Hemodynamic effects of intermittent compression as a countermeasure to orthostatic stress

by

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Author's Declaration

This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Statement of Contributions

The author contributions for Chapters 3 and 4 are described below.

Chapter 3: Travis Gibbons, Kathryn A. Zuj and Richard L. Hughson conception and design of research; Travis Gibbons and Alexandre H.L.F. Cruz performed experiments; Travis Gibbons and Alexandre H.L.F. Cruz processed the data, Travis Gibbons wrote the initial draft of the manuscript; Travis Gibbons and Richard L. Hughson drafted the manuscript; Travis Gibbons and Richard L. Hughson edited and revised the Chapter; Travis Gibbons and Richard L. Hughson approved the final version of the Chapter.

Chapter 4: Travis Gibbons and Richard L. Hughson conception and design of research; Travis Gibbons and Kathryn A. Zuj performed experiments; Travis Gibbons and David C. Kingston processed the data; Travis Gibbons and Richard L. Hughson interpreted the results of the experiments; Travis Gibbons wrote the initial draft of the manuscript; Travis Gibbons and Richard L. Hughson drafted the manuscript; Travis Gibbons and Richard L. Hughson edited and revised the Chapter; Travis Gibbons and Richard L. Hughson approved the final version of the Chapter.

Abstract

Upright posture can be associated with symptoms of dizziness, light-headedness and blurred vision, which are fundamentally caused by impaired cerebral hemodynamics. As gravity acts along the body's longitudinal axis, pooling of blood in the dependent vasculature of the lower body results in a decrease in venous return and compromises the heart's ability to perfuse the brain. The improvement of venous return has been a therapeutic focus to increase tolerance of the upright position. The use of static external compression to limit venous pooling while standing has yielded mixed results. Intermittent external compression of the lower legs provides an alternative strategy to redirect blood from the extremities back to the heart. The main objective of this thesis was to assess how the application of intermittent compression of the lower legs affected the local, central and cerebral hemodynamic responses to orthostatic stress. To accomplish this, a preliminary method comparison study was conducted to address potential shortcomings in the commonly used Modelflow method to accurately estimate dynamic fluctuations in stroke volume during orthostatic stress. The results of this method comparison study indicated that the Modelflow method could not accurately estimate stroke volume in the conditions elicited by orthostatic stress. Intermittent compression of the lower legs attenuated the orthostatic-induced reductions in blood pressure, cerebral blood flow velocity and indices of cerebral oxygenation, while enhancing blood flow to the legs. Thus, the use of intermittent compression of the lower legs as a countermeasure to orthostatic stress may improve the tolerance of certain conditions in which brain blood flow is compromised.

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LIST OF ACRONYMS

ANOVA	Analysis of variance
BIA	Bioelectrical impedance analysis
$\dot{Q}$	Cardiac output
CO_2	Carbon dioxide
CBF	*CBF*
ECG	Electrocardiography
EMG	Electromyography
ESM	End-stress mean
$ETCO_2$	End-tidal CO_2
HHb	Deoxygenated hemoglobin
HUT	Head-up Tilt
ICA	Internal carotid artery
IOH	Initial orthostatic hypotension
LBNP	Lower body negative pressure
MAP	Mean arterial pressure
MCA	Middle cerebral artery
MCAv	Middle cerebral artery velocity
NIRS	Near-infrared spectroscopy
O_2Hb	Oxygenated hemoglobin
$PaCO_2$	Arterial partial pressure carbon dioxide
POTS	Postural orthostatic tachycardia syndrome
SBL	Standing baseline
SFA	Superficial femoral artery
SFAv	Superficial femoral artery velocity
SS	Squat-to-stand
SV	Stroke volume
SV_{ALL}	Stroke volume average over all three methods
SV_{BIA}	Stroke volume via bioelectrical impedance analysis
$SV_{U/S}$	Stroke volume via Doppler ultrasound
SV_{MF}	Stroke volume via modelflow
TCD	Transcranial Doppler
TCR	Thigh-cuff release
THb	Total hemoglobin
TPR	Total peripheral resistance
$TPR_{U/S}$	Total peripheral resistance via Doppler ultrasound
TSI%	Tissue saturation index
VA	Vertebral artery

CHAPTER 1: *INTRODUCTION*

The brain disproportionately receives 12% of cardiac output ($\dot{Q}$) even though it comprises only 2% of the body's weight (Williams & Leggett, 1989). Cerebral blood flow (*CBF*) is rigorously regulated by various mechanisms to ensure a balance between cerebral metabolic demand and supply, however, acute (Levine, Giller, Lane, Buckey, & Blomqvist, 1994a; Ogoh et al., 2005) and chronic (Fraser et al., 2015; Rajagopalan, Raine, Cooper, & Ledingham, 1984) alternations in $\dot{Q}$ can lead to changes in *CBF*. The act of standing up against gravity is a significant challenge for the cardiovascular system as around 70% of blood volume shifts to below the heart (Rowell, 1993). The location of the brain with respect to the heart in the upright posture makes it especially susceptible to the effects of gravity (Rowell, 1993).

Syncope, which is defined as a sudden loss of consciousness and postural tone, can be provoked by any condition that jeopardizes *CBF* and cerebral oxygenation (van Lieshout, Wieling, Karemaker, & Secher, 2003). Active standing elicits a transient and substantial decrease in arterial blood pressure, caused by an imbalance between arterial inflow and outflow (Wieling, Krediet, van Dijk, Linzer, & Tschakovsky, 2007). This transient decrease in arterial blood pressure has been associated with subsequent decreases in *CBF* that must be counteracted by both cerebral and systemic reflexes in order to restore adequate blood flow to the brain to prevent syncope and ischemic damage.

Moving into the upright position results in the accumulation of blood in veins of the lower body, effectively removing a significant amount of blood from central circulation. The peripheral vasculature of the legs and abdomen act a reservoir for blood below the level of the heart, creating a challenging situation in which the blood must somehow be driven against a hydrostatic gradient back to the heart. In the healthy population, autonomic reflexes and skeletal muscle contraction

counteract some of this venous pooling and effectively combat the reduction in cardiac filling in the upright position. Additionally, cerebral autoregulatory reflexes rapidly respond to changes in perfusion pressure by decreasing cerebrovascular resistance in an attempt to maintain adequate flow (Lewis et al., 2013). However, vulnerable populations exist that have difficulties recovering from the initial drop in blood pressure and experience reduced venous return, $\dot{Q}$ and/or *CBF* for prolonged periods. These diverse populations include the elderly (Gupta & Lipsitz, 2007; Nagaya, Hayashi, Fujimoto, Maruoka, & Kobayashi, 2015; Rutan, 2014), highly-trained endurance athletes (Bedford & Tipton, 1987; Fadel et al., 2001; Raven et al., 1988), heart-failure patients (Fraser et al., 2015) and post-flight astronauts (Hughson, Shoemaker, & Arbeille, 2014).

Time-dependent analysis of compensatory reflexes to blood pooling in dogs show that autonomic responses to orthostasis develop fully after approximately 20 – 40 seconds (Guyton, 1973). Thus, the activity of the muscle pump in returning blood flow to the heart in the maintenance of brain blood flow is essential at the onset of standing (Guyton, 1973). Research has shown that external muscle compression effectively simulates muscular contraction, and that intermittent contraction more effectively sustains increases in $\dot{Q}$ when compared to static tetanic contraction (Guyton, 1973). It has been argued that sustaining $\dot{Q}$ during active standing fully compensates for the decrease in peripheral resistance, thus combating the magnitude of arterial hypotension and cerebral hypoperfusion (Krediet et al., 2005).

The association between $\dot{Q}$ and *CBF* during orthostatic stress provides valuable information regarding one's ability to adapt to rapid alterations in blood pressure. Recent research conducted in our laboratory has shown that intermittent compression of the lower limbs causes significant increases in stroke volume (*SV*) during standing plantarflexion exercise (Prince, Zuj, Hughson, & Peterson, 2017). These findings present the possibility that decreases in *SV* and $\dot{Q}$

experienced during orthostatic stress may be counteracted by intermittent external compression of the lower legs at the onset of standing. Assessing the effectiveness of intermittent compression as a countermeasure to orthostatic stress provides an opportunity to better understand the complex relationships between local, central and cerebral hemodynamics, as well give insight into the potentiality of external compression as a therapeutic tool during conditions in which *CBF* is compromised.

The present thesis project attempts to provide a better understanding of how *SV* dynamically responds to active orthostatic stress. To investigate this, *SV* was measured using three methodologies (described further below) – the Modelflow method (SV_{MF}), aortic Doppler ultrasound ($SV_{U/S}$) and bioelectrical impedance analysis (SV_{BIA}) – during a squat-to-stand (*SS*) transition and a standing bilateral thigh occlusion release (*TCR*). Additionally, this project provides insight into the efficacy of intermittent external compression as a potential countermeasure to orthostatic stress by assessing local, central and cerebrovascular responses with and without the application of a novel, intermittent compression system. It was hypothesized that the *SS* transition and *TCR*, would elicit a significant drop in arterial blood pressure and peripheral resistance, which will result in a *SV* method discrepancy brought upon by a violation of model assumptions inherent to the Modeflow method. It was also hypothesized that the reduction in blood pressure elicited by the *SS* transition and *TCR* will coincide with reductions in *CBF* velocity and indices of cerebral oxygenation. Finally, it was hypothesized that intermittent compression of the lower legs would improve the central and cerebral hemodynamic response to orthostatic stress and present a potential therapeutic strategy for individuals vulnerable to orthostatic stress.

CHAPTER 2: ***REVIEW OF RELATED LITERATURE***

2.1 Orthostasis

Orthostasis is defined as the maintenance of an upright posture, but has been stretched to broadly include other conditions that decrease arterial blood pressure and/or compromise *CBF*, such as *TCR*. Maintaining an upright posture provides a considerable challenge for human beings because of the location of the brain with respect to the heart and the distensibility of vasculature below the heart (Rowell, 1993). When standing upright, the weight of the blood in the circulatory system causes an increase in hydrostatic pressures in the lower part of the body, resulting in the 'pooling' of blood in the highly compliant vessels of the lower body (Guyton, 1973). Although static pooling of blood does not actually occur, a decreased rate of venous efflux results in an accumulation of blood in the dependent vasculature of the lower body. Unlike quadruped animals in which the majority of blood volume circulates at or above heart level, human beings circulatory anatomy provides a unique situation in which over 70% of blood volume is distributed below the heart when in the upright position (Rowell, 1993).

The amount of blood volume translocated into dependent organs by gravity depends on the compliance of the vasculature (Rowell, 1993). The legs and splanchnic region contain the most compliant veins in the body and will accommodate large amounts of blood with minimal changes in transmural pressure. These reservoirs of blood provide a buffer to large alterations in blood volume, but create a unique challenge in returning blood to the right atrium when in the upright position (Rowell, 1993). Relaxed standing causes an accumulation of blood below the thorax until eventually the heart does not receive enough blood to effectively pump blood throughout the body.

The location of the brain above the heart and its high metabolic demand leave it particularly susceptible to the effects of orthostasis (Rowell, 1993). The importance of maintaining adequate *CBF* is emphasized by the numerous regulatory mechanisms that work to ensure brain blood flow remains within a narrow range (Tzeng & Ainslie, 2014). However, acute changes in $\dot{Q}$ caused by orthostatic stress can cause marked decreases in *CBF* (Levine, Giller, Lane, Buckey, & Blomqvist, 1994b; Ogoh et al., 2005). Without specific and immediate regulatory mechanisms in place, humans would succumb to cerebral ischemia upon the onset of standing.

2.1.1 Physiological Effects and Adaptations

2.1.1.1 Fluid Shifts

Immediately after the onset of standing, whether passive or active, there is a shift of blood away from thoracic region and into the capacitance vessels of the lower body. This shift in blood volume is caused by increased hydrostatic pressure within the circulatory system (Guyton, 1973; Rowell, 1993). The highly distensible veins of the lower body accommodate most of this blood because of their ability to increase in capacity with minimal changes in pressure, as opposed to their arterial counterparts at comparable branches of the vascular tree (Rowell, 1993). The compartmentalization of blood during orthostatic stress has previously been measured using a longitudinal shift in body center of gravity (Brown, Goei, Greenfield, & Plassaras, 1966), water plethysmography (Musgrave, Zechman, & Mains, 1969), segmental radioisotope activity (R. H. Murray, Krog, Carlson, & Bowers, 1967) and most commonly, bioelectrical impedance analysis (*BIA*) (Diedrich & Biaggioni, 2004; Montgomery, Hanish, & Marker, 1989; Stewart, McLeod, Sanyal, Herzberg, & Montgomery, 2004). Typically, the assessment of fluid shifts during orthostasis is done using lower body negative pressure (*LBNP*) and/or passive upright tilting because of difficulties acquiring reliable measurements during active movement.

BIA, which will be described further in chapter *2.2.2.2*, measures changes in the flow of electrical current through an identified body segment that occur due to alterations in blood volume within that body segment (Montgomery et al., 1989). Upright tilting at 30° and 60° above horizontal increases impedance in the thorax, while simultaneously decreasing impedance in the pelvis, thighs and calves, corresponding to a shift in fluid from the thorax into the lower body. Montgomery et al. also noted a much greater fluid shift into the pelvis (~1500mL) when compared to both the thighs (~200mL) and calves (~150mL) at 60° head-up tilt (Montgomery et al., 1989). Similarly, Diedrich et al. showed a fluid shift of 551 mL of blood into the abdomen, 82 mL into each thigh and 9 mL into each calf, showing that the splanchnic region is by far the greatest reservoir for blood during passive orthostasis (Diedrich & Biaggioni, 2004). Also, Brown et al. showed a significant headward shift in body center of gravity upon the release of *LBNP* at -70 mmHg with thigh cuffs inflated to +70 mmHg prior to the release of *LBNP* (Brown et al., 1966). The subsequent release of the upper leg thigh cuffs resulted in a second headward shift in the center of gravity (Brown et al., 1966). These results indicate that significant pooling occurs in both the legs and buttock/pelvic regions as blood is displaced from the thorax during orthostatic stress.

One must consider that although *LBNP* simulates head-up tilt (*HUT*) in comparable thoracic hypovolemia and unloading of the cardiopulmonary and arterial baroreceptors, the gravitational differences in *HUT* have been shown to produce different regional vascular properties (Hughson et al., 1994; Kitano, Shoemaker, Ichinose, Wada, & Nishiyasu, 2005). Wieling et al. suggested that increased splanchnic pooling during actual orthostasis may result in more stimulation of vascular sub-diaphramatic receptors that may play a role in the orthostatic response by instigating vasoconstriction and augmenting of the carotid sinus baroreflex (Wieling, de Lange, & Jardine, 2014). Testing this hypothesis, Taneja et al. compared compartmental blood flow shifts

in both *LBNP* and *HUT* (Taneja et al., 2007). It was discovered that both *LBNP* and *HUT* produced comparable changes in blood flow out of the thorax and into the pelvis and lower extremities, however, the change in blood volume in the splanchnic region was directionally opposite, with a decrease in blood volume of ~20% with *LBNP* and an increase in blood volume of ~10% with *HUT*. The association between increased splanchnic pooling and reduced orthostatic tolerance (Stewart et al., 2004) suggests that *LBNP* testing in the supine position may not be a suitable diagnostic tool for the determination of orthostatic hypotension.

Segmental fluid shifts during active transition into the upright position are complicated by the activation of the muscle pump and temporary increases in mean systemic pressure (Guyton, 1973). Guyton et al. have shown that instantaneous muscle tensing (especially in the abdominal muscles) can cause increases in mean systemic pressure by 400%, transiently increasing the pressure gradient for venous return (Guyton, Douglas, Langston, & Richardson, 1962). Increasing the pressure gradient between the venous circulation and right atrium creates an increase in $\dot{Q}$ immediately after assuming the upright position (Guyton, 1973). However, shortly following the active transition, the shift in blood volume below the heart attenuates venous return, and $\dot{Q}$ has been shown to decrease by 20% (Taneja et al., 2007).

2.1.1.2 Mean arterial pressure during orthostatic stress

The relatively unimpeded shift of blood into the lower body following active standing is a consequence of the force of gravity along the hydrostatic column of the circulatory system (Hill, 1894). Initially, the veins in the lower body are under-filled and the skeletal muscle pump is unable to counterbalance the force of gravity, which effectively shifts large volumes of blood inferiorly (Hill, 1894). Shortly after this transition, blood remaining in the heart and lungs is pumped in declining amounts as venous return decreases (Stewart & Clarke, 2011). The small reservoir of

blood in the heart and lungs support left ventricular filling transiently, but the maintenance of arterial blood pressure is short-lived (Stewart & Clarke, 2011).

Maintaining blood pressure reflects a balance between the rate of blood volume entering arterial circulation and the amount of resistance provided by the vascular system (Wieling et al., 2007). Reduced cardiac filling decreases *SV* and a lagging sympathetic peripheral response creates a transient window in which arterial blood pressure is reduced. This transient decrease in arterial blood pressure upon active standing is strongly influenced by a reduction in total peripheral resistance (*TPR*) (Smit, Halliwill, Low, & Wieling, 1999; Sprangers, Wesseling, Imholz, Imholz, & Wieling, 1991; Tschakovsky, Matusiak, Vipond, & McVicar, 2011), as calculated by quotient mean arterial pressure (*MAP*) and $\dot{Q}$. It has been shown that immediate decreases in *MAP* are exclusive to active standing as opposed to passive tilting, indicating that muscular contraction plays a significant role in this initial response (Wieling et al., 2007). Presently, three potential mechanisms responsible for this immediate decrease in resistance have been proposed to play a contributing role: the muscle pump (Sheriff, Rowell, & Scher, 1993; Tschakovsky & Sheriff, 2004), muscle activity-dependent locally mediated vasodilation (Tschakovsky et al., 2011), and cardiopulmonary baroreceptor-mediated sympathetic withdrawal (Zuj, Harvey, Wheaton, & Hughson, 2006).

During an active transition into the upright position, the muscle pump compresses venous vessels, propelling blood cranially against the hydrostatic gradient. This creates a transient period in which the venous pressure is reduced, thereby increasing the arteriovenous pressure gradient and promoting arterial efflux (Pollack & Wood, 1949). This mechanism represents a mechanically-mediated decrease in *TPR*, often considered 'virtual conductance', because it is not the consequence of vasodilation, but instead a local change in the arteriovenous pressure gradient

(Sheriff & Nådland, 2007). This temporary increase in conductance upon standing is sufficient to create a transient imbalance between arterial inflow and outflow. Support of this hypothesis is shown in the simulated in vivo model of Sheriff and Van Bibber in which the mechanical influence of muscle contraction was studied in the absence of the associated metabolic responses (Sheriff & Van Bibber, 1998). Using a model that isolated a pig hindlimb transected with a terminal aorta and inferior vena cava, they showed that the mechanical forces produced by muscular contraction and relaxation alone were sufficient to generate a significant flow of blood through the muscle (Sheriff & Van Bibber, 1998). Additionally, Tschakovsky et al. and Nadland et al., showed that muscle compression and contraction increases arterial outflow only under conditions in which a venous hydrostatic gradient is present (Nådland, Walløe, & Toska, 2009; Tschakovsky, Shoemaker, & Hughson, 1996). This indicates that reducing venous hydrostatic pressure via the muscle pump provides an opportunity for greater arterial outflow generated by the force of gravity in the upright position. However, this efflux of arterial blood would be greatest following the first muscle contraction before the venous hydrostatic column refills. This does not correspond precisely with research in active standing, which shows the nadir in *MAP* occurring after approximately 7 seconds of standing (Rossberg & Peňaz, 1988).

Another potential mechanism thought to contribute to this transient reduction in *MAP* is locally-mediated rapid vasodilation (Tschakovsky et al., 2011). Support for this mechanism can be seen in the work of Anrep and von Saalfeld, who demonstrated that the amount of vasodilating substances released from the muscle corresponds to the intensity of muscle contraction (Anrep & von Saalfeld, 1935). These investigators studied the mechanical effects of the muscle pump by draining the blood from a contracting muscle into a resting vascular bed. The resulting

vasodilation in the resting vascular bed indicated the presence of vasoactive substances within the surrounding vasculature during muscle contraction.

Tschakovsky et al. related this vasodilatory response to increased blood flow in their research with external muscle compression and active muscle contraction in the absence of a venous pressure gradient in an attempt to isolate the effect of rapid vasodilation (Tschakovsky et al., 1996). It was demonstrated that a single forearm cuff inflation elicited an immediate elevation and subsequent decay in local blood flow, whereas a single forearm muscle contraction elicited an immediate elevation in local blood flow which was then sustained for three cardiac cycles, further indicating the existence of vasodilatory substances with sustained effects (Tschakovsky et al., 1996). Additionally, Tschakovsky et al. isolated the impact of vasoactive substances by demonstrating that contraction intensity directly correlated to the immediate hyperemia experienced following a single isometric contraction above heart level (Tschakovsky et al., 2004). This method effectively minimized the contribution of the muscle pump to blood flow by (1) eliminating the hydrostatic venous column, (2) using isometric contractions, and (3) quantifying different contraction intensities while maintaining the amount of venous emptying (Tschakovsky et al., 2004). More recently, Tschakovsky et al. were able to quantify relative increases in lower limb-localized vascular conductance and total vascular conductance after rapidly standing from a squat position (Tschakovsky et al., 2011). It was discovered that the increase in total vascular conductance could be entirely accounted for by increases in vascular conductance in the lower limbs (Tschakovsky et al., 2011). The ability of the lower limb vasculature to account for the total increases in total vascular conductance supports the theory that the initial orthostatic hypotension (*IOH)* experienced upon standing could be the consequence of rapid lower limb vasodilation-mediated decreases in *TPR*. Although this work provides supporting evidence for the existence of

locally-mediated vasodilation, it does not disprove the influence of the muscle pump in the immediate hyperemia response to exercise. Also, the intrinsic mechanisms responsible for rapid vasodilation are not well-understood. Several potential mechanisms have been proposed (Crecelius, Kirby, Luckasen, Larson, & Dinenno, 2013; Crecelius, Richards, Luckasen, Larson, & Dinenno, 2013; Knot, Zimmermann, & Nelson, 1996; Mohrman & Sparks, 1974; P. Murray & Sparks, 1978), however, this field of study warrants further investigation.

