B for a figure of the raw RHI data). Hamburg et al. have reported that maximal RHI can be observed between 90-120 seconds into the post-occlusion period (Hamburg et al., 2008). Since the RHI is not normally distributed (Hamburg & Benjamin, 2009), the software computed the natural logarithm of the RHI (Ln-RHI) which was used for comparison across groups and trials. The EndoPAT 2000 also calculated the augmentation index (AI; Please refer to appendix C for a figure of AI calculation), a marker of systemic arterial stiffness, which is expressed as a percentage (Wilkinson et al., 2000) and calculated via the following equation:

$$AI = (P2-P1)/P1 \times 100\ [\%]. \qquad \text{(Eq 4)}$$

P1 is the first pulse amplitude or the "early systolic peak" and P2 is the second pulse amplitude or "the late systolic peak". The EndoPAT 2000 also calculated the AI at a normalized HR of 75 bpm which serves to allow for comparisons across different populations and postures (Durmus et al., 2014).

2.4 Statistical Analysis

Anthropometric data were compared via a 1- way analysis of variance (ANOVA) between the sexes. Hemodynamic averages were taken at baseline and at the time of maximal brachial artery dilation (FMD) or the time of maximal finger blood volume (EndoPAT) after cuff-release/reperfusion. In the tilted posture, the baseline values were taken while in the tilted posture prior to the beginning of the vascular assessment. Changes in hemodynamics were calculated and were defined as the change from baseline to the time of maximum dilation to investigate the influence of hemodynamics on vascular function. In the FMD testing, time of max dilation was determined by an automated software (Cardiovascular Suite, Quipu, Italy), while in the EndoPAT testing, time of max dilation was determined visually upon examination of the report in Appendix B. Hemodynamic averages and change scores are reported as mean ± standard deviation. A 2-way mixed model ANOVA was used to compare data across the 2 postures (sex and posture as factors, posture is a repeated measure).

Analysis of group variables (i.e., FMD, SR, $SR_{AUC,}$ Ln-RHI), and the comparison of hemodynamic variables at baseline was done via a 2-way mixed model ANOVA while accounting for sex and posture (supine and upright) as factors with posture acting as the repeated measure. The normality of distribution was assessed via the Spiro-Wilks test of normality. Tukey's post hoc analysis was used when significance was found. Significance was defined as $P<0.05$. All statistical analyses was performed via Sigmaplot 13.2 (San Jose, California, USA) statistical software.

Chapter 3: Results

3.1 Participant characteristics

A total of 19 women and 15 men were included in the study. Between September 2019 and March 2020, 10 women and 8 men completed the FMD testing. Women self reported their ethnicities as: Middle Eastern (n=6), Caucasian (n=2), south Asian (n=1) and Haitian (n=1), while men self reported their ethnicities as : Middle Eastern (n=6), south Asian (n=1) and Asian (n=1). Women and men had similar ages, weight and BMI (P=0.17, P=0.43, P=0.95, respectively; Table 1). Men had a significantly greater height and estimated VO_2 max (P=0.04, P=0.02, respectively; Table 1). Between January 2020 to March 2020 and from September to November 2020, 9 women and 7 men completed the EndoPAT testing. Women self reported their ethnicities as: Middle Eastern (n=6), Caucasian (n=2), African (n=1) and South Asian (n=1), while men self resported their ethnicities as : Middle Eastern (n=5) and Caucasian (n=2). No significant differences were observed between groups in terms of age and BMI (P= 0.34, P= 0.35, respectively). However, men had significantly greater weight, height and estimated VO_2 max (P=0.03, P=0.01, P=0.001 respectively; Table 1).

Table 1: Anthropometrics of women and men who completed FMD or EndoPAT testing.

	FMD				**EndoPAT**		
	Women	**Men**	***P* value**		**Women**	**Men**	***P* value**
N	10	8			9	7	
Age (years)	20±1	21±1	*0.17*		22±3	21±2	*0.34*
Weight (kg)	73±15	80±21	*0.43*		62±9	79±17	***0.03***
Height (m)	1.6±0.1	1.7± 0.1	***0.04***		1.6±0.1	1.8±0.1	***0.01***
BMI (kg/m^2)	26± 4	27± 7	*0.95*		23±2	25±7	*0.35*
# of times of exercise/ week	1.7±1.3	3.3±1.4	***0.03***		1.7±1.6	2±1.2	*0.76*
VO_2 max estimate (ml/kg/min)	43±2	48±6	***0.02***		37±3	46±5	***0.001***

Values are means ± SD; BMI, body mass index. VO_2: maximum oxygen consumption

3.2 FMD

When comparing baseline hemodynamics at the beginning of the FMD protocol in the supine and tilt posture, we observed that in the tilt posture both sexes significantly increased HR and decreased SVi, MAP and SBP (P=0.007, P= 0.001, P=0.03, P= 0.002, respectively) compared to supine. There was no effect of tilt on DBP (P=0.9), Qi (P=0.7) or TPRi (P=0.7). No main sex effect was observed for MAP, HR, DBP, and SBP (P=0.8, P=0.8, P=0.5, P=0.1, respectively). Men had a significantly higher Qi (P=0.04), lower TPRi (P=0.01), and higher SVi (P=0.007) in comparison to women (P=0.01) (Table 2).

Table 2: Hemodynamics of women and men prior to starting the FMD protocol in the supine and tilt posture.

	Women		Men		
	Supine	**Tilt**	**Supine**	**Tilt**	**Significance**
HR (bpm)	69±8	86±8	67±8	86±9	Sex (P=0.8) **Posture (P=0.001)** Interaction (P=0.8)
MAP (mmHg)	86±9	80±8	86±5	80±9	Sex (P=0.8) **Posture (P=0.03)** Interaction (P=0.8)
SBP (mmHg)	114±11	105±12	123±6	107±8	Sex (P=0.1) **Posture (P=0.001)** Interaction (P=0.3)
DBP (mmHg)	71±9	68±9	73±6	69±8	All P>0.05
Qi ($L/min/m^2$)	3.4±0.3	3.3±0.2	3.9±0.6	3.8±0.7	**Sex (P=0.04)** Posture (P=0.7) Interaction (P=0.7)
SVi ($ml)/(m^2$)	45±8	40±5	57±10	47±10	**Sex (P= 0.001**) **Posture (P= 0.007)** Interaction (P= 0.5)
TPRi ($mmHg.min/L)/(m^2$)	7.3±2.7	7.3±1.9	5.9±1.2	5.7±1.1	**Sex (P= 0.01)** Posture (P= 0.7) Interaction (P= 0.8)

Values are means ± SD.HR is heart rate, MAP is mean arterial pressure, SBP is systolic blood pressure, DBP is diastolic blood pressure, Qi is cardiac output index, SVi is stroke volume index, TPRi is total peripheral resistance index.