It has also been suggested that the act of standing up produces an increase in right atrial pressure great enough to elicit a peripheral reflex withdrawal of sympathetic tone capable of triggering the reduction in *MAP* seen with active standing (Wieling et al., 2007). The contraction of lower body muscles has been shown to increase intra-abdominal pressure by 43±22 mmHg and right atrial pressure by 10 – 15 mmHg due to the large bolus of blood immediately translocated into the heart (Tanaka, Sjöberg, & Thulesius, 1996; Wieling et al., 2007). It is hypothesized that this transient increase in right atrial pressure temporarily activates cardiopulmonary baroreceptors (Tanaka et al., 1996). It should be noted that the loading of cardiopulmonary baroreceptors plays a significant role in reflex withdrawal of peripheral vasomotor tone in the maintenance of arterial blood pressure (Hughson et al., 2004). Zuj et al. investigated the cardiopulmonary baroreflex gain in response to active standing and found a significant inverse correlation with *MAP*, indicating an association between cardiopulmonary baroreceptor loading and decreasing *TPR* (Zuj et al., 2006).

2.1.1.4 Heart Rate Response

Following the active transition into the upright position, an abrupt drop in *MAP* results in the unloading of arterial baroreceptors (Cooper & Hainsworth, 2002), resulting in a rapid withdrawal of parasympathetic tone to the heart, followed by a more delayed increase in sympathetic tone (Arnold & Shibao, 2013). This shift in autonomic nerve traffic increases both

cardiac rate and contractility in an attempt to compensate for the decreased arterial pressure (Arnold & Shibao, 2013).

Although the typical reflex response to orthostatic stress is denoted by a rapid elevation in heart rate (*HR*), the relative importance of tachycardia in the orthostasis adjustment as a compensatory mechanism is not as clear (Convertino, 2014; Wieling et al., 2014). A recent review of neurohumoral mechanisms associated with orthostasis by Convertino supports the theory that the initial tachycardia experienced upon standing is associated with increased orthostatic tolerance (Convertino, 2014). However, in a commentary on Convertino's 2014 paper, Wieling et al. proposed that the impact of tachycardia is much less significant in the response to orthostasis than is the impact of increased sympathetic tone to the peripheral vasculature (Wieling et al., 2014). Wieling et al. suggest that control of the central venous reservoir and the ability to return accumulated venous blood to the heart is the primary contributor supporting orthostatic tolerance (Wieling et al., 2014). Support for this theory can be demonstrated in patients with sympathetic vasomotor lesions and intact vagal *HR* control who experience symptoms of orthostatic hypotension despite presenting with postural tachycardia (Wieling et al., 2014). Similarly, Joyner described the disconnect between *HR* responses and orthostatic tolerance in patients with postural orthostatic tachycardia syndrome (*POTS*) (Joyner, 2011). It has been shown that patients with *POTS* often experience reduced $\dot{Q}$ and orthostatic presyncope upon standing caused by diminished diastolic filling time as a consequence of the reduced *R-R* intervals, and interestingly, treatment for *POTS* is often aimed toward reducing postural tachycardia. Wieling et al. also stated that the correlation between maximal sympathetically-mediated heart rate and orthostatic tolerance observed by Convertino may just be a marker for increased sympathetic nerve traffic to resistance vessels, which provide the primary defense against hypotension (Wieling et al., 2014). In support

of these findings, Cooper et al. found that while cardiac responses were equivocal in those with and without orthostatic intolerance, the vascular resistance response triggered by the unloading of arterial baroreflexes (neck suction) during *HUT* was enhanced in those with good orthostatic tolerance (Cooper & Hainsworth, 2002). One should note that the reflex mechanisms occurring during orthostasis are composed of a complex network of feedback loops on top of feed-forward control mechanisms that likely act in coordination to provide the body with the most effective means to provide adequate perfusion to the heart and brain (Convertino, 2014).

2.1.1.5 Brain Blood Flow

The brain's high oxygen demand requires an uninterrupted supply of blood in order to maintain cerebral function (Lewis et al., 2013; Williams & Leggett, 1989). During homeostatic conditions *CBF* remains relatively constant due in large part to cerebral autoregulation (Tzeng & Ainslie, 2014). Although present in many vascular beds, the brain's ability to autoregulate blood flow is particularly well-developed and can maintain roughly 50 mL/min of blood per 100 grams of brain tissue with cerebral perfusion pressure ranging between ~60 and 160 mmHg (Lassen, 1959). This ability to maintain adequate blood flow under a wide range of perfusion pressures is critically important in protecting the brain from both cerebral edema caused by elevated blood flow and conversely, cerebral ischemia caused by insufficient blood supply (Tzeng & Ainslie, 2014). Additionally, the regulation of *CBF* is critical for arterial carbon dioxide ($PaCO_2$) balance and extracellular pH stasis, as non-polar CO_2 molecules have the ability to diffuse across the cerebrovascular blood-brain barrier, thus shifting the pH of the cerebrospinal fluid (Andrews, Bringas, Alonzo, McComb, & Muizelaar, 1994; Lambertsen, Semple, Smyth, & Gelfand, 1961; Willie, Tzeng, Fisher, & Ainslie, 2014).

However, upon standing, a near simultaneous reduction in *CBF* occurs alongside the reduction in arterial blood pressure even when cerebral perfusion pressure remains within the 'static cerebral autoregulatory range' (Lewis et al., 2013). This transient decrease in *CBF* is due to a brief delay in the dynamic cerebral autoregulatory reflex and represents a short window of vulnerability in which symptoms of presyncope can manifest during this initial orthostatic hypotensive state (Lewis et al., 2013). Although reflexive decreases in cerebrovascular resistance occur within the first ~5 seconds of stepwise drops in cerebral perfusion pressure, full restoration of middle cerebral artery velocity (*MCAv*) might not occur for close to 10 seconds (Aaslid, Lindegaard, Sorteberg, & Nornes, 1989; Hughson, Edwards, O'Leary, & Shoemaker, 2001; Yang et al., 2015). On top of this, systemic blood pressure might not reach near-baseline values until approximately 30 seconds of upright standing (Finucane et al., 2014; Sprangers et al., 1991). Thus, the initial stages of standing are a critical period in which cerebral hypoperfusion and syncope can occur (Lewis et al., 2013).

This initial decrease in *CBF* with active orthostasis appears to be influenced by the immediate drop in *MAP* caused by reactive hyperemia in the lower body independent of sympathetic control of *TPR* (Lewis et al., 2013). In a study looking at *IOH* and presyncope, Lewis et al. used an alpha-1 adrenergic blockade model to show that the initial decrease in *MAP* and *MCAv* occurs as a consequence of rapid muscle hyperemia upon active standing, likely associated with localized vasoactive metabolites. On the contrary, the normalization of *MCAv* to baseline values depended on the feedback reflex response of sympathetic activity within the periphery (Lewis et al., 2013). It was hypothesized that systemic alpha-1 adrenergic blockade during active standing caused a drop in arterial blood pressure below the static cerebral autoregulatory range, resulting in progressively decreasing *CBF* and eventually presyncope (Lewis et al., 2013). Thus,

it appears as though a lagging *dynamic* cerebral autoregulatory response results in the initial decrease in *CBF*, whereas prolonged decreases in cerebral perfusion pressure below the *static* cerebral autogregulatory range results in an inability to normalize *CBF* to baseline values.

This is supported by Levine et al. who suggests that cerebral autoregulation can maintain adequate *CBF* during small fluctuations in blood pressure with moderate sympathetic activation (Levine et al., 1994b). However, when the reduction in central blood volume becomes severe and sympathetic activation is further enhanced, adrenergic-mediated vasoconstriction of cerebral arterioles overrides autoregulatory metabolic vasodilation, as is shown with reduced mean *CBF* occurring before the onset of hypotension. Levine suggested that adrenergic-mediated vasoconstriction of cerebral arterioles in response to orthostatic stress may exacerbate the decrease in *CBF* associated with systemic hypotension (Levine et al., 1994b).

It should also be noted that the reduction in *MCAv* upon standing is likely influenced by a transient decrease in $\dot{Q}$ (Lewis et al., 2013). This association is apparent in a study conducted by Ogoh et al., in which dynamic cerebral autoregulation was impaired in response to acute thoracic hypovolemia when the reflexive tachycardia response was blocked by subsequent bolus injections of metoprolol tartrate and glycopyrrolate (Ogoh, Tzeng, Lucas, Galvin, & Ainslie, 2010). As opposed to control subjects, the inhibition of reflexive tachycardia resulted in a decreased $\dot{Q}$ and decreased rate of regulation of *CBF* (0.353 ± 0.033 s^{-1} vs. 0.255 ± 0.021 s^{-1}; mean ± standard deviation) (Ogoh et al., 2010)]. Additionally, van Lieshout et al. have shown that reductions in *MCAv* are paralleled by reductions in $\dot{Q}$ when moving from supine to standing, despite unchanged blood pressure. With the initiation of lower body muscle tensing, *MCAv* and $\dot{Q}$ increased in unison, once again independent of blood pressure (van Lieshout, Pott, Madsen, Goudoever, & Secher,

2001). Altogether, it is likely that rapid changes *MAP* and $\dot{Q}$ affect dynamic cerebral autoregulation, however, the direct mechanisms responsible for these effects are presently unclear.

Furthermore, recent studies have discovered that there are likely regional differences in dynamic cerebral autoregulation in response to orthostatic stress and that distal arteries of the neck [internal carotid artery (*ICA*) and vertebral artery (*VA*)] contribute significantly to total changes in cerebrovascular conductance and resistance (Ainslie, 2012; Sato et al., 2012). Sato et al. discovered that blood flow in the *ICA* and *MCAv* were reduced during *HUT*, whereas *VA* blood flow was maintained, indicating increased vascular conductance in the *VA* (Sato et al., 2012). It was hypothesized that the maintenance of blood flow into the basilar region of the brain may be instrumental in regulating systemic circulation during orthostatic stress (Sato et al., 2012).

2.1.1.7 Prolonged Orthostasis

The immediate drop in blood pressure during active orthostasis signals a cascade of reflex responses that work to correct blood pressure and $\dot{Q}$ allowing for the maintenance of upright posture (Ainslie, 2012). Recovery from this initial hypotension requires increases in *TPR* and a well-maintained $\dot{Q}$ (Lewis et al., 2013). The tachycardia response attempts compensate for the immediate decrease in *SV*, while increased peripheral sympathetic tone attempts to correct the locally-mediated decrease in peripheral resistance. These integrated carotid and cardiopulmonary baroreceptor responses act in coordination to improve arterial inflow (recovery of $\dot{Q}$) and reduce arterial outflow (increase peripheral resistance), respectively. Arterial pressure recovers to near-baseline values when the venous system is sufficiently filled and venous valves are open creating a continuous hydrostatic column on the venous side of circulation (Tschakovsky et al., 2011). This counterbalances the initial arteriovenous pressure disparity, marking the onset of a new steady

state adapted to the upright position. However, as orthostasis is maintained, the venous pressure-volume relationship is still affected by gravity (Tschakovsky et al., 2011)

Increased luminal pressure associated with high hydrostatic pressures will cause an accumulation of blood in highly compliant organs. With time, venous compliance actually increases due to the viscoelastic properties of the veins themselves (Rowell, 1993). With a step-change in transmural pressure, the increase in compliance is delayed, a process often referred to as 'delayed compliance', 'stress-relaxation' or 'creep' (Rowell, 1993). Thus, as orthostasis is maintained, the amount of blood volume that can be stored in compliant veins tends to increase in an exponentially decaying manner (Johnson & Hanson, 1963).

Additionally, increases in local capillary luminal pressure promotes filtration of plasma from the circulation into interstitial space until a luminal-interstitial pressure equilibrium is reached through the formation of edema. This process reaches a steady-state after ~30 minutes and can produce decreases in circulatory plasma volume of up to 10% (Grubb, 2005). In response to decreased plasma volume associated with prolonged orthostasis, a series of neurohormonal responses are activated dependent on the degree of volume depletion (Grubb, 2005). One such humoral response is the renin-angiotensin-aldosterone system (Rowell, 1993). It is suggested that renin release from the kidneys is of particular importance during extended periods of orthostatic stress during which sympathetic nervous activity gradually becomes unable to maintain adequate peripheral resistance (Rowell, 1993).

Although the direct mechanisms responsible for the release of renin are not well understood, its presence within the renal afferent arteriole is significantly increased during salt deprivation and hypovolemia (Rowell, 1993). The release of renin, a proteolytic enzyme, from juxtaglomerular cells cleaves a decapeptide fragment from an alpha-2 globulin called

angiotensinogen, forming the inactive angiotensin-1. Angiotensin-1 is converted into the active angiotensin-2 as is passes from the renal vein through the microcirculation of the lungs (Rowell, 1993). In the active form, angiotensin-2 directly acts on the smooth muscle of arterioles causing vasoconstriction, while also indirectly raising vascular resistance and stimulating the heart through its potentiating effects on the sympathetic nervous system (Rowell, 1993). It has been shown that the presence of angiotensin-2 amplifies sympathetic effects by increasing the availability of norepinephrine at adrenergic effector sites. It is believed that angiotensin-2 plays a significant role in the preservation of peripheral resistance through its potentiating effects on sympathetic activity even when its plasma concentration is too low to exert direct effects on vascular smooth muscle. Additionally, angiotensin-2 has more gradual and long-term effects that last from hours to days, through its stimulation of the synthesis and release of the mineralocorticoid hormone, aldosterone (Rowell, 1993). Aldosterone is released from the adrenal cortex and acts to increase the reabsorption of sodium by the collecting duct in Henle's loop, while also reducing sodium excretion. Consequently, there is an increase in water retention at the kidneys resulting in an expansion of plasma volume. Simultaneously, angiotensin-2 also acts on the central nervous system to promote water retention through its positive influence in the secretion of anti-diuretic hormone and the promotion of sodium ingestion, each of which act in coordination with aldosterone to increase plasma volume (Rowell, 1993).

The importance of the renin-angiotensin-aldosterone system during orthostasis is limited to longer durations. Shepherd noted that arterial vasoconstriction of the forearm during 10 minutes of 90° *HUT* was sympathetically mediated, with no observable effects coming from circulating hormonal agents (Shepherd, 1963). In addition, Oparil et al. noticed that patients without kidneys maintained on dialysis were able to regulate blood pressure normally during 20 minutes of 80°

HUT (Oparil, Vassaux, Sanders, & Haber, 1970). However, in conditions of salt-deprivation and hypovolemia, which can occur during prolonged orthostasis, the inhibition of angiotensin-1-converting enzyme results in reduced splanchnic vascular resistance and postural hypotension (Oparil & Haber, 1974). Interestingly, the main vasoconstrictor effects of angiotensin-2 are directed toward splanchnic and renal vasculature, as there was no observable effect on forearm vasoconstriction (Stadeager et al., 1990). The role of angiotensin-2's splanchnic-specific vasoconstriction is likely of significant importance during prolonged orthostasis, in which upwards of ~551mL of blood has been shown to translocate to the splanchnic region during upright tilt (Diedrich & Biaggioni, 2004).

2.1.2 Presyncope and Syncope

Syncope is described as a sudden loss of consciousness and postural tone, and is provoked by any condition that jeopardizes cerebral oxygenation (van Lieshout, Wieling, et al., 2003). It is further subclassified based on varying pathogenesis and physiological mechanisms, however, the focus of this literature review will be isolated to postural syncope. Postural syncope is characterized by posturally-dependent hypotension that is relieved by recumbency, and is the most commonly occurring subclassification of syncope in adolescents (Stewart et al., 2004). These syncopal episodes are neurally-mediated when the body maladapts to the upright position, and is often termed 'vasovagal' or 'neurocardiogenic' syncope (Grubb, 2005). A reflex withdrawal of sympathetic tone to the heart and peripheral vasculature elicits a dramatic drop in peripheral resistance and $\dot{Q}$, resulting in significant decreases in *CBF* leading up to the syncopal event (Stewart et al., 2004). Returning to a recumbent or supine position eliminates the hydrostatic effect of gravity on the circulatory and allows blood flow to return to the brain, alleviating the symptoms of cerebral hypoperfusion.

Presyncope can be described by a series of symptoms stemming from decreased cerebral oxygenation without a consequent loss of consciousness (van Lieshout, Wieling, et al., 2003). Symptoms of presyncope are common in healthy individuals and consist of light-headedness, fatigue, blurred and fading vision, palpitations, and tingling of the ears (Calkins, Shyr, Frumin, Schork, & Morady, 1995; Graham & Kenny, 2001; Sutton, 1999; van Lieshout, Wieling, et al., 2003). Signs of impending vasovagal faint are facial pallor, sweating, restlessness, yawning, sighing, hyperventilation and pupillary dilation (Sutton, 1999). Presyncope develops into a collapse of postural tone and a loss of consciousness if *CBF* remains reduced for a prolonged period of time.

The occurrence of syncope is potentiated by hypovolemia (Trouern-Trend, Cable, Badon, Newman, & Popovsky, 1999), autonomic failure, and arrhythmia (van Lieshout, Wieling, et al., 2003), as well as cardiovascular deconditioning, which is seen during bed-rest studies and post-spaceflight (Buckey et al., 1996; Hughson et al., 2014). Also, medications intended to regulate blood pressure are also factors that play a role in the body's ability to tolerate orthostatic stress (Arnold & Shibao, 2013).

2.1.4 Initial orthostatic hypotension

IOH is the most frequent orthostatic complaint in adolescents and is responsible of 3.6% of all-cause fainting incidences (Stewart, 2002; Van Dijk et al., 2008). It is associated with symptoms of reduced postural tone, blurred or loss of vision and less commonly, loss of consciousness (Stewart & Clarke, 2011). The signs and symptoms are characterized by rapid onset and short-duration, occurring in synchrony with a transient and substantial drop in blood pressure (≥40 mmHg systolic and/or ≥20 mmHg diastolic) which often has a nadir 8 – 15 seconds after active standing (Stewart & Clarke, 2011; Thomas et al., 2009). Signs of *IOH* are most often

presented following prolonged supine rest and after arising from the squat position (Wieling et al., 2007). The reproduction of these signs are less obvious with passive orthostasis using *HUT* testing (Stewart & Clarke, 2011). The decrease in blood pressure can be observed as the tilt commences, however, the magnitude is much smaller and depends on the rapidity of the tilt (Stewart & Clarke, 2011). Also, the symptoms associated with *IOH* disappear within 20 – 30 seconds, a phenomenon which differentiates *IOH* from delayed orthostatic hypotension, or simply orthostatic hypotension. These differences are due to the fact that *IOH* occurs as the result of a normal delay in the compensation for gravitational loading during postural changes, as opposed to autonomic insufficiencies (Stewart & Clarke, 2011).

2.1.5 Orthostatic Hypotension

Although *IOH* would be considered orthostatic intolerance – which is defined as the development of signs and symptoms when upright which are relieved by recumbency – it is not true orthostatic hypotension (Stewart & Clarke, 2011). True orthostatic hypotension is a sustained reduction of systolic blood pressure of at least 20 mmHg or diastolic blood pressure of at least 10 mmHg within three minutes of standing or upright tilting at 60 ° or more (Stewart & Clarke, 2011). The symptoms of orthostatic hypotension will continue to persist if the upright posture is maintained, and is often associated with autonomic failure or severe autonomic dysfunction, each of which can be life threatening conditions (Smit et al., 1999). It has been suggested that within the healthy population, individual variability in orthostatic tolerance is determined by vasoconstrictor reserve, which is the intrinsic limit on sympathetically-mediated vasoconstriction (Fu, Witkowski, & Levine, 2004). The findings of Fu et al. show that time to presyncope is positively correlated with muscle sympathetic nerve activity, an indicator of neural sympathetic reserve (Fu et al., 2004). Similarly, Stewart et al. found that reduced orthostatic tolerance during

70° HUT was associated with increased splanchnic blood accumulation (Stewart et al., 2004). Thus, insufficient splanchnic-specific vasoconstrictor-reserve may partly explain individual variability to sustained orthostatic stress.

Recently, the work of Thomas et al. has shown that the sympathetic vasoconstrictor reserve thought to be responsible for orthostatic tolerance does not play a role in the magnitude of the initial drop in blood pressure experienced immediately following an active stand (Thomas et al., 2009). It was discovered that a relative intolerance to sustained orthostatic stress (*HUT* and combined *LBNP*) as measured as time to presyncope, was not related to the severity of *IOH* and cerebral hypoperfusion immediately upon standing. It was concluded that the severity of *IOH* does not predict orthostatic tolerance, suggesting that the mechanisms resulting in cerebral hypoperfusion in *IOH* and presyncope are different. Although both *IOH* and presyncope showed similar decreases in *MCAv*, *MAP* and cerebral oxygenation, presyncopal episodes also showed hypocapnia and decreased *MCAv* pulsatility, an index of cerebrovascular resistance (Thomas et al., 2009). Thus, hypocapnia and the subsequent cerebral vasoconstrictor response resulting from *sustained* orthostasis represent a paradoxical reflex that potentiates cerebral hypoperfusion. Contrarily, the influence of hypocapnia during *IOH* is unlikely to be responsible for the observed decreases in *MCAv*. It has been shown that steady state changes in *CBF* occur only after ~45 seconds following step-changes in $PaCO_2$ (Mitsis, Ainslie, Poulin, Robbins, & Marmarelis, 2004; Poulin, Liang, & Robbins, 1998; Thomas et al., 2009). Because symptoms of hypoperfusion associated with *IOH* abate in 20 – 30 seconds, it is more likely that the transient reduction in *MCAv* is due to the initial hypotension and related time delay before the reflexive effects of cerebral autoregulation begin to compensate (Aaslid et al., 1989; Hughson et al., 2001; Thomas et al., 2009; Yang et al., 2015).