FMD was significantly higher in the tilt posture compared to supine in both sexes (P= 0.005), however, no main sex effect was found (P=0.157) (Figure 1 A). Women had a significantly greater average shear rate in comparison to men (P=0.03), which did not differ between postures (P=0.31) (Figure 1 B). There was no effect of sex or posture on the baseline shear rate (P= 0.1, P=0.48, respectively) or the SR_{AUC} (P=0.405, P=0.394, respectively; Table 3). Women had a significantly higher maximum shear rate in comparison to men (P< 0.001), irrespective of posture (P=0.42). Men had a significantly higher baseline brachial diameter and maximum brachial diameter in comparison to women in both postures (P=0.002, P=0.002 respectively), and there was a trend for a greater maximal brachial artery diameter in the upright posture in both sexes (P=0.06) (Table 3).

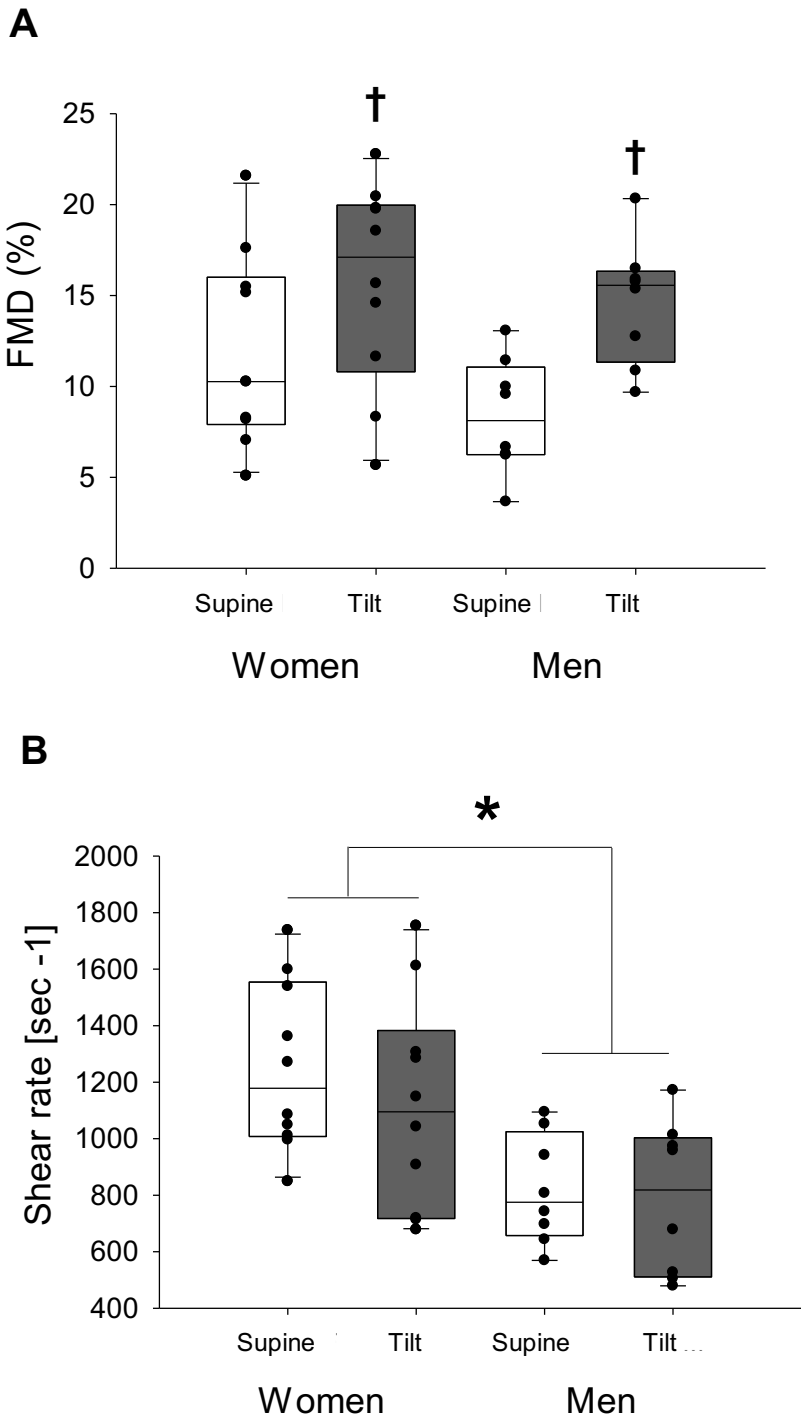

Figure 1: Flow-mediated dilation (FMD; A), and shear rate (B) responses of women and men (n=10 and n=8 respectively) in the supine and tilt posture. White bars indicate supine posture responses, grey bars indicate upright posture responses. †indicates a statistical significant difference due to posture ($P < 0.05$). * indicates a statistical significant difference due to sex ($P < 0.05$).

Table 3: Shear rate and arterial diameter assessments of women and men across the supine and tilt postures in the FMD testing

	Women		Men		
	Supine	**Tilt**	**Supine**	**Tilt**	**Significance**
Shear Rate AUC [sec.-1]	35970 ± 14336	40765 ± 20492	40981± 61906	17509 ± 10644	All P>0.05
Shear Rate Maximum [sec.-1]	1250 ± 299	1116 ± 376	818 ± 192	788 ± 271	**Sex (P=0.001)** Posture (P=0.42) Interaction (P=0.61)
Shear Rate Baseline [sec.-1]	316 ± 181	246 ± 113	206 ± 88	215 ± 61	All P>0.05
Baseline brachial artery diameter (mm)	3.1 ± 0.4	3.3 ± 0.7	3.7 ± 0.2	3.9 ± 0.4	**Sex (P=0.002)** Posture (P=0.43) Interaction (P=0.81)
Maximum brachial artery diameter (mm)	3.6 ± 0.4	3.8 ± 0.7	4.0 ± 0.2	4.4 ± 0.4	**Sex (P=0.002)** Posture (P=0.06) Interaction (P=0.56)

Values are means ± SD. AUC is area under the curve

At the time of cuff release in the FMD trial, there was lower MAP and HR in the tilted posture compared to the supine posture (P=0.002, P=0.04, respectively), yet no sex differences were observed (P=0.3, P=0.2, respectively) (Figure 2). Men had a greater reduction of SBP during the tilt FMD test compared to supine (P=0.001), leading to a greater reduction of SBP during the tilt compared to women (P=0.04). Both sexes had lower DBP, and SVi at the time of cuff release in the tilt posture compared to supine (P=0.002, P=0.02, respectively). There was no effect of posture or sex on TPRi (P= 0.2, P= 0.1, respectively) and on Qi (P= 0.6, P=0.2, respectively) (Table 4).

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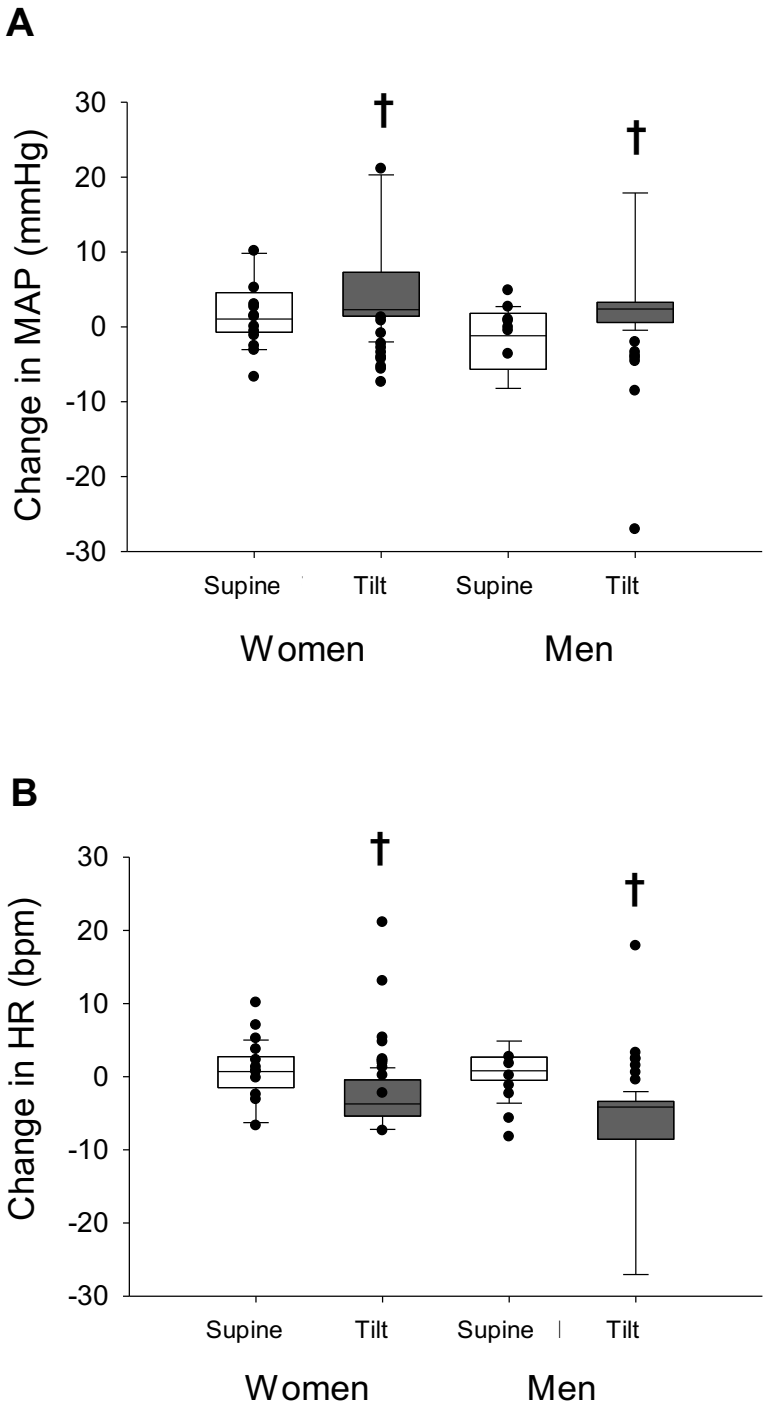

Figure 2: Change in mean arterial pressure (MAP; A) and heart rate (HR; B) from baseline to the time of maximal artery dilation during the FMD protocol in women and men (n=10 and n=8 respectively) in the supine and tilted postures. White bars indicate supine posture responses, grey bars indicate upright posture responses. †indicates a statistical significant difference due to posture ($P < 0.05$).

Table 4: Hemodynamic changes from baseline to the time of maximal dilation of women and men across the supine and tilt in the FMD testing

	Women		Men		
	Supine	**Tilt**	**Supine**	**Tilt**	**Significance**
Δ SBP (mmHg)	3.6±4.2	-3.3±5.4	6.3±3.2	-3.9±8.1†*	Sex (P=0.41) **Posture (P=0.001)** **Interaction (P=0.04)**
Δ DBP (mmHg)	1.2±2.3	-3.2±3.8	-0.5±2.5	-1.6±3.4	Sex (P=0.17) **Posture (P=0.002)** Interaction (P=0.27)
Δ Qi ($L/min/m^2$)	0.08±0.22	-0.02±0.22	0.03±0.29	-0.06±0.24	All P>0.05
Δ SVi ($ml)/(m^2$)	-0.59±5.42	-1.33±1.87	2.42±2.37	-3.95±7.13	Sex (P= 0.89) **Posture (P= 0.02)** Interaction (P= 0.07)
Δ TPRi ($mmHg.min/L)/(m^2$)	0.19± 7.56	-0.02±2.98	-0.040±0.38	-0.14±0.21	All P>0.05

Values are means ± SD.Δ is change from time of baseline to time of maximum dilation during the FMD protocol, SBP is systolic blood pressure, DBP is diastolic blood pressure, Qi is cardiac output index, SVi is stroke volume index, TPRi is total peripheral resistance index.† indicates significant difference between women and men in tilt posture, * indicates significant difference between tilt and supine posture in men.

3.3. EndoPAT

Prior to the beginning of the EndoPAT testing, both sexes had a significantly higher HR (P=0.001) and lower SVi (P=0.001) in the tilt posture in comparison to the supine posture. No main sex effects were observed for HR, SVi, MAP, SBP, DBP and Qi (P>0.05). Lastly, women had a significantly higher TPRi in comparison to men (P=0.001), with no main posture effect observed (P=0.6) (Table 5).

Table 5: Hemodynamics of women and men prior to starting the EndoPAT protocol in the supine and tilt posture.