2.1.1.6 Different Orthostatic Stress Tests

Passive orthostatic stress testing is often used in clinical settings to assess cardiovascular reflexes (Rickards & Newman, 2003). Methods such as *HUT* and *LBNP* are commonly used because of their simplicity and repeatability (Kam, Teo, Gunawan, & Tan, 1995; Protheroe, Ravensbergen, Inskip, & Claydon, 2013; Udani, Bavdekar, & Karia, 2004), however, all orthostatic stress tests cannot be used interchangeably because of the unique physiological responses they elicit. For example, *HUT* does not achieve the large initial drop in blood pressure that is seen with active standing (Rickards & Newman, 2003). This is due to the influence of muscular contraction and increasing arterial outflow, caused by the reactive hyperemic response (Rickards & Newman, 2003; Tschakovsky & Sheriff, 2004). However, *HUT* provides a unique situation by isolating the effect of gravity without the counter balancing effect of muscular contraction. Thus, sustained *HUT* (often combined with *LBNP*) is often used to assess orthostatic tolerance by measuring the time elapsed and physiological changes at the onset of presyncope (Arbeille, Zuj, Shoemaker, & Hughson, 2012; Protheroe, Dikareva, Menon, & Claydon, 2011; Protheroe et al., 2013). However, when used independently, *HUT* and *LBNP* result in different blood pooling patterns, dependent on the positioning of *LBNP* box on the abdomen (Taneja et al., 2007). These unique physiological responses to individual stress tests must be considered when assessing cardiovascular responses to orthostasis.

Alternatively, active orthostatic stress testing is less reproducible and is complicated by a cascade of anti-gravity physiological responses that are elicited during active standing (Stewart & Clarke, 2011). The reactive hyperemic response evokes a temporary imbalance between $\dot{Q}$ and *TPR*, resulting in a transient and substantial decrease in blood pressure (Stewart & Clarke, 2011). Additionally, active standing involves muscular contraction, acting to counterbalance the gravity-

assisted increase in perfusion pressure. It has been shown that prolonged supine rest before active standing exacerbates the drop in blood pressure, *HR* increase and subsequent blood pressure overshoot, likely due to an increased volume of blood translocated into under filled venous vasculature (Ten Harkel, van Lieshout, Van Lieshout, & Wieling, 1990). Similarly, actively standing from a squat results in temporary ischemia of the leg muscles, resulting in a build-up of vasodilatory metabolites and an increase in vascular conductance upon standing (Wieling et al., 2007). Consequently, standing from a squat has shown to induce transient decreases of up to 60 mmHg and 40 mmHg in systolic and diastolic blood pressure, respectively (Rossberg & Peňaz, 1988).

Thigh occlusion release has also been used to simulate the lower-leg hyperemic response that occurs during active and passive orthostasis (Panerai, Saeed, & Robinson, 2015). The *TCR* maneuver results in an accumulation of vasoactive metabolites, leading to a reduction in peripheral resistance and substantial increase in femoral artery blood flow upon occlusion release, similar to that seen with active transitions (Panerai et al., 2015). With *TCR*, a sudden increase in arterial outflow causes a drop in blood pressure that occurs in ~10 – 15 seconds (Panerai et al., 2015). Similar to *LBNP*, the thigh-cuff maneuver does not require gravity. Due to this advantage, the *TCR* maneuver has been used on the International Space Station to assess arterial blood pressure regulation in the absence of gravity (Hughson et al., 2014).

2.2 Methodological Assessment of Orthostatic Stress

2.2.1 Continuous Blood Pressure Monitoring

With the advent of non-invasive, continuous blood pressure monitoring in the 1980's (Finapres®), researchers and clinicians have been able to safely assess central hemodynamic responses at the onset of orthostatic stress (Imholz, Settels, Van Der Meiracker, Wesseling, &

Wieling, 1990; Tanaka et al., 1996; Tanaka, Thulesius, Yamaguchi, & Mino, 1994; Tanaka, Yamaguchi, Matushima, & Tamai, 1999; Yamaguchi, Tanaka, Adachi, & Mino, 1996). Before this, blood pressure measurements could only be made at set intervals and assessing the dynamic fluctuations in blood pressure was limited to invasive measurement techniques. Continuous blood pressure monitoring allows clinicians to discriminate between orthostatic hypotension – often associated with some degree of autonomic failure – and *IOH* – often associated with autonomic integrity and few negative consequences (Stewart & Clarke, 2011). Recently, Finucane et al. brought to light the lack of reliability of orthostatic beat-to-beat blood pressure testing, noting low-to-moderate agreement and considerable intra-subject blood pressure variability between identical stand tests separated by 4 – 12 weeks (Finucane, Savva, & Kenny, 2017). These results suggest that more sensitive orthostatic stress testing and/or blood pressure cut-off criteria are required to reliably identify individuals at risk for orthostasis-related negative outcomes.

2.2.2 Continuous Cardiac Output Measurement

The ability to continuously monitor blood pressure alongside $\dot{Q}$ and *CBF* allows researchers to study integrated reflex mechanisms that play a role in the adjustment to orthostasis. Continuous $\dot{Q}$ is commonly estimated through by pulse contour analysis using non-invasive finger plethysmography (Dyson, Shoemaker, Arbeille, & Hughson, 2010; Shibasaki et al., 2011; Wesseling, Jansen, Settels, & Schreuder, 1993), changes in thoracic bioelectrical impedance (Porter & Swain, 1987; Stout et al., 2006) and aortic Doppler ultrasound (Bouchard et al., 1987; Dyson et al., 2010; Huntsman et al., 1983). Each method is based on different physical principles and has been validated in steady-state conditions against more reliable, yet often more invasive methods (Bogert et al., 2010; Charloux et al., 2000; Huntsman et al., 1983; Marx, Hicks, & Allen, 1987; Matsukawa et al., 2004; Rowland & Obert, n.d.; Tam et al., 2004; Trinkmann et al., 2010).

However, the ability of these devices to accurately estimate dynamic changes in $\dot{Q}$ during non-steady-state or transitional conditions is less clear.

2.2.2.1 Modelflow

The principle of the Modelflow method is based on pressure pulse analyses acquired from arterial pressure waves to predict cardiac *SV* (Wesseling et al., 1993). Due to the commercial accessibility of the Finometer®, Modelflow is one of the most commonly used non-invasive and continuous techniques for estimating *SV*. The underlying methodology uses a three-element model of arterial input impedance to describe the relationship between aortic pressure and flow (Burkhoff, Alexander Jr, & Schipke, 1988; Toorop, Westerhof, & Elzinga, 1987). With the model parameters known, aortic flow is computed from the measured pressure wave by simulating the model (Burkhoff et al., 1988).

Several investigations have validated the Modelflow method during supine rest, *HUT* and exercise when a reference standard measurement was used (Harms et al., 1999; Houtman, Oeseburg, & Hopman, 1999; Sugawara et al., 2003; van Lieshout, Toska, et al., 2003). However, comparing continuous *SV* estimates from Modelflow with aortic Doppler ultrasound revealed consistent beat-to-beat variability in supine postures, but not during a 30° *HUT* (van Lieshout, Toska, et al., 2003). More recently, Modelflow was again compared to aortic Doppler ultrasound during dynamic changes in *TPR* – a parameter that is required to complete the three-element model. Similar to the findings of van Lieshout et al., *SV* estimates were comparable during baseline conditions, but not during acute changes in *TPR* caused by static hand-grip exercise, *HUT*, isoprenaline and norepinephrine infusion, and *LBNP* (Dyson et al., 2010). Modelflow has also been reported to underestimate changes in $\dot{Q}$ during heat stress instigated by *LBNP*, when compared to thermodilution-derived measures of $\dot{Q}$ (Shibasaki et al., 2011). Finally, the recent

findings of Hughson et al. show that when compared to the rebreathing method, *SV* estimates from Modelflow significantly underestimated changes in *SV* provoked by spaceflight (Hughson, Peterson, Yee, & Greaves, 2017). However, because a non-invasive 'gold standard' method for measuring beat-to-beat fluctuations in *SV* does not exist, it is difficult to validate the Modelflow method during transient, non-steady state conditions.

2.2.2.2 Thoracic bioelectrical impedance analysis

Thoracic bioelectric impedance analysis is based on the assumption that changes in electrical resistance within the thorax are related to corresponding changes in intrathoracic blood volume (Thiele, Bartels, & Gan, 2014). The methodology assumes that the heart is situated within a homogenous field of high frequency, low amplitude sinusoidal alternating current, which is supplied by two outer electrodes each placed on the neck and above the umbilicus (Venitz & Lücker, 1984). Two inner electrodes placed just within the emitting electrodes record impedance changes within the thoracic field generated by the outer electrodes (Venitz & Lücker, 1984). The measured impedance changes reflect thoracic volume displacements caused by cardiac ejection. At rest, the average thoracic impedance in a healthy adult is ~25 ohms (Kubicek, Patterson, & Witsoe, 1970). This value decreases by 0.1 – 0.15 ohms during the cardiac cycle and is output as a change in impedance (Z_o) (Kubicek et al., 1970). From the first time derivative of this value, $\Delta Z/\Delta t$, ventricular *SV* can be estimated using the following equation (Kubiceck et al. (1970):

Equation 1 $$\Delta V = \rho\, L^2 / Z_o{}^2\, T(\Delta Z/\Delta t)_{min};$$

where

ΔV	= ventricular *SV* (mL)
ρ	= resistivity of blood at 100 kHz (ohm-cm)
L	= mean distance between the two inner electrodes (cm)
Z_o	= mean body impedance between the two inner electrodes (ohms)

T = ventricular ejection time (s)
$(\Delta Z/\Delta t)_{min}$ = minimum value of $\Delta Z/\Delta t$ occurring during the cardiac cycle (ohms/s)

The resistivity of blood is often estimated with a value between 135 and 150 ohm-cm, however more accurate estimates of resistivity can be achieved if blood hematocrit and body temperature measurements are available (Mohapatra, Costeloe, & Hill, 1977). Additionally, the ventricular ejection time is obtained using the $\Delta Z/\Delta t$ tracing by measuring the time elapsed between the inflection point before the major negative peak to the most positive peak (Figure 1).

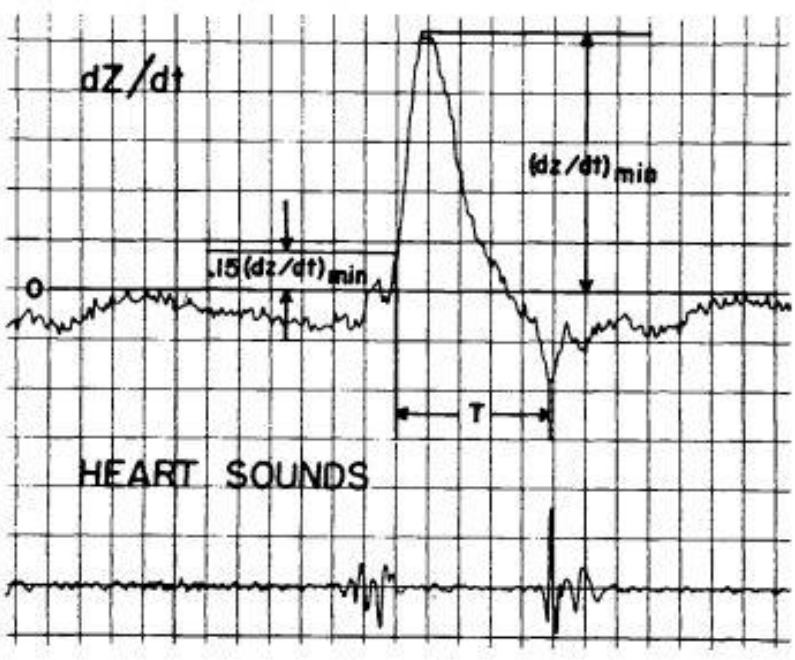

Figure 1. dZ/dt tracing and heart sounds tracing
Ventricular ejection time is measured between the inflection point immediately prior to maximum negative peak and the maximum positive peak. The value of $\Delta Z/\Delta t_{min}$ is measured from the maximum negative peak to the 0 line. Signal in the figure is inverted. [Image from Kubiceck et al. (1970)].

Similarly, the end of ventricular ejection can be determined from the second spike from a heart sound recording (Kubicek et al., 1970). The second heart sound coincides with the closing of the aortic valve and is an indicator of the end of ventricular ejection (Luisada & Portaluppi, 1982).

A conclusive consensus has not yet been reached regarding the accuracy of the *BIA* method in measuring *SV* (Woltjer, Bogaard, & de Vries, 1997). Several investigations have compared *BIA* against the dye dilution method (Gabriel, Atterhog, Oro, & Ekelund, 1976; Judy et al., 1969; Kubicek, Karnegis, Patterson, Witsoe, & Mattson, 1966; Rasmussen, Eriksen, & Andersen, 1977), direct Fick method (Betz, Bastanier, & Mocellin, n.d.; Enghoff & Lövheim, 1979; Naggar, Dobnik, Flessas, Kripke, & Ryan, 1975) and thermodilution (Boer, Roos, Geyskes, & Mees, 1979; Muzi et al., n.d.; Secher, Arnsbo, Andersen, & Thomsen, 1979), with mixed results. In regards to non-invasive methods, *BIA* was compared against aortic Doppler ultrasound, left ventricle M-Mode ultrasound and mechanosphygmography (Breithaupt, Erb, Neumann, Wo, & Be, 1990). A biometric method of principal component analysis was used to find a common 'truth' by integrating each of the non-invasive methods (Breithaupt et al., 1990). Interestingly, it was determined that *BIA* had the highest correlation to the principle component with an R-value of 0.89, suggesting that it had shared the closest correlation with the most common response of all the methods (Breithaupt et al., 1990).

2.2.2.3 Aortic Doppler Ultrasound

Continuous wave Doppler ultrasound of the ascending aorta and ultrasound imaging of the aortic annulus provide the information necessary to estimate blood flow volumes exiting the left ventricle in real time (Bouchard et al., 1987). Flow velocity tracings are achieved by directing a 2 MHz ultrasound beam towards the aortic valve opening by placing an angulated transducer at the suprasternal notch. Left ventricular *SV* can be calculated from the product of the Doppler velocity integral and the cross-sectional area of the sampling site (Bouchard et al., 1987).

Aortic Doppler ultrasound has shown strong correspondence with Modelflow during supine rest, with regression coefficients ranging from 0.91 – 0.96 (Loeppky, Hoekenga, Greene,

& Luft, 1984). However, deviation from the supine position may violate the assumptions of the aortic Doppler ultrasound method (van Lieshout, Toska, et al., 2003). A shift of the heart within the thorax can skew the angle of insonance of the ultrasound beam, effectively altering the Doppler shift. This has been proposed as a potential source of error when aortic Doppler ultrasound is used in non-supine conditions (van Lieshout, Toska, et al., 2003). van Lieshout et al. found a systematic difference of 10.5% between Modelflow and aortic Doppler ultrasound that developed within the first 10 seconds of 30° *HUT*, however, these differences could be attributed to limitations in both of these non-invasive methods (van Lieshout, Toska, et al., 2003). Also, investigators have shown that aortic Doppler ultrasound requires technical skill and continuous attention to ensure that consistent and repeatable measurements are collected, a requirement that is not necessary for both Modelflow and *BIA* (Eriksen & Walløe, 1990).

2.2.3 Cerebral Blood Flow and Oxygenation

The signs and symptoms associated with syncope and presyncope are caused by cerebral hypoperfusion and reduced cerebral oxygenation (van Lieshout, Wieling, et al., 2003). Thus, the most direct method in assessing the effects of orthostatic stress is through continuous monitoring *CBF* and cerebral oxygenation. Changes in blood flow in the cerebral circulation can be inferred from non-invasive measurements of blood flow velocity of intracranial arteries using transcranial Doppler ultrasound (Claydon & Hainsworth, 2003; Griffiths, Hoggard, Dannels, & Wilkinson, 2001). However, *MCAv* measurements can only estimate *CBF* due to the inability to track middle cerebral artery diameter changes that have been shown to occur during moderate changes in perfusion pressure and alterations in arterial blood gases (Hoiland & Ainslie, 2016). With mean flow velocity measurements, researchers can also estimate cerebrovascular resistance with the quotient of mean cerebral artery pressure and mean *CBF* velocity (Claydon & Hainsworth, 2003).

CBF can also be measured more directly (and invasively) with the use of arterial and venous blood sampling using the Kety-Schmidt method (Severinghaus et al., 1966) and with the use of magnetic resonance imaging (Calamante, Thomas, Pell, Wiersma, & Turner, 1999). Additionally, oxygen concentration within the cortical tissue of the prefrontal cortex can be estimated using near-infrared spectroscopy, providing a relational quantification of oxy- to deoxy-hemoglobin content (Murkin & Arango, 2009).

2.2.4 Fluid Shifts

Autonomic and vascular disorders can be assessed by measuring fluid shifts during orthostatic stress (Stewart et al., 2004). Segmental *BIA* provides information on the compartmentalization of blood during orthostasis. Because the accumulation of blood in highly dependent regions (i.e. splanchnic bed) is associated with decreased orthostatic tolerance (Stewart et al., 2004), the tracking of fluid shifts during orthostasis has the potential to be a valuable assessment tool in determining the localized mechanisms associated with orthostatic hypotension.

2.3 Vulnerable Populations

2.3.1 Elderly

It has been hypothesized that one of the reasons the elderly are vulnerable to orthostatic stress is because of decreasing baroreceptor sensitivity that occurs with aging (Rutan et al., 1992). *HR* appears to increase less in the elderly when compared with the young at the onset of orthostatic stress (Rutan, 2014). Increases of 20 beats or more upon standing was seen in only 315 of 4931 (6.4%) participants in a study by Rutan et al., suggesting an attenuation of the compensatory arterial baroreflex response mechanism (Rutan, 2014). It has also been reported that decreases in blood pressure upon standing are exaggerated post-prandially in the elderly (de Biase et al., 1988; Lipsitz, Nyquist, Wei, & Rowe, 1983). It was hypothesized that reduced baroreflex-mediated

splanchnic vasoconstriction leads to slowed venous blood efflux from the gut following meals, causing an exaggerated reduction venous return and $\dot{Q}$ (Lipsitz, 1985). Additionally, the heart becomes stiff and less-compliant with age, resulting in decreased diastolic filling (Gupta & Lipsitz, 2007). Consequently, 30% of community-dwelling individuals over the age of 75 years, and 50% of frail elderly individuals in nursing homes have orthostatic hypotension (Gupta & Lipsitz, 2007). This represents a critically vulnerable population as orthostatic hypotension in the elderly has been linked to falls, fractures, transient ischemic attacks, syncope and myocardial infarctions (Gupta & Lipsitz, 2007).

In addition, the prevalence of essential hypertension increases with age and treatment with antihypertensive drugs can exacerbate orthostatic hypotension (Pinto, 2007; Vagaonescu, Saadia, Tuhrim, Phillips, & Kaufmann, 2000). The combination of these conditions (supine *hype*r-tension and orthostatic *hypo*-tension) within a single patient reflect a unique therapeutic dilemma, as pharmacological treatments for either condition may worsen the other (Naschitz, Slobodin, Elias, & Rosner, 2006). However, it has been shown that particular combinations of antihypertensive drugs, namely peripheral vasodilators - such as alpha receptor antagonists and nondihyrdropyridine calcium channel antagonists - may worsen orthostatic hypotension when compared to other pharmacological treatments (Fotherby & Iqbal, 1997; Mader, 1989; Naschitz et al., 2006). On the contrary, antihypertensives such as angiotensin converting enzyme inhibitors, angiotensin receptor antagonists and beta adrenoreceptor antagonists are less likely to exacerbate orthostatic hypotension (Mader, 1989). The implementation of non-pharamcological treatment options for orthostatic hypotension may help to address this paradoxical problem.

2.3.2 Endurance Athletes

Chronically elevated central blood volume in endurance-trained athletes has the potential to attenuate the responsiveness of central volume and arterial pressure receptors (Bedford & Tipton, 1987; Fadel et al., 2001; Raven et al., 1988). Additionally, increased vasculature in the working muscles provides a greater reservoir for the pooling of blood below the heart upon standing (Lind-Holst et al., 2011). In a recent study by Lind-Holst et al., it was discovered that endurance-trained individuals experienced greater drops in blood pressure and indices of *CBF* in response to *TCR*, when compared to sex-matched untrained individuals (Lind-Holst et al., 2011). Interestingly, endurance-trained individuals had a delayed increase in cerebrovascular conductance following rapid changes in perfusion pressure, suggesting a delayed onset of cerebral autoregulation. This less-effective dampening of oscillations in blood pressure likely contributes to the prevalence of initial orthostasis-related symptoms in endurance athletes (Lind-Holst et al., 2011).

2.3.3 Heart Failure Patients

Substantial decreases in systolic blood pressure and an impaired tachycardia response upon orthostatic stress potentiate the effects of orthostasis in heart failure patients (Fraser et al., 2015; Potocka-Plazak & Plazak, 2001). It has been postulated that the combination of medications plus the disease itself are responsible for the magnitude of decreases in blood pressure associated with *HUT* in individuals with congestive heart failure (Potocka-Plazak & Plazak, 2001). An attenuated tachycardia response and decreased cardiac contractility cause an imbalance between arterial inflow and outflow during orthostatic stress, resulting in substantial decreases in *MAP* and associated reductions in *CBF* (Cornwell & Levine, 2015).

2.3.4 Post-Flight Astronauts

Following short- and long-duration exposure to microgravity, astronauts frequently return to earth with reduced physical fitness, altered autonomic reflex responses, and reduced cardiac muscle mass (Hughson et al., 2014). *HR* variability, an indicator of cardiovascular health, has also seen to be reduced in post-flight astronauts, reflecting a decreased regulation of cardiovascular control (Hughson et al., 2014). When comparing pre-, mid- and post-flight baroreflex response slopes during paced breathing, Hughson et al. noticed significant reductions in only the post-flight condition, suggesting that prolonged exposure to microgravity may reduce the effectiveness of the arterial baroreflex to compensate for changes in blood pressure upon active standing on earth (Hughson et al., 2014).

2.3.5 Fighter Jet Pilots

The increased hydrostatic component on the circulatory system caused by temporary exposure to hypergravitational forces can be combatted by using the anti-G straining maneuver (Kobayashi, Tong, & Kikukawa, 2002). Pilots that are exposed to these high gravitational forces are trained to initiate these straining maneuvers during air-to-air combat maneuvering to prevent dramatic and potentially deadly shifts of blood away from the brain (Kobayashi et al., 2002). The straining maneuver consists of a continuous and maximal contraction of lower body and abdominal skeletal muscles and the intermittent closing of the respiratory tract at the glottis (Comens, Reed, & Mette, 1987). This maneuver improves G-tolerance by elevating arterial blood pressure and promoting blood flow to the head (Chen, Wu, & Kuo, 2004). However, the release of maximal isometric contraction results in exercise-induced hyperemia to the active muscles, effectively pulling large volumes of blood out of central circulation. This hyperemic response has the potential to cause significant decreases in central blood volume and blood pressure (Wieling et al.,

2007). Unpublished pilot data from our lab has shown near-instantaneous drops in *MAP* of near ~100 mmHg with concomitant decreases in *MCAv* following the release of the anti-G straining maneuver. However, one must consider that cerebral perfusion pressure will not elevate to the same degree when extreme *Gz* forces act upon the body during the realistic conditions in which the straining maneuver is commonly employed.

2.4 Countermeasures to Orthostatic Stress

The symptoms associated with orthostatic hypotension are a direct consequence cerebral hypoperfusion and reduced cerebral oxygenation (Figueroa, Basford, & Low, 2010; van Lieshout, Wieling, et al., 2003). Thus, effective countermeasures to orthostatic stress target different mechanisms to achieve improved *CBF*. Common therapies in clinical settings usually consist of a combination of vasoconstrictor drugs, volume expansion tactics, compression garments and counter-pressure maneuvers (Figueroa et al., 2010). In both the elderly and patients with cardiovascular disease, pharmacological treatments focusing on increasing peripheral vasoconstriction and blood volume can be hazardous due to their influence on resting blood pressure in the supine position (Figueroa et al., 2010). For this reason, non-pharmacological treatment options that improve orthostatic tolerance without increasing supine blood pressure should be considered first (Figueroa et al., 2010).

Several countermeasures to orthostatic stress focus on physical maneuvers meant to generate counter-pressures that can oppose gravitational venous pooling (Wieling et al., 2015). One of the most commonly used physical counter-pressure maneuvers is active muscle tensing. It has been demonstrated that those with lower intramuscular calf pressure (6 - 9mmHg) have a higher tendency to faint during *HUT* when compared to those with higher intramuscular calf pressure (15 – 24mmHg) (Mayerson & Burch, 1940). Lower body muscle tensing, which consists

of tensing of leg, buttock and abdominal skeletal muscles, has been shown to be effective in increasing blood pressure in patients with orthostatic hypotension mediated by autonomic failure, as well as during vasovagal reactions (Henderson, Oughterson, Greenberg, & Searle, 1935; Wieling & Leshout, 2009). In a study by Krediet et al., it was discovered that participants who engaged in lower body muscle tensing immediately following a *SS* transition had mean blood pressure nadirs at 88 mmHg, while in control subjects it was 69 mmHg (Krediet et al., 2007). It was hypothesized that the attenuated drop in blood pressure was the result of blunting the immediate decrease in *TPR* that is experienced at the onset of active standing (Krediet et al., 2007; Tschakovsky et al., 2011).

An alternative counter-pressure maneuver is muscle pumping (Wieling et al., 2015). The muscle pump is capable of translocating blood against the hydrostatic pressure gradient and back toward the heart. In a recent study in elderly persons, Nagaya et al. discovered that passive dorsi- and plantar-flexion increased cerebral oxygenation in the supine position, without altering *MAP* or *HR*, suggesting an interrelationship between cerebral oxygen availability and passive activation of the muscle pump (Nagaya et al., 2015). Interestingly, recent work by Bernardi et al. has revealed connection between carotid sinus baroreceptor modulation and postural sway (Bernardi, Bissa, DeBarbieri, Bharadwaj, & Nicotra, 2011). It was hypothesized that orthostatic hypotension may result in a reflex response which activates muscle contraction resulting in postural sway in an attempt to improve venous return.

Alternative to self-controlled physical counter-pressure maneuvers, static external compression devices are also commonly used to combat peripheral pooling during orthostatic stress (Lucas, Ainslie, Morrison, & Cotter, 2012; Smit et al., 2004; Stenger et al., 2013; Stenger, Brown, Lee, Locke, & Platts, 2010). Static compression of the abdomen has been shown to be the

most effective in improving orthostatic tolerance, presumably because of the high capacitance of the splanchnic region (Denq et al., 1997). However, when combined with external compression of the thighs and calves, orthostatic tolerance can be further improved (Denq et al., 1997). Isolated abdominal compression and all compression (abdomen + thigh + calves) were the only conditions that resulted in increased orthostatic blood pressure and *TPR*, indicating an association between elevated *TPR* and improved orthostatic tolerance (Denq et al., 1997). However, the use of abdominal compression has been reported to be uncomfortable, difficult to dawn and doff, and was associated with poor patient compliance (Benkö, Cooke, McNally, & Mollan, 2001; Raju, Hollis, & Neglen, 2007).

The use of graded calf compression garments are suggested to be the most effective means of lower leg static compression, presumably because gravity results in the highest hydrostatic pressures at the ankle (Protheroe et al., 2011). A recent study conducted by Protheroe et al. investigated the efficacy of graduated compression socks with a 35 – 29 mmHg pressure gradient measured from the ankle to knee (Protheroe et al., 2011). During 60° *HUT* and incremental increases in *LBNP*, it was discovered that graduated compression stockings had no effect on orthostatic tolerance when compared to a randomized control placebo. It was also noticed that both groups experienced comparable cardiovascular responses to 60° *HUT*, indicating an ineffectiveness of graduated compression stockings at attenuating the magnitude of venous pooling. In a recent study by Lucas et al., it was noticed that the use of commercially-available compression stockings delayed the onset of the blood pressure nadir following active standing, indicating that the resistance applied by the compression stockings was capable of slowing that rate venous pooling during orthostatic stress. However, the delay in venous pooling had no effect on magnitude of the *MAP* nadir, nor did it alter cerebral hemodynamics (Lucas et al., 2012).

Recent work coming out of out lab has shown that lower-leg active compression timed to the local diastolic phase of each cardiac cycle is capable of increasing the local blood flux and *SV* during standing plantarflexion exercise, and during exercise recovery (Zuj, Prince, Beentjes, Hughson, & Peterson, 2017). Similarly, Guyton et al. noticed that tetanic stimulation of lower-body muscles in the upright position elicited a transient increase in $\dot{Q}$ by 30 – 40%, before returning to baseline values, whereas intermittent stimulation of lower-leg muscles elicited a sustained increase of the same magnitude (Guyton, 1973). Presently, the efficacy of intermittent lower-leg compression as a countermeasure to orthostatic stress has not been tested.

CHAPTER 3: *COMPARISON OF IMPEDANCE CARDIOGRAPHY, MODELFLOW, AND AORTIC DOPPLER ULTRASOUND ESTIMATES OF STROKE VOLUME DURING ACUTE CHANGES IN BLOOD PRESSURE*

3.1 Introduction

Continuous monitoring of *SV* and $\dot{Q}$ provides clinicians and researchers valuable information regarding tissue perfusion, oxygen delivery and cardiac contractility. The use of pulse contour analysis to estimate *SV* is commonly used by researchers because of its continuous and non-invasive nature, as well as its ease of use in a wide variety of testing conditions. The commercial accessibility of Finometer® devices has provided researchers with the ability to obtain estimates of cardiac *SV* and $\dot{Q}$ continuously and in real-time. The Modelflow method, which is employed by Finometer® devices, has been validated in supine (Wesseling et al., 1993), standing (Matsukawa et al., 2004), steady-state exercise (Faisal, Beavers, Robertson, & Hughson, 2009; Sugawara et al., 2003) and head up- and down-tilt (Harms et al., 1999). Under certain conditions of acute or chronic changes in arterial compliance, deviations of the Modelflow method from other *SV* estimation methods have been observed (Dyson et al., 2010; Hughson et al., 2017; Shibasaki et al., 2011). There is limited information available that compares continuous *SV* estimates against the Modelflow method with the same high time-resolution. However, van Lieshout et al. did compare Modelflow with Doppler ultrasound *SV* measured from the aorta, reporting differences between the methods were less than the physiological beat-by-beat variability, but that tilting 30 degrees head-up produced a systematic difference of 11% (van Lieshout, Toska, et al., 2003).

The Modelflow method is based on a three-element model that relates pulse wave contours acquired from arterial pressure to aortic flow. This model relies on assumptions used to determine interrelationships between aortic characteristic impedance (Z_o), arterial Windkessel

compliance (*Cw*) and *TPR* (Wesseling et al., 1993). Absolute measures of *SV* by the Modelflow method requires calibration with a more direct method to account for the high degree of variability in the size and properties of the human aorta, however, it has been reported that Modelflow's precision in tracking changes is unaffected by this variability (Harms et al., 1999; Jellema, Imholz, Van Goudoever, Wesseling, & van Lieshout, 1996; van Lieshout, Toska, et al., 2003; Wesseling et al., 1993). Questions of Modelflow's validity in non-steady-state conditions stem from the assumption that *TPR* changes relatively slowly when compared to *R-R* interval (Wesseling et al., 1993). The acute changes in peripheral resistance with standing from a squat position (Tschakovsky, Matusiak, Vipond, & McVicar, 2011) and at the onset of exercise (Ishii et al., 2016; Wieling, Harms, ten Harkel, van Lieshout, & Sprangers, 1996) might violate this required assumption. Both the *SS* transition and bilateral thigh occlusion release have been shown to induce dynamic decreases in blood pressure and peripheral resistance (Krediet et al., 2007; Rueckert & Hanson, 1995; Tschakovsky et al., 2011). For this reason, it was chosen to continuously monitor *SV* using three different methodologies – Modelflow (SV_{MF}), aortic Doppler ultrasound ($SV_{U/S}$) and bioelectrical impedance analysis (SV_{BIA}) – each based on different physical properties. Doing so, the attempt was made to better understand the immediate *SV* responses to orthostatic stress and identify potential method biases during dynamic changes in blood pressure and *TPR*.

It was hypothesized that: (1) *SV* would increase immediately upon standing due to a transient increase in venous return before dropping to a new, lower steady-state volume, (2) *SV* would increase with upright bilateral thigh-occlusion release due to a concomitant decrease in arterial blood pressure, and (3) there would be significant method bias immediately following both orthostatic stresses due to a rapid change in physiological state.

3.2 Methods

3.2.1 Subjects

Nine male (25.2 ± 3.2 years of age, mean ± standard deviation) and six female (20.5 ± 1.8 years of age) healthy university students (body mass index: 23.2 ± 3.2 kg/m^2) gave written informed consent to participate in the experiment (Appendix A). All subjects completed a health status form and a Physical Activity Readiness Questionnaire (PAR-Q & You) to ensure inclusion criteria were met (Appendix B and C). An information letter was sent to each participant prior to arriving on testing day to provide familiarization of the specifics of the testing protocol (Appendix D). One subject was unable to complete the protocol due to an episode of postural presyncope during the thigh-cuff release protocol. Study protocols and procedures were approved by the University of Waterloo, Clinical Research Ethics Committee (ORE# 21433) and conformed to the Declaration of Helsinki.

3.2.2 Measurements

Subjects were instrumented with monitoring equipment upon entry into the lab. Data collection was continuous and progressed throughout the duration of the testing period. *HR* (Pilot 9200, ColinMedical Instruments, San Antonio, TX, USA), arterial blood pressure, and *SV* estimated by pulse contour analysis with height correction on the left arm and hand continuously held at heart level (Finometer®, FMS, Amsterdam, The Netherlands), and blood velocity in the ascending aorta assessed by pulsed wave Doppler ultrasound (Multigon Industries, Mt Vernon, NY, USA) were collected using a data-acquisition system (PowerLab, ADInstruments, Colorado Springs, CO, USA). For $SV_{U/S,}$ the pulsed Doppler 2 MHz probe was positioned in the suprasternal notch and directed towards the aortic root with the sample volume immediately above the aortic valve to obtain the maximal velocity during systole and an angle of insonance with forward blood

flow within 15°, in accordance with Tibbals et al. (Eriksen & Walløe, 1990; Tibbals, Osborne, & Hockmann, 1988). The diameter of the base of the aorta was measured by echo Doppler ultrasound (Mindray M5, Shenzen Mindray Bio-medical Electronics, Shenzen, China). *SV* was also measured by impedance cardiography (Minnesota Impedance Cardioraph 304B, Surcom, Minneapolis). Disposable surface electrodes were used and the specific positioning was adapted from the methods of Bernstein (Bernstein, 1986). Two neck electrodes were placed just below the ears along the midaxillary line, with another two electrodes placed 3 cm directly inferior. The first thoracic electrodes were positioned on the midaxillary line at the xiphisternal joint, with the bottom two electrodes placed 3 cm inferior. An illustration depicting the placement of electrodes can be seen in Figure 2. Superficial femoral artery blood velocity (*SFAv*) was continuously measured using Doppler ultrasound (WAKIe, Atys Medical, Soucieu en Jarrest, France). A 4 MHz Doppler probe was positioned on the medial thigh and was held in place with an elastic bandage throughout the study.

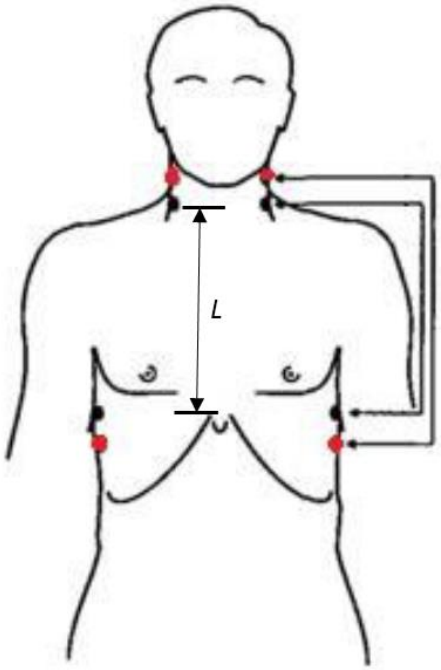

Figure 2. Placement of emitting and receiving electrodes for determination of SV_{BIA}
The red circles represent emitting electrodes, while the black circles represent receiving electrodes. The window between the black electrodes represents the segment in which changes in blood volume were measured. The length of this window (L) was measured for the calculation of SV using the Kubiceck quation (**Equation 1**). Image adapted from Woltjer et al., 1997.

3.2.3 Experimental Protocol

The testing of each subject was completed during a single visit lasting approximately 2 hours. Subjects were requested to refrain from ingesting caffeine or participating in heavy exercise for 5 hours prior to testing, and to avoid alcohol and tobacco for 24 hours prior to testing. Once instrumented, subjects stood quietly in the upright position for 3 minutes while baseline measurements were recorded. *SS* transitions and *TCR* maneuvers were randomly ordered. The *SS* test involved a 3-minute squat followed by a rapid transition into the standing position, while the *TCR* maneuver involved 3 minutes of thigh occlusion before rapid cuff release. Subjects remained standing quietly for an additional 2 minutes in both trials. A 3-minute seated period between trials

allowed subjects to recover from the previous orthostatic stress test. The *SS* protocol is outlined in Figure 3 and the *TCR* protocol in Figure 4. The *SS* transition and *TCR* maneuvers were each repeated 3 times.

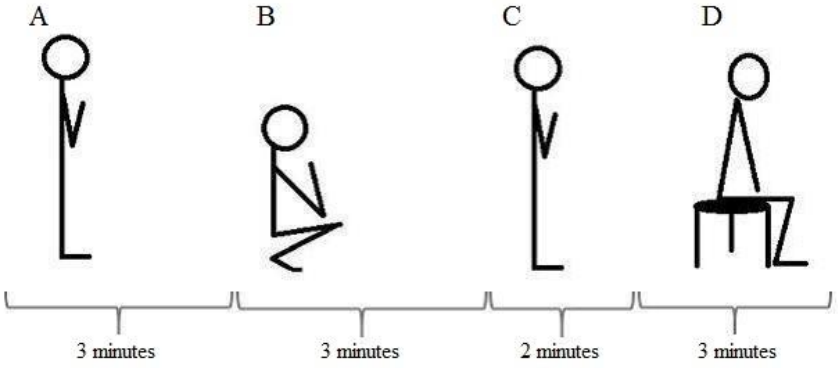

Figure 3. Squat-to-stand transition
(A) Participants stood quietly in the upright position while baseline data were recorded. (B) Participants lowered into the squat position, ensuring that their backs were flat against a wall. (C) Participants quickly transitioned into the standing position without the aid of their hands. (D) A period of seated rest separated trials to ensure the participants fully recovered.

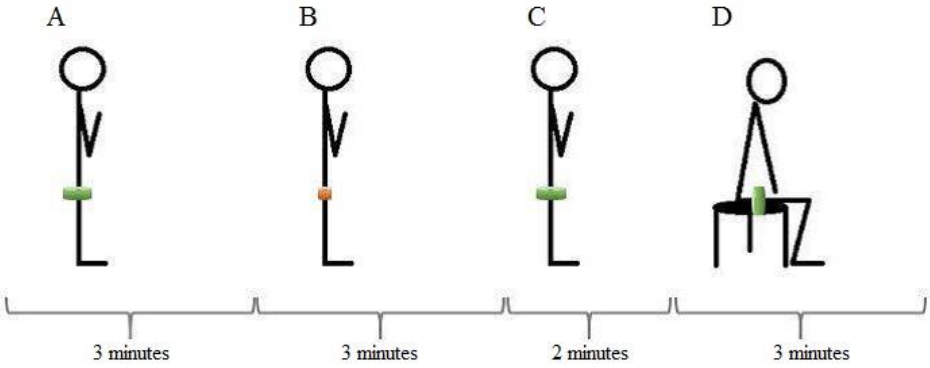

Figure 4. Thigh-cuff release maneuver
(A) Participants stood quietly with the cuffs deflated while baseline data were recorded. (B) Rapid inflation of the thigh-cuffs occluded blood flow into and out of the leg. (C) Rapid deflation of the thigh-cuffs released blood flow occlusion. (D) A period of seated rest separated trials to ensure participants fully recovered.

3.2.3.1 Squat-to-Stand Test

It has been suggested that prolonged squatting results in muscle ischemia caused by partial arterial occlusion due to isometric contraction and hyperflexion of the hip and knee, which results in an accumulation of vasoactive metabolites and an efflux of arterial blood upon standing (Convertino et al., 1998; Tschakovsky et al., 2011). Several studies have described different *SS* protocols, with variations in duration and technique (Convertino et al., 1998; Marfella et al., 1994; Rossberg & Peňaz, 1988; Tschakovsky et al., 2011). Preliminary testing revealed that a 3-minute deep squat was a satisfactory stimulus to elicit an immediate drop in blood pressure of over 30 mmHg without causing excessive discomfort for the participant. Subjects lowered into the squatting position and were instructed to squat as deeply as possible without using their hands for balance. Once in the squat position, subjects pressed their backs erect against a wall to maintain upper body positioning that was similar to standing. Maintaining verticality in the upper body was ensured to eliminate potential $SV_{U/S}$ measurement errors caused by tilting of the heart within the thorax during posture changes, resulting in a skewing of the ultrasound insonation angle (van Lieshout, Toska, et al., 2003). At the 3-minute mark, subjects were instructed to stand quickly and remain upright for an additional 2-minutes. Vertical upper body positioning was maintained throughout the transition.