	Women		Men		
	Supine	**Tilt**	**Supine**	**Tilt**	**Significance**
HR (bpm)	72±8	85±8	69±8	85±9	Sex (P=0.7) **Posture (P=0.001)** Interaction (P=0.7)
MAP (mmHg)	83±9	84±8	85±5	84±9	All P>0.05
SBP (mmHg)	113±11	112±12	123±6	121±8	All P>0.05
DBP (mmHg)	69±9	70±9	71±6	71±8	All P>0.05
Qi ($L/min/m^2$)	3.8±0.3	3.6±0.2	4.0±0.6	4.0±0.7	All P>0.05
SVi ($ml)/(m^2$)	55±8	44±5	57±10	46±10	**Sex (P= 0.2**) **Posture (P= 0.001)** Interaction (P= 0.9)
TPRi ($mmHg.min/L)/(m^2$)	7.7±2.7	8.4±1.9	5.7±1.2	5.6±1.1	**Sex (P= 0.001)** Posture (P= 0.6) Interaction (P= 0.4)

Values are means ± SD.HR is heart rate, MAP is mean arterial pressure, SBP is systolic blood pressure, DBP is diastolic blood pressure, Qi is cardiac output index, SVi is stroke volume index, TPRi is total peripheral resistance index.

Men had a significantly higher Ln-RHI value in both postures compared to women (P=0.035). Both sexes tended to have lower Ln-RHI values in the tilt posture in comparison to the supine posture (P=0.06) (Figure 3 A). Men had a significantly lower AI in both postures compared to women (men supine: 0.17± 0.03, men tilt: 0.23±0.04 vs women supine:0.09±0.03, women tilt: 0.13±0.03, P= 0.048) with no effect of posture (P=0.18). However, when normalized to HR, there were no significant difference observed in AI@75 bpm between sexes or postures (P=0.08, P=0.151) (Figure 3 B).

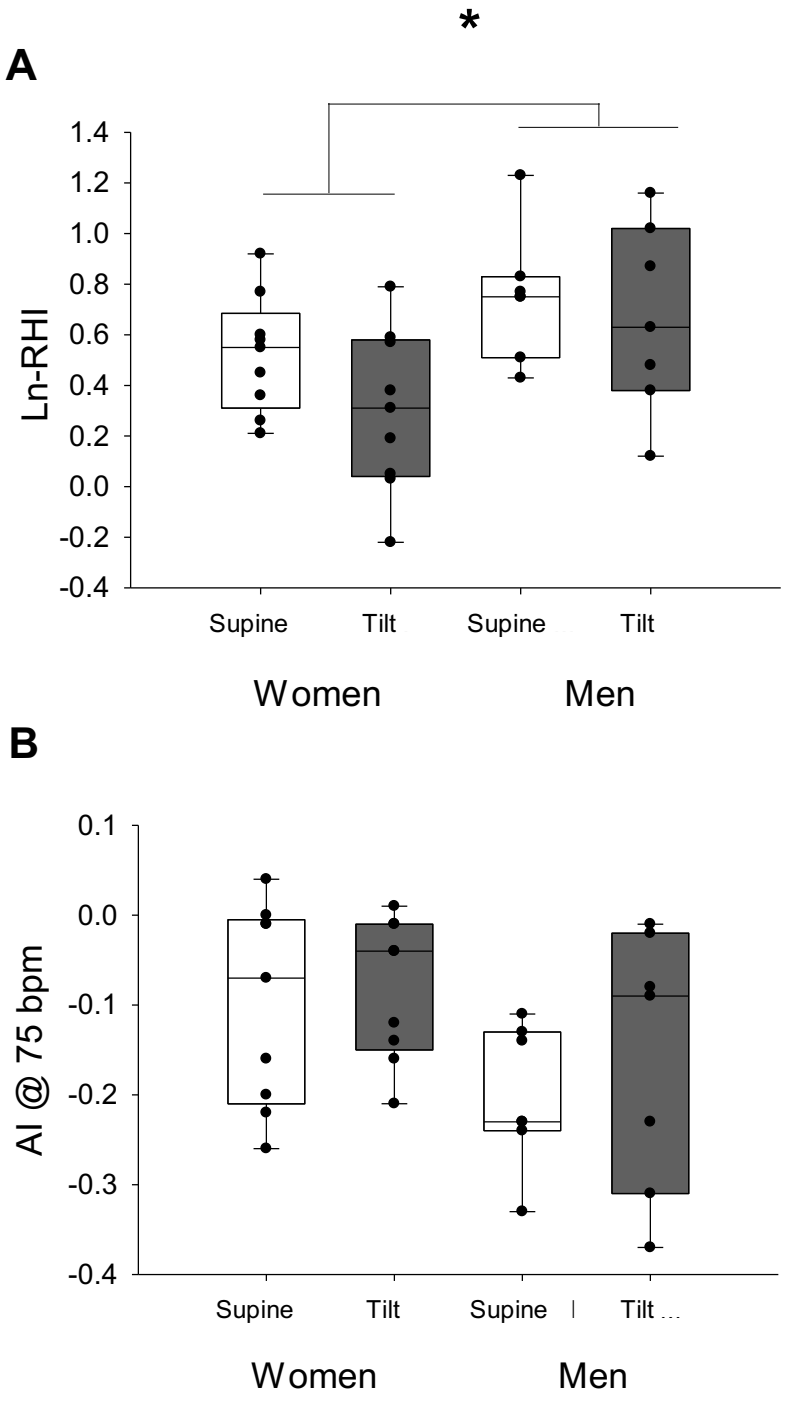

Figure 3: Natural logarithm of the reactive hyperemia index (Ln-RHI; A) and augmentation index at 75 beats per minute (AI@ 75bpm; B) in women and men (n=9 and n=7 respectively) in the supine and tilt postures.* indicates a significant difference between sexes ($P < 0.05$).

During the EndoPAT testing, there were no significant main effects of sex or posture on the change of MAP from baseline to the time of maximal response (P=0.38, P=0.48) (Figure 4 A). Both sexes tended to have a greater change of HR during tilt than in the supine posture (P=0.07), while no significant sex differences were found (P=0.72) (Figure 4 B). Both sexes had an attenuated response of SBP in the tilt posture in comparison to the supine posture (P= 0.02), while no main sex effect was found (P=0.67). There were no main sex or posture effects in regards to the change in DBP or Qi (P>0.05 for both). (Table 6). Men had a greater SVi response regardless of posture in comparison to women (P=0.03), and the SVi response tended to be smaller in the tilt position compared to supine in both men and women (P=0.07). Men tended to experience an increase in TPRi in comparison to women who experienced a reduction in TPRi across both postures. (P=0.07), while no main effect of posture was observed (P=0.67) (Table 6).

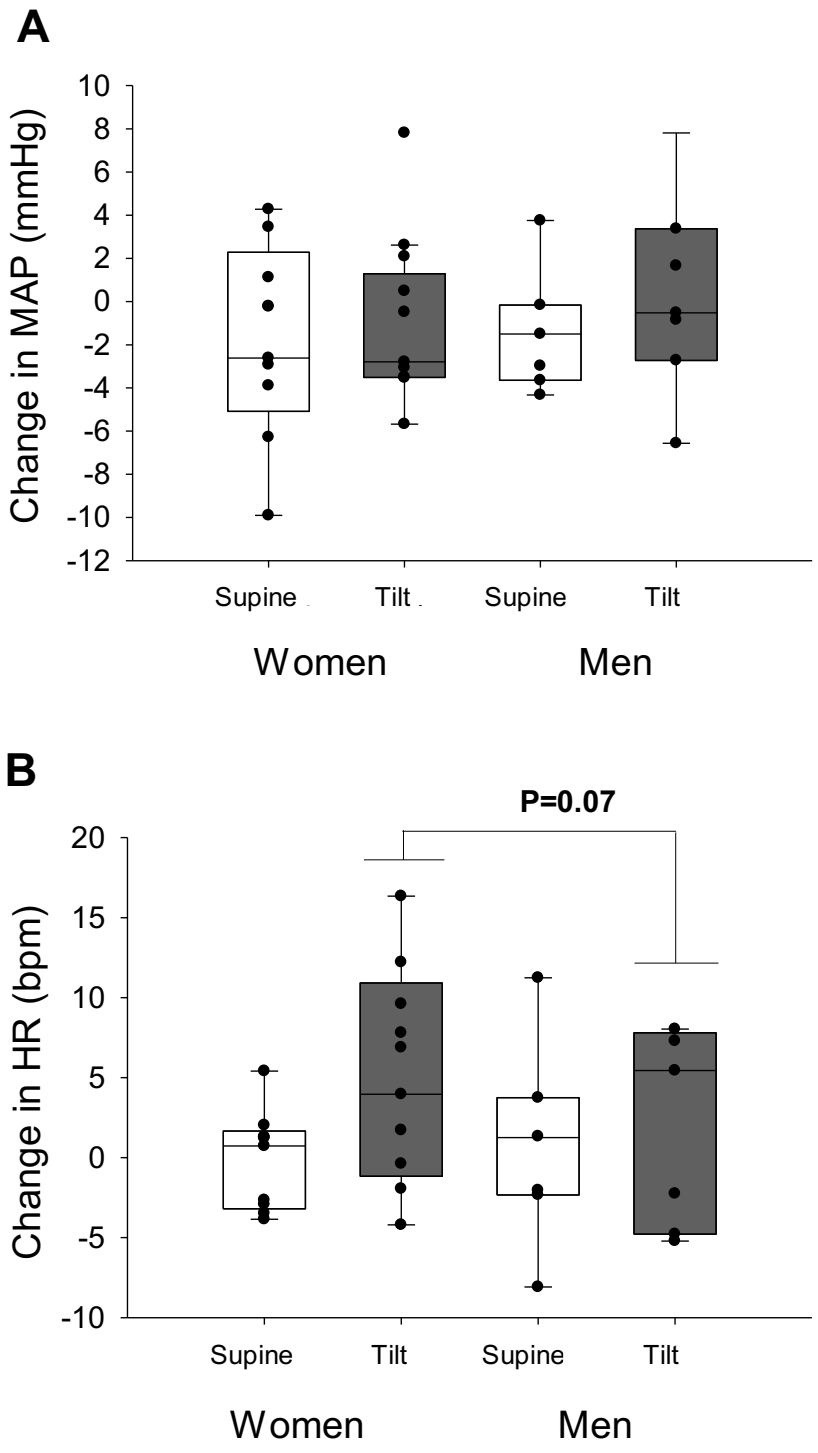

Figure 4: Change in mean arterial pressure (change in MAP; A) and heart rate (change in HR; B) from baseline to maximal EndoPAT response in women and men (n=9 and n=7 respectively) in the supine and tilt posture. White bars indicate supine posture responses, grey bars indicate upright posture responses

Table 6: Hemodynamic changes from baseline to the time of maximal dilation during the EndoPAT protocol in women and men across the supine and tilt postures

	Women		Men		
Variable	**Supine**	**Tilt**	**Supine**	**Tilt**	**Significance**
Δ SBP (mmHg)	2.3±1.6	-2.8±1.5	2.2±1.8	-1.2±1.7	Sex(P=0.67) **Posture (P=0.02)** Interaction (P=0.61)
Δ DBP (mmHg)	0.07±0.86	-1.8±0.9	-1..9±0.9	-8.4±0.8	All P > 0.05
Δ Qi (L/min)	0.045±0.185	0.107±0.325	0.157±0.328	0.282±0.106	All P > 0.05
Δ SVi (ml/min^2)	0.565±1.990	-1.635±2.103	1.854±2.300	0.229±2.933	**Sex (P=0.03)** Posture (P=0.07) Interaction (P=0.61)
Δ TPRi (mmHg.min/ L/)/(m^2)	-0.375±0.332	-0.451±0.844	0.265±0.508	0.939±0.374	Sex (P=0.07) Posture (P=0.6) Interaction (P=0.64)

Values are means ± SD. AI is augmentation index, Δ is change from time of baseline to time of max dilation, SBP is systolic blood pressure, DBP is diastolic blood pressure, Qi is cardiac output index, SVi is stroke volume index, TPRi is total peripheral resistance index

Chapter 4: Discussion

4.1 Summary

During tilt, FMD was augmented in both sexes. While both sexes had similar baseline shear stress and area under the curve, women had a significantly higher maximum shear rate in comparison to men. From baseline to the time of maximal brachial artery dilation during the FMD testing, both sexes had an attenuated change in MAP and HR during tilt compared to supine. Men had a significantly reduced SBP in the tilt posture in comparison to the supine posture leading to lower SBP during tilt compared to women. Both sexes had attenuated DBP and SVi responses in the tilt posture in comparison to the supine posture The change in Qi and TPRi was similar between sexes in both postures.