3.2.3.2 Thigh-Cuff Release Maneuver

The sudden release of inflated thigh cuffs induces transient arterial hypotension (Fadel et al., 2001; Ogoh et al., 2010; Panerai et al., 2015) which is exaggerated with *HUT* (Sato et al., 2012). Several studies have employed different approaches to the *TCR* maneuver, with varying durations of occlusion, inflation pressure, and postures (Aaslid et al., 1989; Hamilton et al., 2012; Ogoh et al., 2010; Panerai et al., 2015; Sato et al., 2012). Preliminary testing revealed that bilateral

arterial occlusion for 3-minutes in the standing position elicited a drop in *MAP* by approximately 15 mmHg. The magnitude of inflation pressure was individually determined by monitoring *SFAv* just inferior to the thigh cuffs. The minimum inflation pressure that could sufficiently occlude *SFA* blood flow was used for each subject. Following three minutes of occlusion, rapid deflation of the cuffs was achieved with a rapid cuff deflator (Hokanson model RD2). Subjects remained standing quietly for an additional 2 minutes while data were continuously collected. Thigh-cuff occlusion was applied to each participant's legs before data collection commenced to familiarize each subject with the procedure in an attempt to alleviate any apprehensions.

3.2.4 Data Analysis

Arterial blood pressure, *ECG*, *BIA* and *SFAv* were collected at 1000 Hz (PowerLab, ADInstruments, Colorado Springs, Colorado, USA) and recorded using LabChart software (LabChart, v7.3.7, ADInstruments, Colorado Springs, Colorado, USA). The *ECG R-R* interval was used to calculate continuous *HR*. $\dot{Q}$ was calculated from the product of *HR* and *SV*. *MAP* was continuously computed as the mean value of the reconstructed brachial artery pressure wave form over each cardiac cycle. *TPR* was calculated as the quotient of *MAP* and $\dot{Q}$ using the equation:

Equation 2 $$TPR = MAP / (SV*HR) ;$$

where *TPR* is total peripheral resistance (in mmHg/mL/min), *MAP* is the mean arterial pressure (in mmHg), *SV* is stroke volume (in mL) and *HR* is heart rate (in beats per minute). All analysis made use of *TPR* acquired using $SV_{U/S}$ and is noted as $TPR_{U/S}$ unless otherwise stated.

The outer envelope of the velocity tracing from the aortic Doppler ultrasound was integrated within each cardiac cycle to acquire blood flow distance travelled per beat. The aortic annulus was measured by echo Doppler ultrasound by capturing cines of >15 cardiac cycles for

off-line analysis. Image-J (National Institutes of Health) open source image processing software was used to measure the diameter of the aortic annulus during ventricular ejection with the placement of calipers at the base of the aortic valves. Measurements were only taken when the aortic valves were visible and maximally opened. $SV_{U/S}$ was calculated during the final minute of the 3-minute baseline stand and from the final minute of the squat and thigh-occlusion until the subject regained the seated position at the end of the trial. The equation used to calculate $SV_{U/S}$ was:

Equation 3 $$SV_{U/S} = V_a * A_{Aorta};$$

where $SV_{U/S}$ is the stroke volume measured by Doppler ultrasound (in mL), V_a is the integrated aortic blood velocity (in cm), and A_{Aorta} is the cross-sectional area of the base of the aorta (in cm^2).

Beat-to-beat SV_{MF} was calculated by the Modelflow method using the Finometer® device through the analysis of the finger pressure waveform using a three-element (Z_o, C_w, and TPR) non-linear equation, dependent on the pressure-area relationship of the aorta. Age, sex, height and weight of each subject was input into the Finometer® device prior to the commencement of testing. These parameters are used to determine individual aortic pressure-area relationships. The pressure-area relationship provides a means for computing Z_o and C_w, while TPR is estimated from the model. The equations used to generate aortic flow are:

Equation 4 $$Z_o = \sqrt{(\rho/AC)}$$

Equation 5 $$C = \Delta A/\Delta P$$

Equation 6 $$C_w = lC;$$

where ρ is the density of blood, A is the aortic cross-sectional area, C is the compliance per unit length of the aorta, P is pressure and l is the length of the aorta.

Beat-to-beat SV_{BIA} was calculated using the equation of Kubicek (Kubicek et al., 1966). The equation proposed by Kubiceck utilized the mean and the first derivative of the impedance signal measured between the two sets of inner electrodes. The equation used was:

Equation 1 $$\Delta V = \rho\, L^2 / Z_o^{\,2}\, T(\Delta Z/\Delta t)_{min};$$

where

ΔV	= ventricular SV (mL)
ρ	= resistivity of blood at 100 kHz (ohm-cm)
L	= mean distance between the two inner electrodes (cm)
Z_o	= mean body impedance between the two inner electrodes (ohms)
T	= ventricular ejection time (s)
$(\Delta Z/\Delta t)_{min}$	= minimum value of $\Delta Z/\Delta t$ occurring during the cardiac cycle (ohms/s)

The ventricular ejection time was obtained using the $\Delta Z/\Delta t$ trace by measuring the time elapsed between the inflection point before the major positive peak to the most negative peak (Figure 5). A surrogate blood resistivity value of 150 ohm-cm was used for each subject, as individual hematocrit values were not collected.

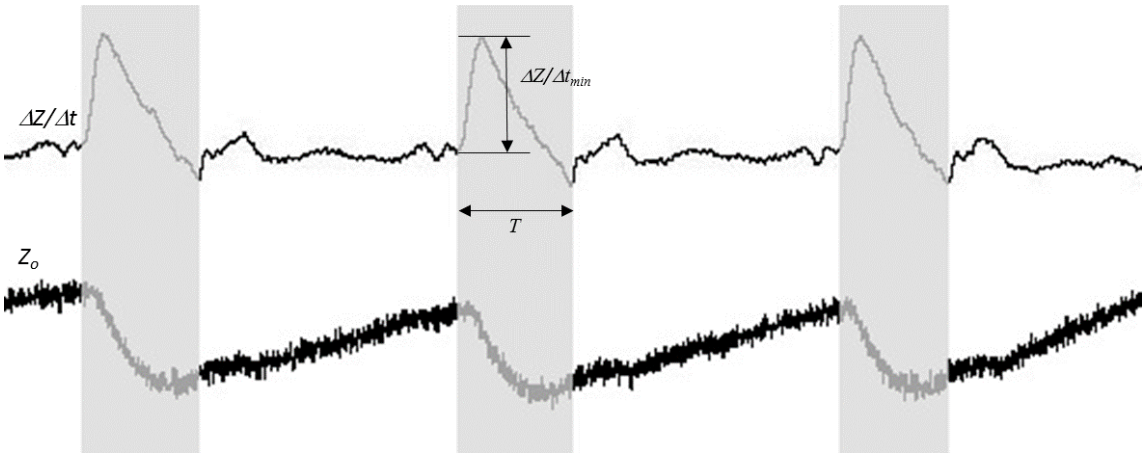

Figure 5. Raw bioelectrical impedance signals for determination of SV_{BIA}
Three cardiac cycles of raw, inverted bioelectrical impedance data collected from a single subject. The area highlighted in grey represents ventricular ejection (*T*).

Intra-subject trials were averaged before all subjects were cumulatively averaged. *SS* and *TCR* trials were time-aligned by manually identifying and aligning the rapid increase in *SFAv* triggered by standing and the release of circulatory occlusion. All beat-by-beat variables were interpolated at 1 Hz for comparative analysis. The data in the figures were smoothed using a 5-second running average.

3.2.4.1 Data Exclusion Criteria

All *TCR* that did not elicit at ≥10 mmHg drop in blood pressure were eliminated from analysis (4/42). Additionally, trials in which reliable aortic Doppler ultrasound signals could not be acquired were also eliminated from analysis. These were often trials in which several beats were missed or unclear during the transition period. SV_{BIA} was not available for one subject due to technical problems with one of the bioelectrical impedance channels.

One subject experienced dizziness during the first *TCR* trial and did not continue testing. Additionally, one subject was unable quickly stand from the squat position and was eliminated from all *SS* analysis. This subject's *TCR* data were included in the analysis.

3.2.5 Statistical Analysis

Shapiro-Wilk normality tests and Brown-Forsythe equal variance tests were performed on all of the variables during the standing baseline period to justify the use of parametric statistical testing (SigmaPlot 13.0, Systat Software, San Jose, CA, USA). A 2x2 (orthostatic stress test by time) repeated measures ANOVA was used to compare *HR*, *SFAv*, *MAP* and $TPR_{U/S}$ at 10 second averages collected at the end of standing baseline, end-squat/end-occlusion, and 30 seconds and 60 seconds post-squat and *TCR*, and at the *HR* and *SFAv* peaks and the *MAP* and $TPR_{U/S}$ nadirs. Two-tailed paired T-tests compared the timing of *HR* and *SFAv* peaks and *MAP* and $TPR_{U/S}$ nadirs between the two orthostatic stress tests.

A comparison of the absolute *SV* measures from each methodology was made during standing baseline, end-stress, 1-minute post-stand and *TCR*, and 2-minutes post-stand and *TCR* using the last 10-seconds of each of the individual epochs. These particular epochs were compared using a 2x2 (method by time) repeated measures ANOVA with Holm-Sidak post hoc testing for both orthostatic stress tests.

The dynamic change in *SV* at the onset of standing and *TCR* was assessed using end-squat and end-occlusion normalized values presented as percent changes. Interpolated values to 1-second intervals were compared for the first 60 seconds following the stand and *TCR* using a 2x2 (method by time) repeated measures ANOVA with Holm-Sidak post-hoc testing. All main effects for method, as well as all interaction effects are presented in Figures 6 and 7. Similarly, a 2x2

repeated measures ANOVA was used to compare $SV_{MF} - SV_{U/S}$ method bias provoked by the *SS* transition and *TCR* maneuver during the first 20 second of each orthostatic stress.

3.3 Results

3.3.1 Hemodynamic responses during the squat-to-stand transition and thigh cuff release

Table 1 summarizes the *MAP*, *HR*, $TPR_{U/S}$ and *SFAv* responses to both orthostatic stress test. There were no differences in standing baseline values between the two orthostatic stress tests. By the end of the squat, *MAP* had increased, while *HR* and *SFAv* decreased (*MAP*: P<0.001, *HR*: P<0.001, SFAv: P=0.034). Engaging in the squat position trended toward a decrease in $TPR_{U/S}$ when compared to standing baseline (P=0.054). Three minutes of standing bilateral thigh occlusion increased *HR* (P=0.001), without altering *MAP*, $TPR_{U/S}$ or *SFAv*. Three minutes of squatting resulted in a greater decrease in *HR* and $TPR_{U/S}$, and a greater increase in *MAP* when compared to 3 minutes of standing bilateral thigh cuff occlusion (All: P<0.001). The peak *HR* and *SFAv* responses following the *SS* transition and *TCR* were significantly greater than all other time points (*HR*, *SFAv*: P<0.001). Similarly, *MAP* and $TPR_{U/S}$ nadirs following the *SS* transition and *TCR* were different from all time points (*MAP*, $TPR_{U/S}$: P<0.001). The *MAP*, *HR* and *SFAv* responses were exaggerated during the *SS* condition, while the $TPR_{U/S}$ nadir was similar between conditions (*MAP*, *HR*, *SFAv*: P<0.001; $TPR_{U/S}$: P=0.43). At 60s and 90s post-stand and *TCR*, *MAP*, *HR*, $TPR_{U/S}$ and *SFAv* were different from their respective peaks and nadirs and were not different from standing baseline values. At 60s post-stand, *MAP* remained lower and *SFAv* remained higher in the *SS* condition when compared to *TCR* (*MAP*: P=0.05, *SFAv*: P=0.041).

Table 1. Hemodynamic responses to squatting and return to standing, and thigh occlusion and subsequent release.

	Standing Baseline	End Squat/ End Cuff	Peak/ Nadir	60-s Post-stand/*TCR*	90-s Post-stand/*TCR*
MAP, mmHg					
SS	93.8 ± 8.3$^{\dagger\#}$	103.9 ± 10.6†	67.5 ± 9.3$^{\#}$	96.3 ± 10.3$^{\dagger\#}$	92.9 ± 9.6$^{\dagger\#}$
TCR	94.5 ± 6.8†	97.0 ± 7.8*†	79.0 ± 10.8*$^{\#}$	94.3 ± 9.6*†	93.7 ± 10.0†
HR, beats/min					
SS	76.4 ± 12.9$^{\dagger\#}$	69.7 ± 11.2†	103.2 ± 13.7$^{\#}$	75.5 ± 11.5$^{\dagger\#}$	75.3 ± 11.3$^{\dagger\#}$
TCR	73.9 ± 11.5$^{\dagger\#}$	81.3 ± 11.3*†	91.4 ± 9.0*	73.5 ± 10.1$^{\dagger\#}$	75.1 ± 9.6$^{\dagger\#}$
$TPR_{U/S}$, mmHg*(L/min)$^{-1}$					
SS	26.1 ± 7.8†	23.4 ± 7.4†	14.2 ± 4.0$^{\#}$	26.7 ± 7.8†	26.5 ± 7.6†
TCR	25.8 ± 6.9†	26.4 ± 5.5*†	13.6 ± 3.9$^{\#}$	25.3 ± 5.3†	27.1 ± 6.6†
SFAv, cm/s					
SS	9.0 ± 5.0$^{\dagger\#}$	0.1 ± 0.5†	66.9 ± 21.7$^{\#}$	15.9 ± 4.4$^{\dagger\#}$	11.6 ± 4.4$^{\dagger\#}$
TCR	8.4 ± 4.4†	3.1 ± 4.5†	46.3 ± 15.5*$^{\#}$	11.2 ± 5.0*†	10.3 ± 6.7†

Values are means ± SD. *MAP*, mean arterial pressure; *HR*, heart rate; $TPR_{U/S}$, total peripheral resistance via aortic Doppler ultrasound; *SFAv*, superficial femoral artery velocity; *SS*, squat-to-stand transition; *TCR*, thigh-cuff release. All values are 10s averages. *Significantly different from *SS* within time period; †significantly different from peak/nadir within condition; $^{\#}$significantly different from end-squat/end-cuff within condition. All P<0.05.

Figure 6 is a visual representation comparing the hemodynamic responses during the *SS* transition and *TCR*, with values expressed as changes from end-squat and end-cuff occlusion. After arising from the squat position, the *MAP* nadir and *HR* peak occurred after 10.4s and 14.5s, respectively. Following *TCR*, the *MAP* nadir occurred at 8s and the *HR* peak at 10.4s, both of which were significantly earlier than the *SS* transition (*MAP*: P=0.009, *HR*: P=0.005). The $TPR_{U/S}$ nadirs and *SFAv* peaks occurred at similar times following the stand and *TCR* ($TPR_{U/S}$: P=0.81, *SFAv*: P=0.61).

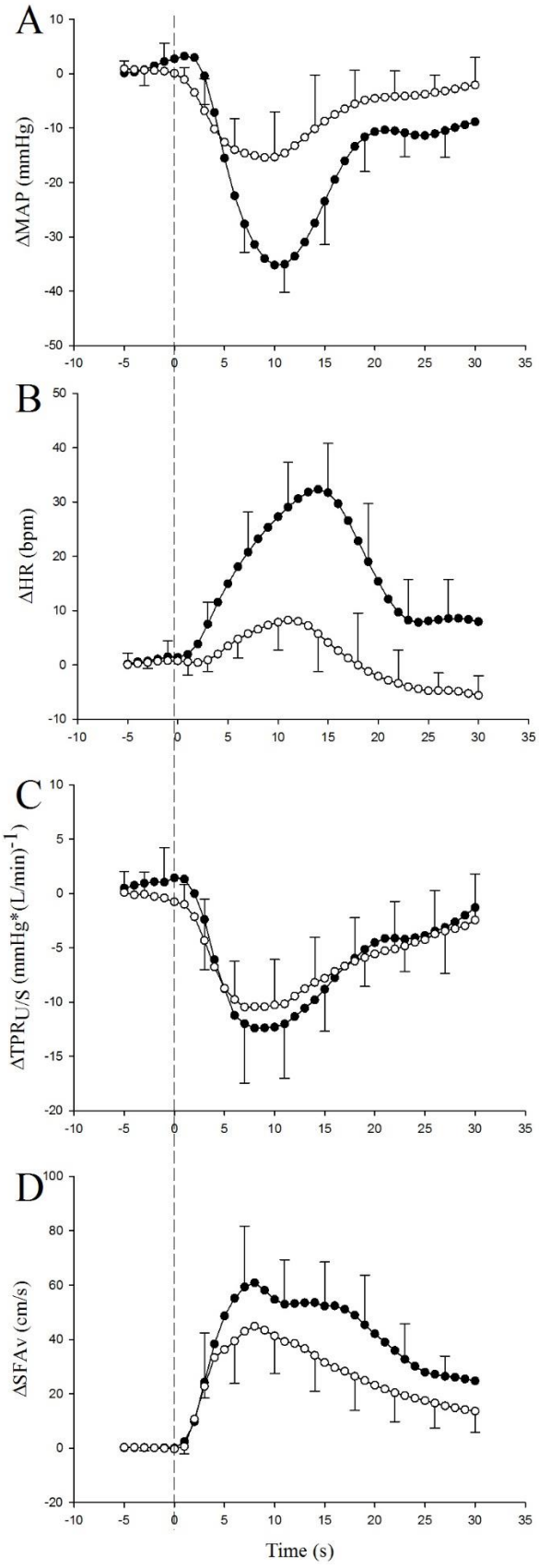
A
ΔMAP (mmHg)
B
ΔHR (bpm)
C
ΔTPRU/S (mmHg*(L/min)-1)
D
ΔSFAv (cm/s)
Time (s)

Figure 6. Hemodynamic responses elicited from the *SS* transition and *TCR*
All points (mean ± SD) represent second-by-second means from -5 to +30 seconds, with time = 0 (dashed line) representing when the subjects attained the standing position and the time of *TCR*. Error bars are displayed from- and to-0 every 4 seconds for clarity. *SS*, squat-to-stand; *TCR*, thigh-cuff release; *MAP*, mean arterial pressure (A); *HR*, heart rate (B); $TPR_{U/S}$, total peripheral resistance assessed with aortic Doppler ultrasound (C); *SFAv*, superficial femoral artery velocity (D).

3.3.2 Absolute stroke volume responses from all methods during the squat-to-stand transition and thigh cuff release

A comparison of absolute *SV* estimates at fixed points during the SS transition and TCR can be seen in Figure 7. *SV* estimations from all methods were similar between *SS* and *TCR* conditions during standing baseline (P=0.46), and *SS* and *TCR* standing baseline data were pooled together. Slight discrepancy's in standing baseline values between *SS* and *TCR* conditions are a consequence of a different number of subject included in *SS* and *TCR* analysis.

During the 3-minute standing baseline of the *SS* trial, SV_{MF}, $SV_{U/S}$ and SV_{BIA} had similar estimates of 67.4 ± 18.8mL, 54.3 ± 16.5mL and 69.1 ± 38.3mL, respectively (SV_{MF} vs. $SV_{U/S}$: P=0.56; SV_{MF} vs. SV_{BIA}: P=0.81; $SV_{U/S}$ vs. SV_{BIA}: P=0.55). By the end of the 3-minute squat, SV_{MF} (+14.7mL), $SV_{U/S}$ (+22.4mL) and SV_{BIA} (+11.3mL) estimates were elevated to similar levels from their respective standing baseline values (SV_{MF}, $SV_{U/S}$: P<0.001, SV_{BIA}: P=0.005) (Figure 7A). There was no main effect of method, nor time by method interactions detected during the *SS* transition (method: P=0.08; method by time: P=0.55). Three minutes of bilateral thigh cuff occlusion resulted in a decrease in $SV_{U/S}$ (-12.4mL, P<0.001) and SV_{BIA} (-13.3mL, P<0.001), while SV_{MF} remained similar (-6.8mL, P=0.11). By the end of the first minute post-*SS* transition, all *SV* methods had estimates that were similar to their respective standing baseline values and remained that way until the end of the second minute. One minute post-*TCR*, $SV_{U/S}$ was significantly lower than standing baseline (-15.7mL) and remained lower until the end of the second minute (-19.1mL;

1-min post-*TCR*: P=0.026, 2-mins post-TCR: P=0.002). SV_{MF} and SV_{BIA} returned to near-standing baseline values after 1-minute had elapsed post-*TCR*. In the *TCR* condition, $SV_{U/S}$ was significantly lower than SV_{BIA} at all time points, and significantly lower than SV_{MF} at time points after standing baseline (Figure 7B).

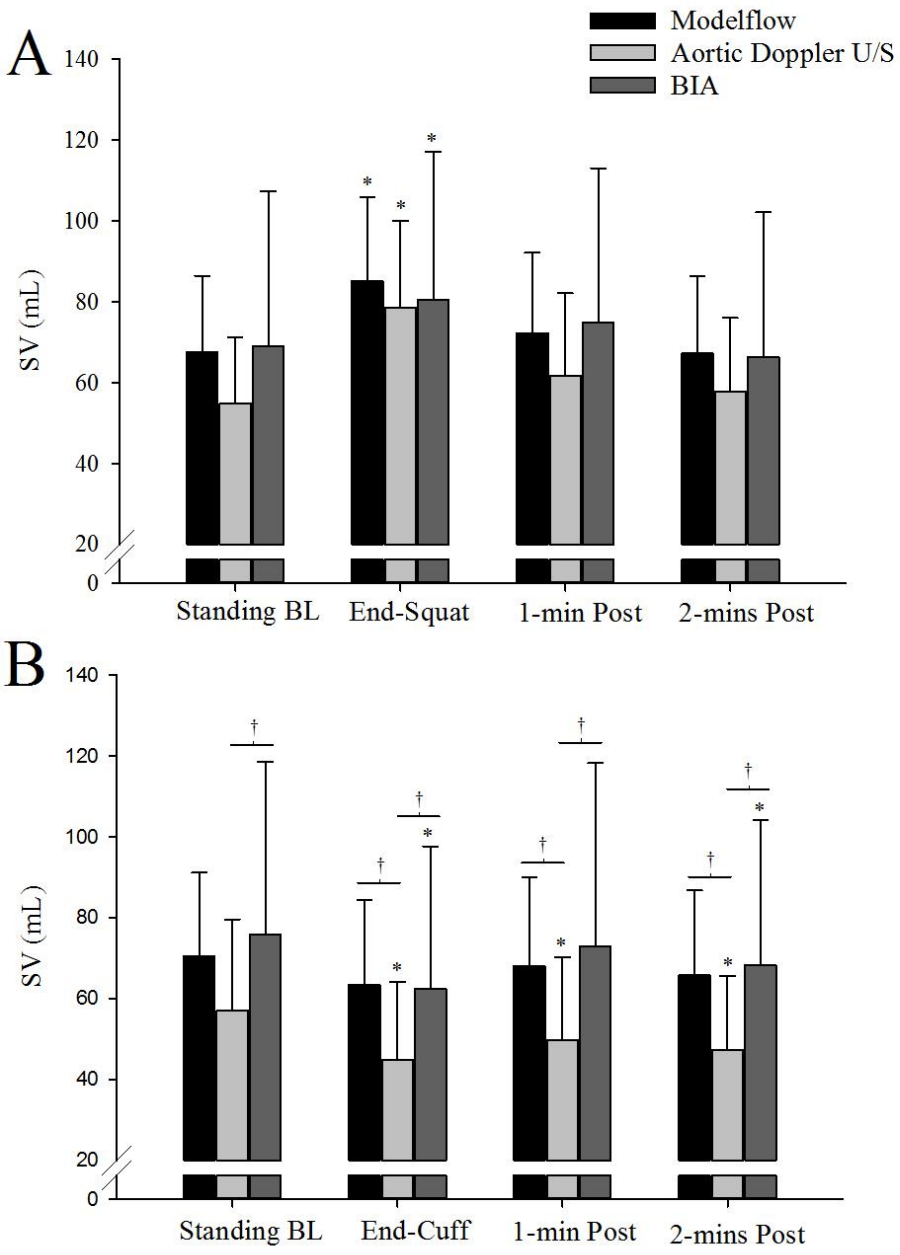

Figure 7. Absolute *SV* responses at fixed time points from all methodologies
SV method comparison during the *SS* transition (A) and during standing bilateral *TCR* (B). All measurements are means ± SD collected during the last 10 seconds of each time point. *SBL*, standing baseline; End-Squat, 10s average during final seconds of 3-minute squat; End-Cuff, 10s average during final seconds of bilateral thigh cuff occlusion; 1-min Post,1 minute following stand/thigh cuff release; 2-mins Post, 2 minutes following stand/thigh-cuff release. *Significantly different from standing BL within *SV* method; †significant method discrepancy between *SV* methods.

3.3.3 Comparison of dynamic stroke volume responses

The second-by-second *SV* analysis of the *SS* transition revealed an immediate increase in $SV_{U/S}$, whereas SV_{MF} and SV_{BIA} decreased (Figure 8A). This resulted in method bias between $SV_{U/S}$ and SV_{MF} from 3 – 11s after the stand, and $SV_{U/S}$ and SV_{BIA} from 5 – 11s after the stand. From the consistent *SV* overshoot at around ~21 seconds, SV_{BIA} had an exaggerated response when compared to $SV_{U/S}$, with significant method bias occurring at several points between 21 and 58s. Method bias between SV_{MF} and SV_{BIA} was not detected during the first 60s following the stand. The *TCR* maneuver elicited an immediate increase in *SV* measured from each of the three methodologies (Figure 8A). The initial increase was exaggerated by $SV_{U/S}$ and was significantly greater than SV_{MF} from 3 to 7s, and with SV_{BIA} at non-continuous points between 4 and 11s following *TCR.* Early method bias existed between SV_{MF} and SV_{BIA} at 3 seconds and at non-continuous points between 34 and 44s.

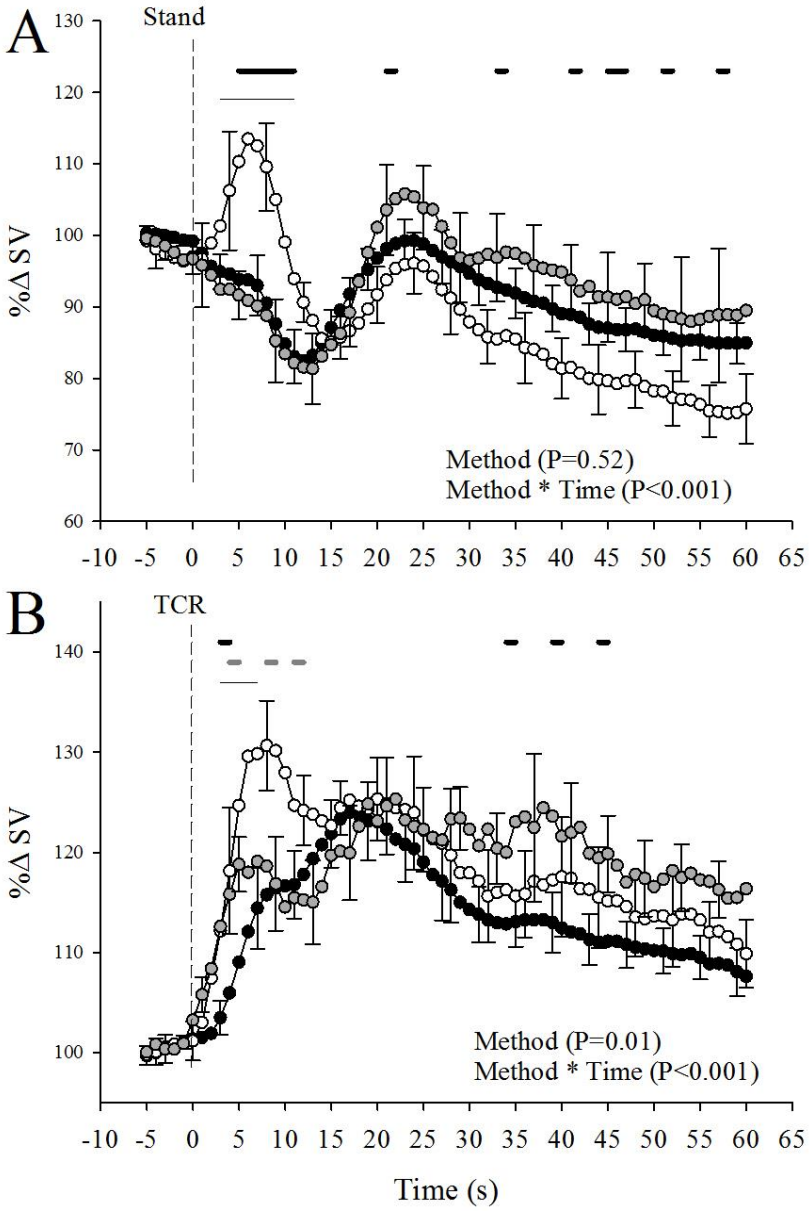

Figure 8. Comparison of the dynamic *SV* response to the *SS* transition and *TCR*
SV response during the *SS* transition (A) and *TCR* maneuver (B). *SV* was normalized to end-squat and end-occlusion data from -15 to -5s prior to standing and *TCR* and is presented as percent change from 100% at 1-second intervals. Filled black circles represent SV_{MF}, unfilled circles represent $SV_{U/S}$ and the filled gray circles represent SV_{BIA}. A pairwise comparison with time by method interactions (2-Way RM ANOVA with Holm-Sidak post-hoc testing) identified the seconds with method discrepancy. In panel A, the uppermost thick horizontal lines delineate time points with $SV_{MF} - SV_{BIA}$ method bias, while the lower thin horizontal line delineates $SV_{MF} - SV_{U/S}$ bias. In panel B, the uppermost thick black line delineates $SV_{U/S} - SV_{BIA}$ method bias, the middle grey line for $SV_{MF} - SV_{BIA}$ bias, and the lowest thin horizontal line for $SV_{MF} - SV_{U/S}$ bias. All data points are mean ± SEM with error bars every 4s for clarity.

Further emphasis was placed on the initial $SV_{MF} - SV_{U/S}$ method bias that occurred immediately after each orthostatic stress. Figure 9 illustrates the relationship between *HR*, $TPR_{U/S}$ and $SV_{MF} - SV_{U/S}$ method bias during the first 20s of the *SS* transition and the *TCR* maneuver. Panel A compares the magnitude of response elicited by each test, while panels B and C separates and scales the responses of each orthostatic stress test. The symbols highlighted in red represent significant method bias between SV_{MF} and $SV_{U/S}$ ($P<0.05$). A 2-Way repeated measure ANOVA revealed no difference between the magnitudes of method biases elicited by the *SS* and *TCR* tests over time ($P=0.731$).

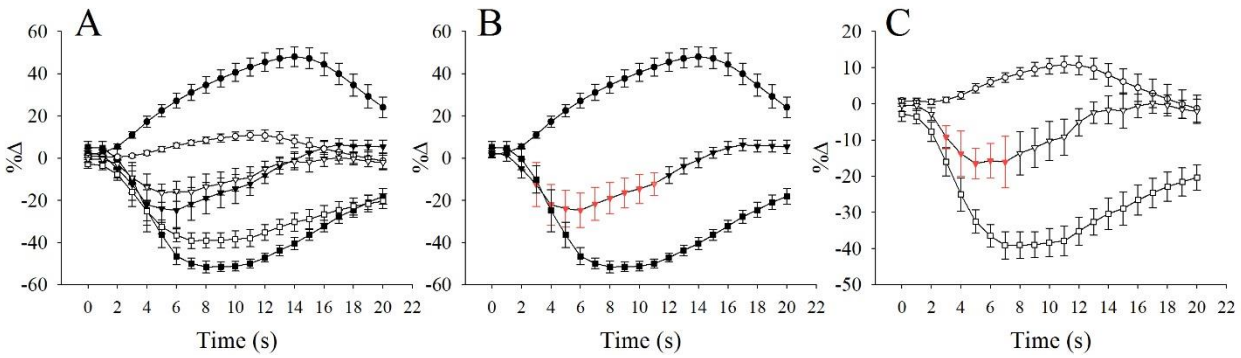

Figure 9. *HR*, $TPR_{U/S}$ and method bias comparisons
HR (circles), $TPR_{U/S}$ (squares) and method bias (inverted triangles) assessed during the first 20 seconds of the *SS* transition (filled black symbols) and *TCR* maneuver (unfilled symbols) (A). Panel B depicts the responses from the *SS* transition, while panel C shows the scaled responses to the *TCR* maneuver. Symbols highlighted in red represent time points in which significant $SV_{MF} - SV_{U/S}$ method bias occurs ($P<0.05$). All data points (mean ± SEM) are represented as percent change from end-squat and end-occlusion means.

3.4 Discussion

In alignment with our initial hypothesis, significant method bias occurred immediately following the *SS* transition and the release of bilateral thigh cuffs. Early SV_{MF} and $SV_{U/S}$ method discrepancy suggest that the dynamic changes in blood pressure and peripheral resistance at the onset of both stresses likely contributes to Modeflow estimation error.

Differences in the hemodynamic responses to the SS transition and TCR

Both orthostatic stress tests elicited significant drops in *MAP* and $TPR_{U/S}$ (Figure 6). These immediate drops in *MAP* and $TPR_{U/S}$ resulted in reflexive increases in *HR* and a substantial increase of arterial blood flow into the leg, as indicated by *SFAv*. It is important to note that the magnitudes of change of *MAP*, *HR* and *SFAv* were significantly greater with the *SS* transition (Table 1). Differences in *MAP*, *HR* and $TPR_{U/S}$ at the end of 3 minutes of squatting and 3 minutes of bilateral thigh occlusion also show that the two orthostatic stress tests induce a dramatically different physiological state prior to the *SS* transition and *TCR*. Additionally, the *MAP* nadir and *HR* peak occurred significantly earlier in the *TCR* condition (Figure 6). Therefore, when assessing the *SV* responses from each orthostatic stress it is important understand the differences in the physiological responses elicited by the *SS* transition and the *TCR* maneuver.

SV responses assessed by each of the three methodologies further highlight differences between the orthostatic stress tests. Squatting for 3 minutes resulted in a consistent increase in *SV* from each of the methods, which then decreased to standing baseline values within 1 minute of attaining the upright position (Figure 7A). Thus, the drop in *SV* at the end of the first minute of standing is likely influenced by the fact that *SV* was much greater in the end-squat position. In contrast, 3 minutes of standing bilateral thigh cuff occlusion resulted in a significant decrease in *SV*, which did not fully recover to standing baseline values by the end of the first or second minutes of standing post-*TCR* (Figure 7B). Similar to the *SV* response triggered by standing from the squat position, the changes in *SV* brought upon by the release of thigh occlusion are likely influenced by the altered hemodynamic state brought upon by 3 minutes of squatting and thigh occlusion, respectively (Figure 7). Thus, when the dynamic, second-by-second *SV* response to

each orthostatic stress is discussed in the following sections, one must understand that the end-squat and end-occlusion hemodynamic states are considerably different, and likely influence the immediate and prolonged *SV* response upon standing and *TCR*.

SV methodology comparisons at fixed intervals during SS transition and TCR

In contrast to the *SS* condition, absolute *SV* estimates from aortic Doppler ultrasound were lower than that of SV_{MF} and SV_{BIA} at the end of thigh occlusion, and 1 and 2 minutes post-*TCR* (Figure 7B). The method bias that occurred during the *TCR* trial may be the result of a greater degree on physiological instability at the fixed times that the methods were compared when compared to the *SS* trial. $SV_{U/S}$ was significantly decreased from standing baseline at the end of thigh occlusion and 1- and 2-minutes post-*TCR*, whereas *SV* returned to near-standing baseline within 1 minute of standing from the squat. Additionally, it could be seen that inflation of the thigh cuffs resulted in a slow but progressive decrease in *SV* that persisted throughout the 3-minute period, culminating in a significant decrease in SV at the end of thigh occlusion. In contrast, moving into the squat position resulted in a relatively rapid increase in *SV,* which stayed consistently elevated throughout the duration of the 3-miunte squat. This might indicate that although the *SS* transition triggered a greater initial orthostatic stress, the end-minute averages used in this analysis represent periods of improved cardiovascular stability when compared to the *TCR* condition. In addition, the method bias during the *TCR* trials and not *SS* may result from a smaller degree of measurement error for $SV_{U/S}$ during the *TCR* trials. The likelihood of motion artifacts biasing the $SV_{U/S}$ signal would be more prominent in the *SS* trials. However, it is unlikely that motion artifacts would result in the significant underestimation of $SV_{U/S}$ that was detected 1- and 2-minutes post-stand. It should also be noted that none of the methods were previously calibrated

with gold-standard methods prior to collection. Thus, although the relative changes in SV_{MF} should be accurate (Wesseling et al., 1993), the absolute measurements will not be as reliable.

Dynamic SV responses to orthostatic stress

The dynamic, second-by-second assessment of *SV* revealed significant method bias within the first ~11 seconds of the *SS* transition, making it difficult to interpret the immediate *SV* response to standing (Figure 8A). In alignment with hypothesis (1), the $SV_{U/S}$ response revealed an immediate ~13% increase in *SV* within the first 6 seconds of attaining the standing position. To our understanding, the only other report of continuous aortic Doppler ultrasound recording during the initial phases of active posture change revealed a 12 ± 11 $mL*m^{-2}$ decrease in stroke index (Tanaka et al., 1996) at the blood pressure nadir. However, the investigators reported that adequate aortic Doppler signals could not be attained within the first 5 seconds of standing, and the first *SV* measurement that was reported occurred 9.5 seconds after standing. In the present study, subjects were instructed to maintain consistent upper body positioning throughout the squat, transition, and standing phases, permitting continuous signal recording and minimizing the risk of poor signal quality. The results from the present study show that by the time of the first measurements reported by Tanaka et al. at 9.5s, *SV* has already begun to decrease to near pre-stand values (Figure 8A).

The legitimacy of this *SV* increase within the first 6 seconds of active standing can be supported by the findings of Wieling et al., who showed an abrupt increase in right atrial pressure at the onset of short bouts of cycling (Wieling et al., 1996). This study revealed that exercise onset elicited an immediate 12 ± 2 mmHg increase in right atrial pressure, which reached a peak within 3 seconds and lasted for 6 – 7 seconds. One would suspect that this abrupt increase in preload

would translate into an increase in *SV*, as per Starling's Law (Starling & Visscher, 1927). As would be expected, this study revealed a 54 ± 7% increase in *SV*, reaching a peak 6 seconds after the onset of exercise. Interestingly, the onset of the increase in *SV*, as assessed by Modelflow, was delayed by 3 seconds when compared to the immediate increase in right atrial pressure. Although the onset of cycling exercise and *SS* transitions represent very different hemodynamic situations, the initiation of exercise in large muscle masses, i.e. the legs, and the absence of a changing gravitational force vector, trigger very similar *MAP*, *TPR* and *SV* responses (Tschakovsky et al., 2011; Wieling et al., 1996). Thus, if a similar increase in right atrial pressure occurred at the onset of standing in the present study, this ~13% increase in *SV* within the first 6 seconds of standing would be entirely reasonable. In addition, similarities in Modelflow bias ($SV_{MF} - SV_{U/S}$) within the first 20 seconds of both orthostatic stress tests indicate that Doppler measurements acquired during, and shortly after the *SS* transition were accurate (Figure 9A).

In accordance with hypothesis (2), the release of bilateral thigh cuff occlusion resulted in an abrupt increase in *SV* as assessed by each of the three methodologies (Figure 8B). This initial increase in *SV* is likely influenced by a drop in afterload, which is largely dependent on arterial blood pressure and peripheral vascular resistance, both of which decreased immediately upon *TCR* (Figure 6A and C) (MacGregregor, Covell, Mailer, Dilley, & Ross Jr., 1974; Sonnenblick & Downing, 1963). Differences in *SV* methodology, as well as heterogeneity in *TCR* protocols (body position, inflation pressure, and occlusion duration) have led to mixed results in the magnitude of *SV* response to *TCR* (Deegan et al., 2008; Ichinose, Watanabe, Fujii, Kondo, & Nishiyasu, 2013; Lind-Holst et al., 2011). To the best of our knowledge, this is the first study to examine the dynamic *SV* response to standing bilateral thigh-cuff release. With this in mind, the $SV_{U/S}$ increase of ~32% seen in the present study is comparable to that of Ichinose et al., who showed a peak *SV*

increase of ~39% following 9 minutes of supine thigh occlusion on top of 2 minutes of unilateral arm occlusion using the same aortic Doppler ultrasound technique to measure *SV* (Ichinose et al., 2013) (Figure 8B). Deegan et al. measured *SV* with both Modelflow and left ventricular echocardiography during a 2-minute *TCR* test in supine and seated positions. Their results, however, show almost no change in *SV* within the first 20 seconds from either method, but note minimal agreement between estimates from Modelflow and left ventricular echocardiography (Deegan et al., 2008). On the contrary, using a similar 2.5-minute seated *TCR* test, Lind-Holst et al. found that Modelflow *SV* increased 45% and 21% in trained and untrained males, respectively, which compares to the 24% increase in SV_{MF} observed in the present study. Of note, the significantly greater increase in *SV* seen in trained athletes occurred alongside much greater decreases in *MAP* and systemic vascular resistance, supporting the notion that *SV* is inversely related to afterload upon *TCR*.

Accounting for SV method bias during rapid changes in blood pressure and TPR

In alignment with hypothesis (3), the assessment of the dynamic responses to the *SS* transition reveals significant method bias with aortic Doppler ultrasound immediately after the assumption of the upright position (Figure 8A). The early method bias between $SV_{U/S}$ and SV_{MF} occurs from 3 to 11 seconds after standing, a period in which there is substantial a drop in $TPR_{U/S}$ and *MAP*. Beyond the 11-second mark, there is method agreement between $SV_{U/S}$ and SV_{MF} that persists for the duration of the first minute. This supports the hypothesis that it is the dramatic decrease in vascular resistance to highly compliant tissues of the legs and abdomen that triggers this transient Modelflow bias. Interestingly, SV_{MF} and SV_{BIA} displayed very similar *SV* patterns and were not significantly different at any point during the first 60 seconds after standing. That

being said, it is difficult to ascribe reasoning as to why these methods displayed such similar trends during the *SS* transition, as they operate under completely different physical principles. One possible explanation is a transient imbalance in pulmonary blood flow and thoracic aortic blood flow upon standing from the squat position. One major assumption of the *BIA* is that the aorta is the main source of the change in impedance. However, immediately upon standing from the squat position there is a temporary increase in $SV_{U/S}$ that coincides with a drop in arterial pressure. The transient increase in blood flow returning to the right heart may counteract the impedance change detected in the aorta if the aorta is not the main source of the impedance change detected by *BIA* (de Sitter, Verdaasdonk, & Faes, 2016). Thus, the lack of sensitivity to detect the initial increase in *SV* by *BIA* might be a limitation of the method caused by infiltration of impedance from sources other than the aorta. However, further investigation is required to better understand the limitations of SV_{BIA} during dynamic changes in physiological state.

The early method bias at the onset of bilateral thigh cuff release is also in accordance with hypothesis (3). The underestimation of SV_{MF} is apparent when compared to both $SV_{U/S}$ and SV_{BIA} (Figure 8B). Similar to the *SS* transition, this early phase of *TCR* represents a period in which there is a transient, yet dramatic shift away from physiological homeostasis. Interestingly, although the *MAP*, *HR* and *SFAv* response is exaggerated in the *SS* trials, the magnitude and timing of the $TPR_{U/S}$ drop is not significantly different between the *SS* transition and *TCR* maneuver. This coincides with a similar magnitude of method bias elicited by both orthostatic stress tests (Figure 9A). However, it does appear that the slightly greater drop in $TPR_{U/S}$, or perhaps the compounding influence of greater blood pressure instability, results in a longer duration of method bias in the *SS* trial. This can be seen in the prolonged duration of $SV_{MF} - SV_{U/S}$ bias during the *SS* trial, which

persists until 11 seconds post-stand, compared to 7 seconds post-*TCR*, as well as the slight elongation of the Modelflow bias loop in Figure 9B.