Both women and men tended to have lower Ln-RHI in the tilt posture versus the supine posture. Men had a significantly higher Ln-RHI, but not AI@75bpm, in comparison to women across both postures. From baseline to the time of maximal EndoPAT dilation response, both sexes had similar changes in MAP when comparing both postures, yet there was a trend for a greater HR response. While both sexes had a similar Qi response across both postures, men had a significantly augmented SVi response across both postures in comparison to women. Men tended to experience an increase in TPRi in comparison to women who experienced a reduction in TPRi across both postures.

4.2 FMD

Initially, we hypothesized that: 1) FMD will be lower in the tilt position because of lower shear stress in the upright posture, 2) women will have a higher FMD in both postures because of their increased vasodilatory capacity. Both hypotheses were not supported by the data.
We found that FMD significantly increased in the upright posture for both men and women, with no significant sex differences. This finding is supported by Guazzi et al. who compared FMD in 21 males, in a supine and a 60° head-up tilt posture and found that FMD increased in the tilt posture (Guazzi et al., 2004). Yet, it is important to acknowledge that they did not include any women.

An important factor to consider when comparing FMD between our subjects is to consider their general endothelial health, as this may be a confounding variable that impacts their FMD. We found that men had a significantly higher VO_2 max estimate in comparison to women. However, the higher bMI in the men may be misleasding as it could indicate a higher lean muscle mass. This is supported by the literature, as multiple studies found that men have a significantly higher VO_2 max in comparison to women (Sharma & Kailashiya, 2016; Sparling, 1980; Tomlin & Wenger, 2001). Furthermore, VO_2 max has been shown to be correlated with endothelial health (Buscemi et al., 2013). Hence, it is important to consider that in our study this cohort of men could have had greater endothelial health in comparison to the women.

Previous studies found that shear stress is the main contributor to the increase of NO production which causes the arterial vasodilation leading to FMD (Horobin et al., 2019). Hence, it is expected that brachial FMD is proportional to shear stress (Pyke & Tschakovsky, 2005). We found that shear stress was similar between both the supine and tilt postures and therefore, there was no enhanced shear stimulus which would have been responsible for the observed increase of FMD. Furthermore, we noticed a significant reduction of MAP in the tilt posture in both sexes which would have been expected to reduce FMD. Hence, our results raise the possibility of the contribution of a confounding variable to the significant increase of FMD across postures.

In 2012, Rubini et al., conducted a study investigating the effect of the upright and supine postures on the metabolic, cardiovascular, and electromyographic activity of anti-gravitational postural muscles such as the soleus and gastrocnemius muscles. Rubini et al., found that in the upright posture electromyographic activity of the soleus and gastrocnemius significantly increases (Rubini et al., 2012a). This indicates increased postural muscle activation and presumably increased metabolite production during the upright posture. These metabolites could then enter the systemic circulation and subsequently enter the ischemic arm upon cuff release causing a vasodilatory effect, which could explain the increased FMD response in the upright posture. Indeed, Almenoff et al., found that lactate stays in circulation for approximately 14 minutes (Almenoff et al., 1989). Hence, it is very possible that metabolites such as lactate stay in the general circulation long enough to enter the ischemic arm upon cuff release and possibly cause the increased non-shear stress dependent vasodilatory effect observed in our results. In our study, the subjects were not fully upright (as in Rubini et al. (2012a)) but tilted to 70° and Guazzi et al. (2004) observed that FMD increased proportionally with increasing degrees of tilt. Hence,

we would expect a smaller increase of FMD during tilt compared to standing due to reduced activation of anti-gravitational muscles. Nevertheless, the hypothetical impact of metabolite build-up is still observed in our study. Future studies are needed to assess any direct relationships between muscle activation, metabolite build and brachial FMD.

Contrary to our expectation due to enhanced vasodilatory capacity in women, we did not observe a sex difference in FMD. However, a recent study by Johns et al. also found that young women and men had similar brachial FMD (Johns et al., 2020). Contrarily, Harris et al., found that healthy females in two phases of the menstrual cycle had a significantly higher FMD versus healthy men (Harris et al., 2012). Further, Shenouda et al., found that young healthy women had a smaller FMD in comparison to men (Shenouda et al., 2018). Therefore, it is evident that the literature presents conflicting results. Johns et al. suggested these discrepancies could be due to the lack of consideration of sex differences in baseline brachial arterial diameter (Johns et al., 2020), as Juonala et al. found that women have greater FMD responses in comparison to men, however after normalizing for baseline diameter, they found no significant differences in FMD between women and men (Juonala et al., 2008). We found that women have a significantly smaller baseline brachial artery diameter in comparison to the men, however, we did not adjust our values for baseline diameter yet found similar results to Johns et al.. Therefore, normalization of FMD to baseline diameter may not be the reason for the discrepancy between studies.

Our findings also highlighted that women had a significantly higher shear rate in comparison to men in both postures. This can be explained by the previously discussed fact that women have small brachial artery diameters with similar blood pressures (see equation 2 on page 14). However, since women in our study had greater shear rate responses without an increase in FMD, it poses the possibility that our cohort of women have decreased endothelial health in comparison to our men cohort. As mentioned earlier, this is supported by our women's significantly lower estimated VO_2 max which is correlated with endothelial health (Buscemi et al., 2013).

4.3 EndoPAT

We hypothesized that 1) Ln-RHI values will be lower in the upright posture because of the increased sympathetic activation associated with the upright posture (i.e. in the upright posture there will be greater α-receptor mediated vasoconstriction counteracting the β2-receptor-mediated dilation), and 2) in both postures, men will have lower LnRHI compared to women because of their higher resting sympathetic activity levels. Both hypotheses were refuted by the data.

While the EndoPAT has been used to assess endothelial function (Moerland et al., 2012), we used it to assess the β2-mediated dilation of the microvasculature, as our lab group previously observed that LnRHI is correlated with dobutamine induced vasodilation (i.e. β2 mediated dilation; Nardone et al., 2019) which is a form of endothelial-independent vasodilation. In the currently study, we found that men exhibited significantly greater Ln-RHI in both postures in comparison to women which was unexpected as Davis et al. found that women have greater Ln-RHI scores in comparison to age-matched men (Davis et al., 2020). This discrepancy between our results and Davis et al. could again be due to the underlying fitness differences of our participants. The men and women in Davis et al.'s study were matched on the history of regular resistance exercise, and we did not control for fitness or physical activity. Hence, fitness differences could have been a confounding variable responsible for the discrepancy between our results and Davis et al.

In both sexes, Ln-RHI tended to be lower in the upright posture (P=0.06). This trend could be due to increased sympathetic tone leading to increased α-receptor-mediated vasoconstriction counteracting β2-receptor mediated vasodilation. However, our results were contradictory to Goswami et al. who found no significant difference in LnRHI due to posture between both sexes (Goswami et al., 2018). This discrepancy could be because Goswami et al. obtained their results after returning to supine posture after a 20-minute stand test and not during the 15-minute tilt test that we performed in our study. Notably, women tended to experience a reduction in TPRi at the time of maximal EndoPAT response whereas men tended to experience

an increase of TPRi. The systematic increase of vasodilation in women could have obscured any interactions between sex and posture during the EndoPAT testing.

4.4 Arterial stiffness

We investigated arterial stiffness in both postures via measuring augmentation index (AI) and AI normalized to a heart rate of 75 bpm (AI@75bpm) and found that while AI decreased in the upright posture, AI @75 bpm was similar in both sexes and across both postures. We relied mainly on AI@75 bpm because AI is inversely proportional to HR (Lantelme et al., 2002) and HR increases in the tilt posture. Hence AI@75 bpm provides us with an accurate representation of arterial stiffness at a standard heart rate that allows for comparison across different groups. Our findings are not consistent with the literature, as Cohen et al., found that arterial stiffness increases in the upright posture due to an increase of blood pressure (Cohen et al., 2020), however, we did not observe any significant change in MAP during the EndoPAT trials. Additionally, Cohen et al. measured arterial stiffness via carotid-femoral pulse wave velocity which is different from our protocol since we used augmentation indices. Increased carotid-femoral pulse wave velocity has been linked to increased sympathetic activity (Nardone et al., 2018) which is known to increase during upright posture. Therefore, the increase seen in Cohen et al. is likely driven by increased sympathetic activity whereas measurements of augmentation indices are less likely to be influenced by autonomic function.

We found no main sex effect in AI or AI@75 bpm. This finding is not supported by the literature, as DuPont et al. found that arterial stiffness is usually higher in men in comparison to women (DuPont et al., 2019). Previously observed lower arterial stiffness observed in women has been attributed to the cardioprotective effect of estrogen and the modulating effect of estrogen on arterial stiffness (DuPont et al., 2019; Mendelsohn & Karas, 1999a). Further, Corrigan et al. found that testosterone has been associated with increased arterial stiffness (Corrigan et al., 2015), yet some studies suggest that testosterone does a vascular protective effect and decreases arterial stiffness (J. C. Smith et al., 2001; Vlachopoulos et al., 2014). We did not measure the plasma concentration of sex hormones for our subjects and cannot draw conclusions based on sex hormone concentrations; however, the women were tested in the low hormone phase of the menstrual cycle which perhaps obscured any sex differences. Further, the

women in this study had significantly lower VO_2 max estimates in comparison to men and VO_2 max is inversely correlated with arterial stiffness (Vaitkevicius et al., 1993).

4.5 Limitations and future studies

Due to the pandemic, we were unable to recruit more participants due to the shutdown of human testing at York University, which would have allowed us to have a considerably higher sample size. Many trends observed in this thesis could have been established as statistically significant changes if a greater sample size was used. Future studies should try to include at least 48 participants (n=24 for each testing protocol).

There were discrepancies in the changes of hemodynamic variables when comparing both protocols (i.e. MAP decreased at the time of dilation in the FMD protocol, but not the EndoPAT protocol; Figure 2A vs. Figure 4A) which could have been due to the fact that subjects in the FMD testing were tilted for a longer period of time before the beginning of the protocol in comparison to the EndoPAT testing. This increased tilt time in the FMD testing was due to the fact that an appropriate brachial artery ultrasound image needed to be obtained prior to the beginning of the protocol. This is not needed for EndoPAT testing, as testing can start immediately after tilt because the finger cuffs are already placed on the subject's index fingers prior to tilting. The prolonged tilt time during the FMD trials could have led to the reduction of MAP over time which was not evident in the shorter EndoPAT testing period. Hence, future studies should ensure that the subjects are tilted for a similar period of time across both protocols.

All subjects in this study used a self-reported questionnaire (Appendix A) to determine their ethnicity, however, we did not examine the effect of ethnicity on postural control. We suggest that future studies should investigate ethnicity as a possible confounding variable in hemodynamic postural control as there has been conflicting evidence on this topic. Tanbakouie et al. found that orthostatic responses in women were similar between different populations (i.e Caucasian, middle eastern, south Asian) (Tanbakouie et al., 2021). However, Hinds and Stachenfeld found that African American women have greater orthostatic tolerance in comparison to Caucasian women (Hinds & Stachenfeld, 2010). Furthermore, Shaw et al. found that African American women are less likelty to suffer from postural orthostatic tachycardia

syndrome (Shaw et al., 2019). Additionally, Light et al. found that African American men tend to have higher TPR and HR during tilt in comparison to white men (Light et al., 1993). Therefore, further investigations are needed

In order to test our hypothesis that the increased FMD in the upright posture is due to an increase of metabolites from the activation of postural muscles, future studies should consider measuring lactate, adenosine, arterial O_2 and CO_2, and electromyography of posture muscles as it has been shown that these variables increase in the upright posture (Rubini et al., 2012a; Shoemaker et al., 2001). Along with these measurements, we suggest conducting a true cardiopulmonary exercise test to determine fitness as we used a VO_2 estimate via the Ainsworth equation (Ainsworth et al., 1993). This could be used as a predictor for endothelial health (Buscemi et al., 2013).

All the women self-reported their menstrual cycle where day 0 was defined as the first day of menstruation; however, we did not measure plasma hormone concentrations of estrogen or progesterone and assumed low concentrations since testing occurred during the early follicular phase (days 2-5). Future studies should investigate the hormone levels of each subject by collecting plasma or saliva samples and performing biochemical measurements. Additionally, we did not measure MSNA as an index of sympathetic activity which we could have correlated to sympathetic mediated vasodilation (i.e. LnRHI). There has been conflicting evidence surrounding sex differences and sympathetic responses to the tilt posture, where Fu et al. found similar responses in both sexes to orthostasis (Fu et al., 2004, 2005), while other studies found differences between both sexes (Kimmerly et al., 2007; Shoemaker et al., 2001). Hence further investigations are needed on this topic.

4.6 Conclusion

Many studies have identified women as having a lower orthostatic tolerance in comparison to men (Convertino, 1998; Fritsch-Yelle et al., 1994; Hordinsky et al., 1981; Montgomery et al., 1977). While we hypothesized that enhanced vasodilatory capacity in the upright posture would contribute to impaired orthostatic tolerance in women, we did not find any evidence to indicate this, and we hypothesize that enhanced FMD in the upright posture in both sexes is due to greater metabolite build-up from postural muscle activation. We also observed a

trend for reduced LnRHI in the upright posture suggesting reduced sympathetically mediated dilation, perhaps due to greater opposition from α-receptor activation. Lastly, there were no observed sex differences in FMD and men were observed to have greater Ln-RHI values in comparison to women. These results indicate that compared to women, the men investigated in this study may have had greater fitness leading to better vascular health.