Further support for the association between decreased in peripheral resistance and Modelflow bias can be seen in conditions in which vascular resistance to highly compliant organs is chronically reduced. Hughson et al. recently showed that Modelflow estimates of *SV* greatly underestimate those of rebreathing during long duration spaceflight, during which blood flow to the highly compliant splanchnic region is increased by 45% and *TPR* is reduced by 39% (Arbeille, Provost, Zuj, & Vincent, 2015; Hughson et al., 2017; Norsk, Asmar, Damgaard, & Christensen, 2015). Additionally, Shibasaki et al. showed that Modelflow estimates of *SV* underestimated changes in *SV* assessed by the more direct thermodilution method during passive heat stress, a condition which is known to increase vascular conductance in the skin (Crandall et al., 2008), as well as the renal and splanchnic vascular beds (Rowell, Brengelmann, Blackmon, Twiss, & Kusumi, 1968). Finally, Dyson et al. showed that Modelflow underestimated *SV* compared to estimates obtained from aortic Doppler ultrasound during acute reductions in *TPR* elicited by isoprenaline influsions (Dyson et al., 2010). However, it should be noted that all of these studies obtained *SV* estimates over several seconds to several minutes, under conditions in which the reduction in *TPR* was prolonged, which is in contrast to the rapid and transient decreases in *TPR* triggered during the active orthostatic stress tests in the present study.

When considering possible mechanisms underlying Modelflow bias, it is important to recognize that both the *SS* transition and *TCR* maneuver elicited changes in *TPR* that were unmatched in magnitude and rate by that of *HR*. As is noted by Wesseling in his paper outlining the original model, this relatively faster change in *TPR* would violate one of the inherent assumptions of the Modelflow method (Wesseling et al., 1993). The Modelflow method computes

aortic flow from three elements: C_w, Z_o, and *TPR*. C_w and Z_o are parameters derived from the pulse contour of the arterial pressure wave using a non-linear arctangent model, whereas *TPR* is unknown, but an outcome of the model, calculated as the quotient of $\dot{Q}$ and *MAP*. Thus, to compute aortic flow, a value of *TPR* generated from the previous cardiac cycle is used for the calculation of *SV* for the subsequent cardiac cycle. During most conditions, *TPR* does change relatively slowly compared heart beat interval, and the Modelflow method accurately tracks changes in *SV* (Sprangers et al., 1991). However, in the present study, the *SS* transition induces 52% decrease in $TPR_{U/S}$ that occurs within the first 8 seconds of attaining the upright position (Figure 9B). During this same 8-second period, *HR* only increases by 35% and does not reach a maximum until 15 seconds post-stand. Similarly, bilateral *TCR* elicits a 40% decrease in $TPR_{U/S}$ within the first 8 seconds, which again is unmatched by *HR* in magnitude and timing, which has a peak increase of 11% 13 seconds post-*TCR* (Figure 9C). It should also be noted that the non-linearity of the model could also be susceptible to the rapid changes in blood pressure induced by both orthostatic stress tests in the present study. The two model parameters that determine systolic inflow, C_w and Z_o, are non-linearly dependent on arterial pressure pulsations, and rapid changes in pressure might impact the assumptions of these equations (**Equations 4, 5 and 6**). Independent of the underlying mechanisms, this study reveals that conditions such as *SS* transitions and bilateral *TCR* that are capable of provoking abrupt decreases in *TPR* and blood pressure can violate the inherent assumptions of the Modelflow method, resulting in *SV* estimations that deviate from the truth.

3.5 Limitations

The main limitation of the present study was the absence of a gold-standard comparative method that could assess *SV* with the same time resolution as Modelflow. Presently, only direct

flow probe measurements can measure *SV* with transient beat-to-beat accuracy and a similar time resolution as Modelflow. Due to the invasiveness of such procedures, it was decided to use aortic Doppler ultrasound measurements while taking great care to control for its proposed sources of error. To control for sources of error pertaining to the insonation angle of the ultrasound signal, subjects were instructed to maintain vertical upper body positioning throughout the duration of the squat, transition and standing phases, as well as throughout the *TCR* maneuver. Maintaining a consistent upper body position allowed for continuous Doppler ultrasound recordings throughout the transition periods. Similarly, sources of error associated with tilting of the heart within the thorax were limited by maintaining an upright position, a potential source of error that has been proposed during *HUT* testing (van Lieshout, Toska, et al., 2003). Similarities in Modelflow bias between the *SS* transition and *TCR* maneuver during the first 20 seconds of the stresses support that aortic Doppler ultrasound accurately tracked *SV* throughout the dynamic *SS* transition (Figure 9A). Additionally, *SV* was measured by *BIA* to compare pairwise method biases between all three methods. Although SV_{MF} and SV_{BIA} were comparable throughout the *SS* transition, it is likely that these similarities are due to different limitations in both methods.

The *SS* transition introduced a large degree of noise into the SV_{BIA} signal that was difficult to control. Although electrodes and wires were taped to the skin, noise caused by excessive movement had to be cleaned from most trials. In most cases, this noise only persisted as long as the duration of the *SS* transition and was not a major source of error beyond the first few seconds. Additionally, the analysis of SV_{BIA} introduces the subjective measurement of ventricular ejection time from $\Delta Z/\Delta t$ signals that are not always obvious. Thus, future work would benefit from the inclusion of a microphone capable of detecting heart sounds to better identify the end of ventricular ejection based on the heart sound that coincides with aortic valve closure.

3.6 Conclusions, Applications and Future Perspectives

This was the first study to compare beat-to-beat estimates of *SV* during major changes in physiological conditions. When compared to *SV* estimates from aortic Doppler ultrasound, Modelflow underestimated *SV* by up to 25% during the first 3 – 11 seconds of the *SS* transition and by up to 16% during the first 3 – 7 seconds of *TCR*. SV_{BIA} and SV_{MF} had similar dynamic responses to the *SS* transition and were comparable during *TCR*, but did have intermittent discrepancy during the first 11 seconds. Although limitations in each method must be considered, this study shows that rapid decreases in $TPR_{U/S}$ and blood pressure elicited by common orthostatic stress tests can generate physiological conditions that violate the assumptions of the Modeflow method and result in significant method bias. These findings provide evidence of the inadequacies of the Modelflow method to track changes in *SV* during dynamic changes in physiological state. The relevance of this work to cardiovascular research is considerable, as the Modelflow method is frequently used under these conditions as it is a convenient method to ascertain information regarding central hemodynamics. This study highlights that estimates of *SV* from the Modelflow method should be interpreted with a high degree of caution dynamic changes in physiological conditions. Similarities between SV_{MF} and SV_{BIA} warrant further investigation into the advantages and limitations of SV_{BIA} during similar transitional states.

CHAPTER 4: *HEMODYNAMIC EFFECTS OF INTERMITTENT COMPRESSION AS A COUNTERMEASURE TO ORTHOSTATIC STRESS*

4.1 Introduction

Symptoms of presyncope, such as dizziness, light-headedness and blurred vision, can be provoked by any condition that jeopardizes *CBF* and cerebral oxygenation (van Lieshout, Wieling, et al., 2003). In the standing position, upwards of 70% of blood volume shifts below heart level, and adequately perfusing the brain presents a challenging task (Rowell, 1993). Orthostatic stress can elicit substantial decreases in arterial blood pressure and $\dot{Q}$ as a consequence of this translocation of blood below the heart (Guyton, 1973). These immediate centralized hemodynamic effects of orthostatic stress have been associated with concomitant decreases in *CBF* velocity and cerebral oxygenation, which are the fundamental causes of orthostatic symptoms (Harms et al., 2011; van Lieshout, Wieling, et al., 2003). Continuing or sustained decreases in *CBF* must be counteracted to restore adequate cerebral oxygen supply in order to maintain consciousness in the upright position.

The translocation of blood volume below the heart is a consequence of delayed venous emptying in highly compliant vessels of the lower body, which effectively removes a significant proportion of blood from central circulation (Rowell, 1993). The peripheral vasculature of the legs and abdomen act as a reservoir for blood, creating a challenging situation in which the blood must be driven against the hydrostatic gradient in order to return to the heart (Montgomery et al., 1989; Rowell, 1993). In the healthy population, autonomic reflexes and skeletal muscle tone counteract some of the venous pooling and combat the reduction in cardiac filling in the upright position. However, a diverse group of populations exist that are vulnerable to the initial drop in blood pressure, and others that have difficulty recovering from the initial hypotensive strain. These

populations include the elderly (Gupta & Lipsitz, 2007; Nagaya et al., 2015; Rutan, 2014), highly-trained endurance athletes (Bedford & Tipton, 1987; Fadel et al., 2001; Raven et al., 1988), heart-failure patients (Cornwell & Levine, 2015; Fraser et al., 2015; Potocka-Plazak & Plazak, 2001) and post-flight astronauts (Hughson et al., 2014).

Time-domain analysis of the compensatory reflexes to blood pooling in dogs shows that autonomic responses to orthostasis develop fully after approximately 20 – 40 seconds (Guyton et al., 1962). Thus, the tonic and dynamic activity of the muscle pump in returning blood to the heart in the maintenance of brain blood flow is of significant importance at the onset of standing (Guyton, 1973). Research has shown that tetanic lower-body muscle contraction can transiently increase $\dot{Q}$ by 30 – 40%, but only intermittent contraction can sustain this increase in the upright position (Guyton, 1973). Additionally, active muscle tensing during orthostatic stress significantly increases $\dot{Q}$, *CBF* velocity and oxyhemoglobin concentration, independent of blood pressure (van Lieshout et al., 2001). Similarly, recent research suggests that passive lower leg muscle movement has the potential to improve indices of cerebral oxygenation without altering blood pressure or *HR* (Nagaya et al., 2015).

The association between $\dot{Q}$ and brain blood flow during orthostatic stress provides valuable information regarding one's ability to adapt to alterations in blood pressure. Recent research conducted in our laboratory has shown that intermittent compression of the lower limbs during standing plantarflexion exercise and standing exercise recovery caused sustained increases in *SV* (Zuj et al., 2017), similar to that seen with intermittent electrical stimulation of the lower body (Guyton et al., 1962). These findings present the possibility that decreases in *SV* and $\dot{Q}$ experienced during orthostatic stress may be counteracted by active external compression of the lower legs at the onset of orthostatic stress. Assessing the effectiveness of lower-leg intermittent

compression as a countermeasure to orthostatic stress provides an opportunity to better understand the complex relationship between local, central and cerebral hemodynamic variables, as well gives insight to the potentiality of external compression as a therapeutic tool for populations vulnerable to orthostatic stress.

The present study assessed local, central and cerebral hemodynamic changes triggered by two orthostatic stress tests (*SS* transition and standing bilateral *TCR*) with and without the application of intermittent external compression of the lower legs. It was hypothesized that: (1) active compression of the lower legs would further enhance the local blood flux through the leg following standing and the release of thigh-cuff occlusion; (2) intermittent compression would improve blood pressure and $\dot{Q}$ regulation immediately following and during the recovery from both orthostatic stresses and (3) these improvements in central hemodynamics would translate into increased *CBF* velocity and indices of cerebral oxygenation.

4.2 Methods

4.2.1 Subjects

Fourteen healthy [body mass index: 23.0 ± 2.4 $kg*m^{-2}$; (mean ± S.D.)] university students (seven female) aged 24.6 ± 3.5 years old participated in the experiment. Written informed consent was signed before the initiation of testing (Appendix A). All subjects were emailed an information letter to familiarize them with the specifics of the study before arriving on testing day (Appendix D). On testing day, subjects were asked to complete a Health Status Form to ensure inclusion criteria were met prior to the commencement data collection (Appendix B). Study protocols and procedures were approved by the University of Waterloo, Clinical Research Ethics Committee (ORE# 21433) and conformed with the Declaration of Helsinki.

4.2.2 Measurements

Upon entering the lab, subjects were instrumented is preparation for collection. Data collection was continuous and progressed throughout the duration of the testing period. *HR* (Pilot 9200,ColinMedical Instruments, SanAntonio,TX,USA), continuous blood pressure by finger photoplethysmography with height correction on the left arm and hand continuously held at heart level (Finometer ®, FMS, Amsterdam, The Netherlands), blood velocity in the superficial femoral artery and blood velocity in the ascending aorta assessed by pulsed wave Doppler ultrasound (Multigon Industries, Mt Vernon, NY, USA) were collected using a data-acquisition system (PowerLab, ADInstruments, Colorado Springs, CO, USA). For the assessment of cardiac *SV*, a pulsed Doppler 2 MHz probe was positioned in the suprasternal notch and directed towards the aortic root with the sample volume immediately above the aortic valve to obtain the maximal velocity during systole and an angle of insonance with forward blood flow within 15°, in accordance with Tibbals et al. (Eriksen & Walløe, 1990; Tibbals, Osborne, & Hockmann, 1988). *SFAv* was continuously measured using Doppler ultrasound (WAKIe, Atys Medical, Soucieu en Jarrest, France). A 4 MHz Doppler probe was positioned on the medial thigh and was held in place with elastic tape throughout the study. *SFA* diameters were measured with Echo Doppler ultrasound on contralateral leg (Mindray M5, Shenzen Mindray Bio-medical Electronics, Shenzen, China). A 2 MHz pulsed-wave transcranial Doppler (*TCD*) probe (TCDX, Atys Medical, Soucieu en Jarrest, France) was placed on the temporal window to measure blood flow velocity in the middle cerebral artery with a collection frequency set to 100 Hz. The *TCD* probe was held in place by eye glasses that allowed for the connection of the probe and counterweight, and was strapped securely around the circumference of the participant's head. Cerebral blood oxygenation was measured by a continuous-wave near infrared spectroscopy (NIRS) device (PortaLite, Artinis, The

Netherlands). The probe was attached to the left side of the forehead throughout the data collection period with a dark elastic headband to limit ambient light from entering the photo diode. In a subset of the participants, exhaled CO_2 was collected using a face mask and analyzed using infrared spectroscopy (Ametek, Thermox Instruments, Pittsburgh, PA, USA). The peak percent concentration of carbon dioxide at the end of an exhaled breath represented the end-tidal CO_2 ($ETCO_2$).

4.2.3 Experimental Protocol

The testing of each subject was completed during a single visit lasting approximately 3 hours. Subjects were requested to refrain from ingesting caffeine for 5 hours prior to testing and avoid alcohol and tobacco for 24 hours prior to testing. Subjects were also instructed not to participate in heavy exercise prior to testing. Once instrumented, subjects stood quietly in the upright position for 3-minutes while baseline measurements were recorded. The orthostatic stress (squat and thigh-cuff occlusion) was initiated following the 3-minute baseline period, and was maintained for 3 minutes. Following the stand/*TCR*, subjects remained standing quietly for an additional 4 minutes. A 3 minute seated period between trials allowed subjects to recover from the previous orthostatic stress. The *SS* protocol is outlined in Figure 10 and the *TCR* protocol in Figure 11. The *SS* transition and *TCR* maneuver were each repeated 4 times and the order of testing conditions was randomized. Two trials of each test utilized intermittent compression of the lower legs during the first 2 minutes of standing.

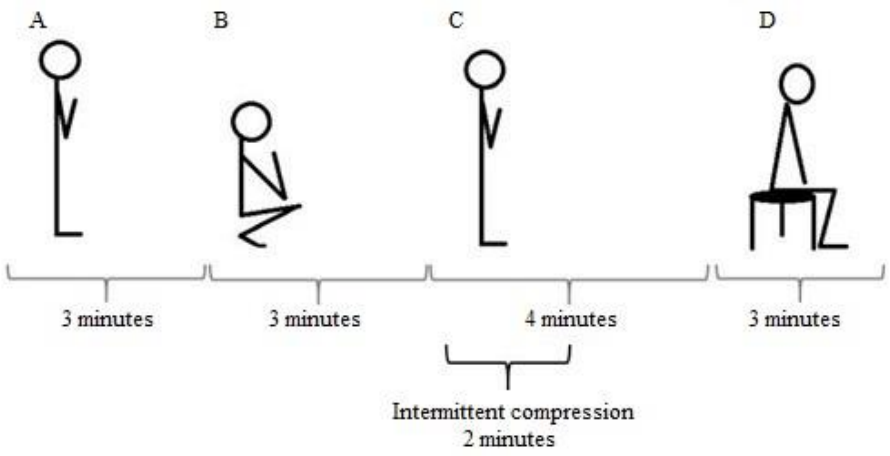

Figure 10. Squat-to-stand transition
Illustration of the *SS* transition. (A) Participants stood quietly in the upright position while baseline data were recorded. (B) Participants quickly lowered into the squat position, ensuring that their backs were flat against the wall. (C) Participants rapidly transitioned into the standing position without using their hands. Intermittent pneumatic compression of the lower legs was manually initiated when the participants attained the upright position. After two minutes of standing quietly, the compression system was deactivated. (D) A period of seated rest separated trials to ensure participants regained a steady-state condition.

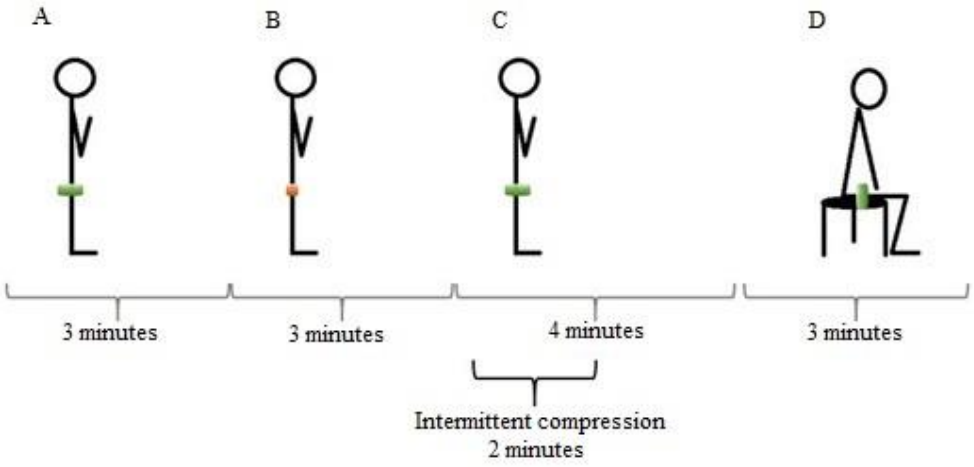

Figure 11. Thigh-cuff release maneuver
Illustration of the TCR maneuver. (A) Participants stood quietly with the cuffs deflated while baseline data were recorded. (B) Rapid inflation of the thigh cuffs occluded leg blood flow. (C) Rapid deflation of the thigh cuffs released blood occlusion. One second after the release of the thigh cuffs, intermittent compression of the lower legs was manually initiated. After two minutes of standing quietly, the compression system was manually deactivated. (D) A period of seated rest separated trials to ensure participants regained a steady-state condition.

4.2.3.1 Squat-stand test

See section *3.2.3.1.*

4.3.2.2 Thigh-Cuff Release Maneuver

See section *3.2.3.2.*

4.2.3.3 Intermittent Compression

Compression was applied to the lower legs using a custom-built, intermittent compression system. The system consisted of five air-bladder containing cuffs which were subsequently and independently inflated in a peristaltic manner to maximize venous flow out of the lower leg. Each compression and relaxation cycle was completed within 200 milliseconds and was easily completed during the local diastolic phase of each cardiac cycle during these conditions.

The sequence and timing of compression cycles was controlled electronically using LabView software (National Instruments Corp., Austin, Texas, USA). The timing of compression was set to activate only during the local diastolic phase of the cardiac cycle. The timing of compression was determined using continuous ECG monitoring and a one-time calculation of pulse wave transit time before the commencement of testing. Pulse wave transit time was calculated by measuring the elapsed time between the *R*-wave of the ECG signal and the end of systolic flow at the *SFA*. During *SS* trials, the compression system was manually activated when the subject attained the upright position. The compression system was activated immediately after the release of bilateral thigh cuff occlusion.

The pressure between the compression cuffs and the lower legs was determined using four pressure sensors located under the top and bottom cuffs of each leg. The pressure sensors consisted of a Picapress® bladder (Microlab Electronica, Italy) connected to an Omega PX390 pressure transducer (Omega Engineering Inc., Laval, Quebec, Canada). The mean pressure

applied was approximately 65 mmHg with a gradient of ~10 mmHg between the ankle and knee. The pressure applied by the cuffs was measured and adjusted once before the start of data collection.

4.2.4 Data Analysis

Analysis of blood pressure, aortic Doppler velocity, ECG, *SFAv*, $\dot{Q}$ and *TPR* was the same as in Chapter 3 and is described in section *3.2.4*.

The outer envelope of the velocity tracing from the aortic Doppler signal was integrated within each cardiac cycle to compute distance travelled per cardiac cycle. $SV_{U/S}$ was presented as percent change from standing baseline as vessel diameters were unavailable to compute absolute flow measurements.