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Appendices

Appendix A: Data collection sheet- FMD study

KURE (ID): _____ **Date**: _____

Sex: □ M □ F **Time:** _____ □ am □ pm

Self-Identified ethnicity: _____

Day of cycle: _____

How many times do you vigorously exercise per week? _____

does the participant use Oral Contraceptives (OC): Y N

Did the participant partake of any of these activities within the last 12 hours?

Drink caffeine (coffee, energy drinks etc.) Y N

Eat any Fatty Food Y N

Smoke (cigarettes, vaping, marijuana) Y N

Drink any alcohol Y N

Heavily exercise Y N

Breakfast/ lunch? Y N

If Yes, what was the meal? _____

Height: _____ **cm** **Weight:** _____ **kg** **Age:** _____

Any known medical conditions? Y N

If Yes, list them: _____

Any use of Medications? Y N

If Yes, list them: _____

Initial Manual BP: _____ **Initial Manual HR:** _____

PS value: _____ **ED value:** _____

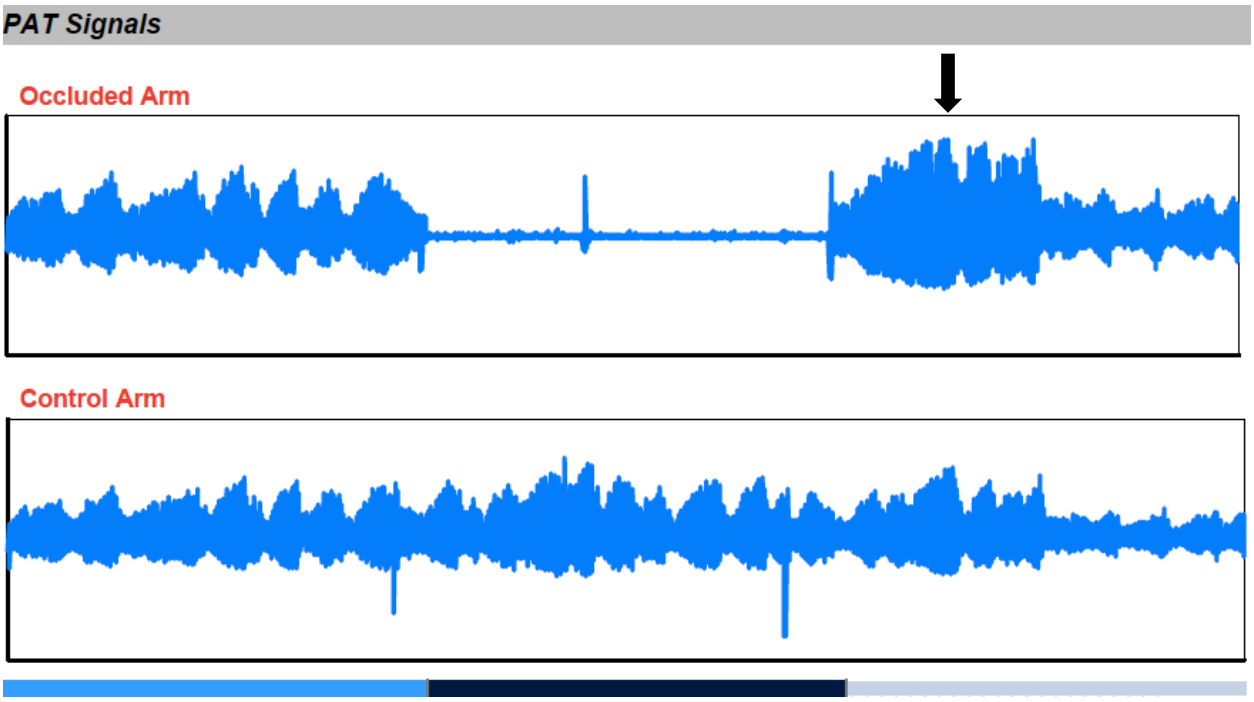
Standing trial:

A 30 min break was taken between trials? Y N

Sitting trial:

Final Manual BP: _____ Final manual HR: _____

Appendix B:



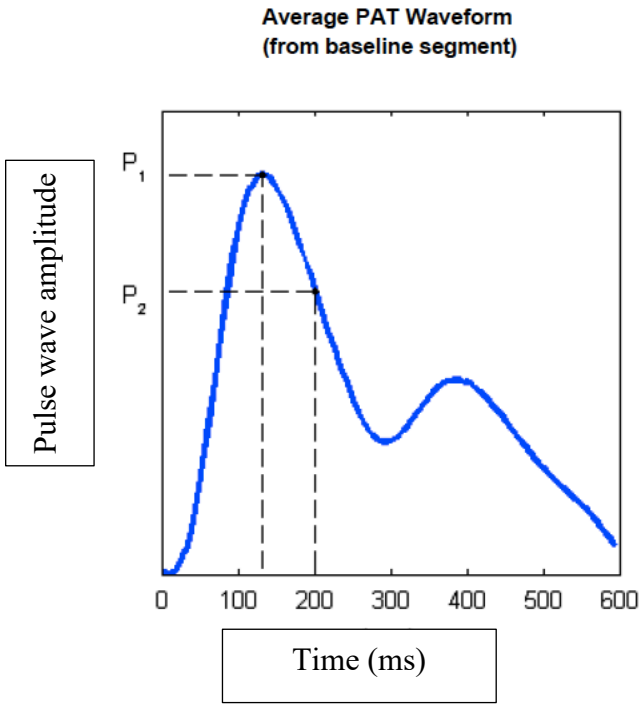
A sample of peripheral arterial tonometry (PAT) signals from our data collection

↓ Arrow indicates maximal dilation after cuff release

Appendix C:

Augmentation Index (AI) - a measure of Arterial Stiffeness

AI: -29%
AI@75bpm: -16%
AI = (P2-P1)/P1 x 100 [%] Averaged - 200 pulses



A sample of Augmentation index (AI) calculations from our peripheral arterial tonometry (PAT) data collection

P1= Pulse wave 1
P2= Pulse wave 2
AI= Augmentation index
AI@75bpm= Augmentation index @75 beats per minute
PAT= Peripheral arterial tonometry