The diameter of the *SFA* was continuously measured from cine loops recorded using echo Doppler ultrasound, while *SFA* velocities were continuously recorded on the contralateral leg. Diameter measurements were recorded during the final minute of the 3 minute baseline standing period and immediately following the stand and *TCR*. All baseline measurements were collapsed to generate a single resting diameter measurement for each subject. Following the stand/*TCR*, the first reliable diameter measurements were recorded within 13 seconds of standing and within 5 seconds of *TCR*. For this reason, *SFA* diameter and *SFA* blood flow are presented from 13 seconds post-stand and 5 seconds post-*TCR*. Diameter measurements were analyzed offline with customized edge-detection software (MAUI Software, Hedgehog Medical, Waterloo, ON, Canada). Each 8-second cine was analyzed separately to ensure the edge-detection software was accurately tracking the intima-intima distance. Due to the intermittent nature of diameter measurements throughout the 4-minute stand, all trials were then spine-fit using a MATLAB spline-fitting function (MathWorks, Natick, MA, USA) before identical trials were collapsed and

averaged. These spline fitted measurements were then averaged throughout all subjects and compared as 15-second binned averages.

Superficial femoral artery blood flow was calculated using the equation:

Equation 7 $$SFA_{Flow} = SFAv * \pi r^2 * 60s/min ;$$

where SFA_{Flow} is blood flow in the superficial femoral artery (in mL/min), *SFAv* is superficial femoral artery blood velocity (in cm/s) and *r* is the radius of the superficial femoral artery of the contralateral leg.

The quantification of absolute changes in oxyhemoglobin (O_2Hb), deoxyhemoglobin (HHb), total hemoglobin (tHb) and tissue saturation index (*TSI%*) were be obtained using NIRS with a collection frequency of 50 Hz. *TSI%* is calculated as the quotient of O_2Hb and tHb (O_2Hb + HHb) and has been interpreted to reflect cerebral oxygenation, as evidence of vasodilation and improved oxygen delivery (Subudhi et al., 2011).

For all variables, intra-subject trials were averaged to generate a mean response per subject. Inter-subject means were then averaged to generate an overall mean response. *SS* and *TCR* trials were time-aligned by manually identifying and aligning the rapid increase in *SFAv* triggered by active standing and *TCR*. With the exception of *SFA* diameter, all variables were averaged over each cardiac cycle and interpolated at 1 Hz for comparative analysis. *SFA* diameters were grouped into 15s averages and compared across the 4-minute stand. A five-second running average was used in the second-by-second figures as a smoothing treatment.

4.2.4.1 Data Exclusion Criteria

Due to an interaction between aortic Doppler ultrasound and *TCD* in 5 subjects, only 9 subjects (6 males) were included in the analysis of variables derived from *SV* (i.e., $\dot{Q}$ and *TPR*).

Although *SV* was measured simultaneously using the pulse contour of the blood pressure waveform (Modelflow), previous testing in our lab verified that *SV* estimates are likely inaccurate during the first ~15 seconds of orthostatic stress testing.

Due to technical issues with the portable *TCD* device, *MCAv* data were not collected for one subject and another subject for only the *SS* trials. Similarly, due to an inordinate amount of noise in the *SFAv* signal associated with poor Doppler resolution, *SFAv* data were not available for 1 subject for both *SS* and *TCR* data, and for a second subject only in the *TCR* trial.

SFA diameters were collected in 13 of the 14 subjects. All 13 tests were analyzed with the customized edge detection software. Frames in which the software did not accurately detect the inner borders of the vessels were removed from analysis. Poor image quality in 2 of the subjects made accurate vessel diameter measurement unreliable. These subjects were removed from analysis, and the total number of subjects included was 9. Similarly, *SFA* flow measurements only included the same 9 subjects.

$ETCO_2$ was measured with a nasal cannula in 12 subjects with a different CO_2 analyzing device (DATEX-OHMEDA 5200 CO_2 Monitor, Mundelein, IL, USA). Due to unreliable output, a new CO_2 analyzing system was used with a full face mask for 3 additional participants (Ametek, Thermox Instruments, Pittsburgh, PA, USA). Due to the unreliability of the first CO_2 analyzer, $ETCO_2$ data were separated based on the system of use. All $ETCO_2$ data presented is from the 3 subjects tested with the full face mask using the Ametek CO_2 monitor.

4.2.5 Statistical Analysis

All statistical computations were made using SigmaPlot version 13.0 software (Systat Software Inc., Chicago, Illinois), with statistical significance set at $P<0.05$ and trends reported for

P<0.10. Data are presented as mean ± standard error for all statistical tests. All variables were linearly interpolated at 1 Hz to allow for time-aligned inter-trial comparisons. Steady state baseline measures were averaged over a 50-second period collected in the last minute of the 3-minute standing baseline period. The last 10 seconds of standing baseline data was excluded to account for participant anticipation of the impending orthostatic stress test.

To capture the dynamic nature of *IOH*, all variables were compared at 1 second intervals from the onset of the stand/*TCR* for the first 60 seconds. Data were then compared as 10-second averages taken from the end of the second, third and fourth minutes. Shapiro-Wilk normality tests and Brown-Forsythe equal variance tests were performed on all of the variables from the averaged data collected during the standing baseline, end-squat/end-occlusion, and at the end of minutes-2, -3 and -4 to validate the use of parametric statistical testing. A 2x2 (compression by time) repeated measures ANOVA design with Holm-Sidak post hoc testing was used to assess the effect of intermittent compression over time. The main effects of compression, as well as the interaction effects of compression by time are presented in the figures. Statistical testing was run on absolute values for all variables except *SFA* diameters, which were run on values normalized as percent change from standing baseline. All figures are presented as percent change from standing baseline, with the standing baseline value representing 100%.

4.3 Results

4.3.1 Local hemodynamic responses to the squat-to-stand transition

Following the *SS* transition, there was a significant increase in blood flow through the *SFA* with the application of intermittent compression (Figure 12A). This increase in blood flow through the *SFA* became apparent 19 seconds post-stand and persisted for the duration of the first minute. The increase in SFA flow was the result of a significant increase in *SFAv* from 19 seconds

post-stand to the end of the first minute, with a brief gap at t = 55 seconds (P=0.05) and t = 57 seconds (P=0.06). Additionally, *SFAv* was significantly less in the compression condition for 1 second immediately following the stand (time = 7 seconds), but quickly surpassed the non-compression condition at the 10-second mark and remained elevated for 2 additional seconds (Figure 12B).

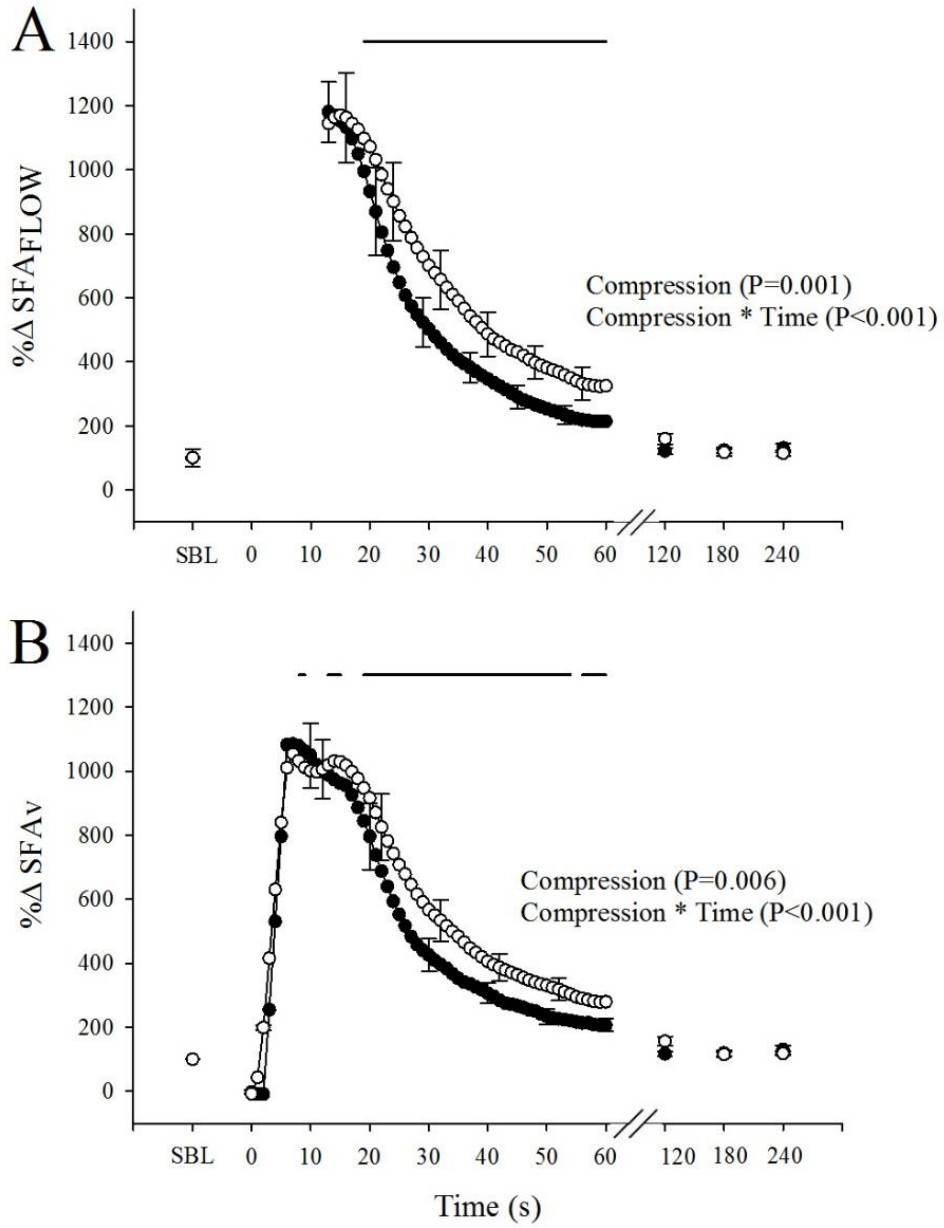

Figure 12. *SFA* flow and velocity in response to the *SS* transition
SFA blood flow (A) and velocity (B) in response to the *SS* transition for both no-compression (filled circles) and compression (unfilled circles) conditions, expressed as percent change from standing baseline (*SBL*). All data points between 0 and 60 seconds represent means ± SEM interpolated at 1 Hz from all subjects, with time = 0 representing to onset of the stand. Intermittent compression was active from 0 to 120 seconds (SFA_{FLOW} data available from 13s onward). Data points at *SBL*, 120, 180 and 240 seconds are averages taken from the last 10 seconds of each time period. The solid bar above each plot shows time points that are significantly different between compression and no-compression conditions as assessed by a 2-Way RM ANOVA with Holm-Sidak post-hoc testing.

The *SFA* diameter was also significantly increased for one minute, 45 seconds after attaining the standing position (Figure 13). *SFA* diameters were not significantly different for the 2 minutes of standing following intermittent compression.

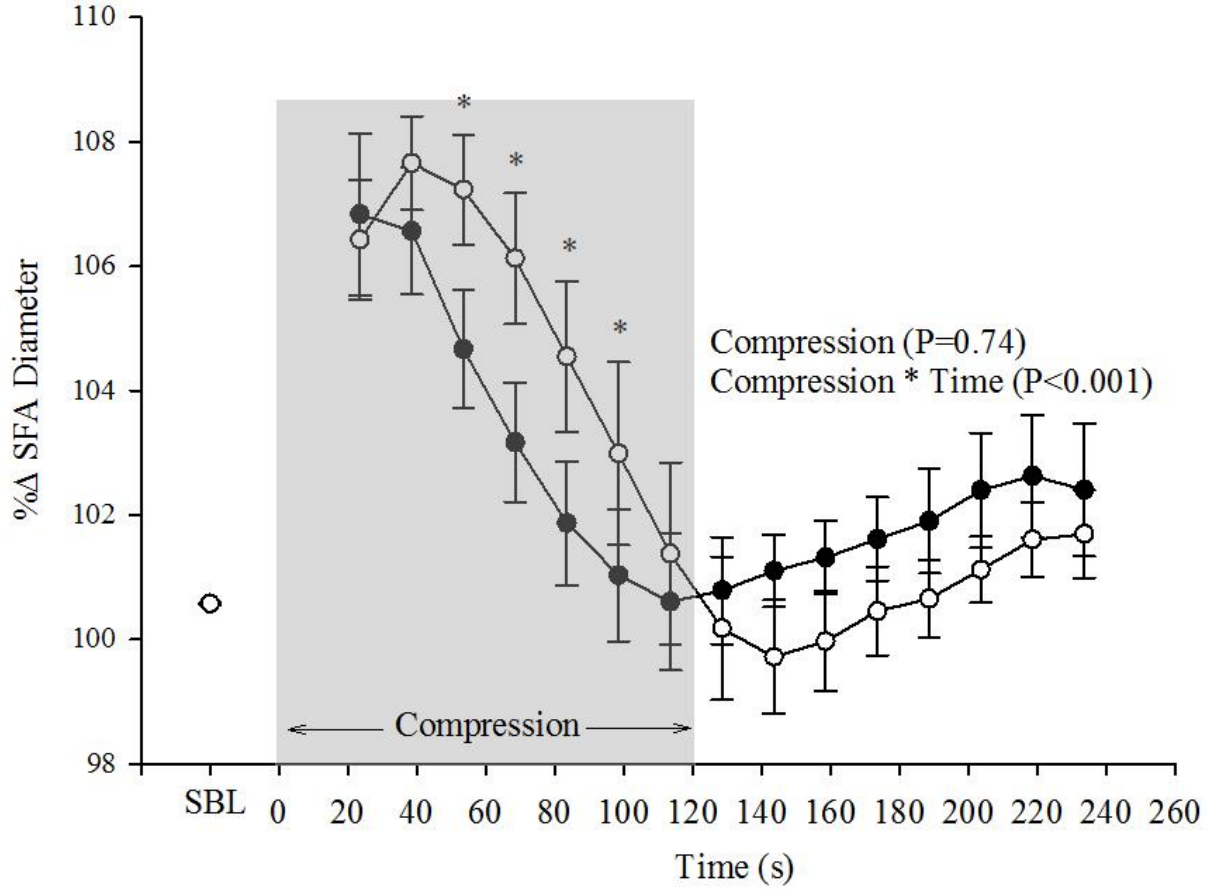

Figure 13. *SFA* diameter response to the *SS* transition
SFA diameter in response to the *SS* transition for both no-compression (filled circles) and compression (unfilled circles) conditions, expressed as percent change from standing baseline. Each data point is a 15-second average ± SEM taken from 13s post-stand to the 4-minute mark. The area shaded in grey represents when the compression cuffs were active in the compression condition. The (*) represents time periods where diameters are significantly different with compression as assessed by a 2-Way RM ANOVA with Holm-Sidak post-hoc testing.

4.3.2 Central and systemic hemodynamic responses to the squat-to-stand transition

The dynamic assessment of central and systemic hemodynamics revealed a significant attenuation of the *HR* response following standing with intermittent compression. The immediate *HR* response was decreased for 3 seconds and at non-continuous points from 30 – 60 seconds post-stand. Additionally, *HR* remained significantly lower at the end of minutes 2 and 4. The end of the third minute showed a trend for decreased *HR* (P=0.054) (Figure 14A). Intermittent compression increased *SV* from 14 seconds post-stand to the end of the first minute, and remained elevated at the end of the second minute. The end of the third minute showed a trend for increased SV, similar to that of *HR* (P=0.097) (Figure 14B). The relative increase in *SV* overshadowed the decrease in *HR*, as is seen by the elevation in $\dot{Q}$ with intermittent compression (Figure 14C). $\dot{Q}$ was significantly greater at non-continuous points from 14 seconds post-stand to the end of the first minute in the compression condition. This increase in $\dot{Q}$ persisted to the end of the second minute (P=0.041). *TPR* was decreased with intermittent compression. This decrease became apparent at the 19-second mark and was significantly lower at non-continuous points to the end of the first minute. At the end of minutes 2 and 3 there was a trend toward decreased *TPR* (minute-2: P=0.079, minute-3: P=0.094), and a significant difference at the end of minute 4 (P=0.041) (Figure 14D). The magnitude of the initial orthostatic hypotensive state was attenuated with compression between 6 and 10 seconds post-stand. *MAP* was similar throughout initial recovery, and became elevated at non-continuous time points from 33 to 58 seconds post-stand (Figure 14E). Irrespective of condition, the SS transition elicited a mean drop in diastolic blood pressure of over 20 mmHg within the first 15s of standing, meeting the criteria for *IOH*.

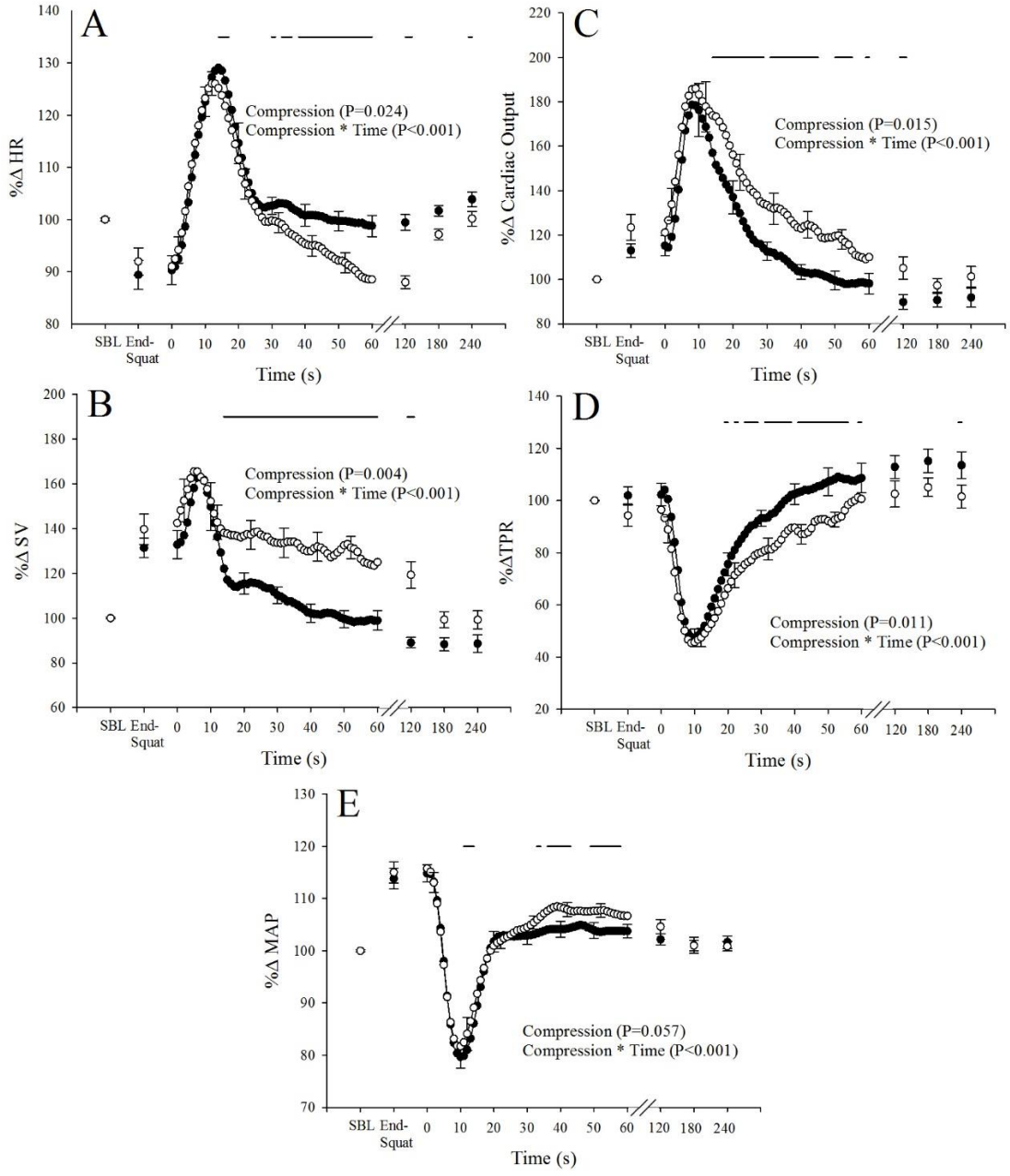

Figure 14. Average central and systemic hemodynamic responses to the *SS* transition
HR (A), *SV* (B), $\dot{Q}$ (C), *TPR* (D) and *MAP* (E) responses to the SS transition for no-compression (filled circles) and compression (unfilled circles) conditions, expressed as percent change from standing baseline (SBL). All data points between 0 and 60 seconds represent means ± SEM interpolated at 1 Hz from all subjects, with time = 0 representing the onset of the stand. Intermittent compression was active from 0 to 120 seconds. Data points at *SBL*, *End-Squat*, 120, 180 and 240 seconds are averages taken from the last 10 seconds of that time period. The solid bar above each plot shows time points that are significantly different between compression and no-compression conditions as assessed by a 2-Way RM ANOVA with Holm-Sidak post-hoc testing.

4.3.3 Cerebral hemodynamic response to the squat-to-stand transition

At the cerebral level, intermittent compression attenuated the initial decrease in *TSI%* shortly after arising from the squat position (time = 11 seconds). *TSI%* was once again elevated from 25 – 46 and again at 53 seconds as well as at 2-minutes post-stand when compared to the no-compression condition (Figure 15A). Intermittent compression exaggerated the initial drop in *MCAv*, which was significantly lower than the no-compression condition 4 seconds post-stand. By the 8-second mark, there was a trend towards and increase in *MCAv* with compression. *MCAv* was elevated with intermittent compression at non-continuous points between 39 and 51 seconds post-stand (Figure 15B). $ETCO_2$ was similar at all time points between compression and no-compression conditions in response to the *SS* transition.

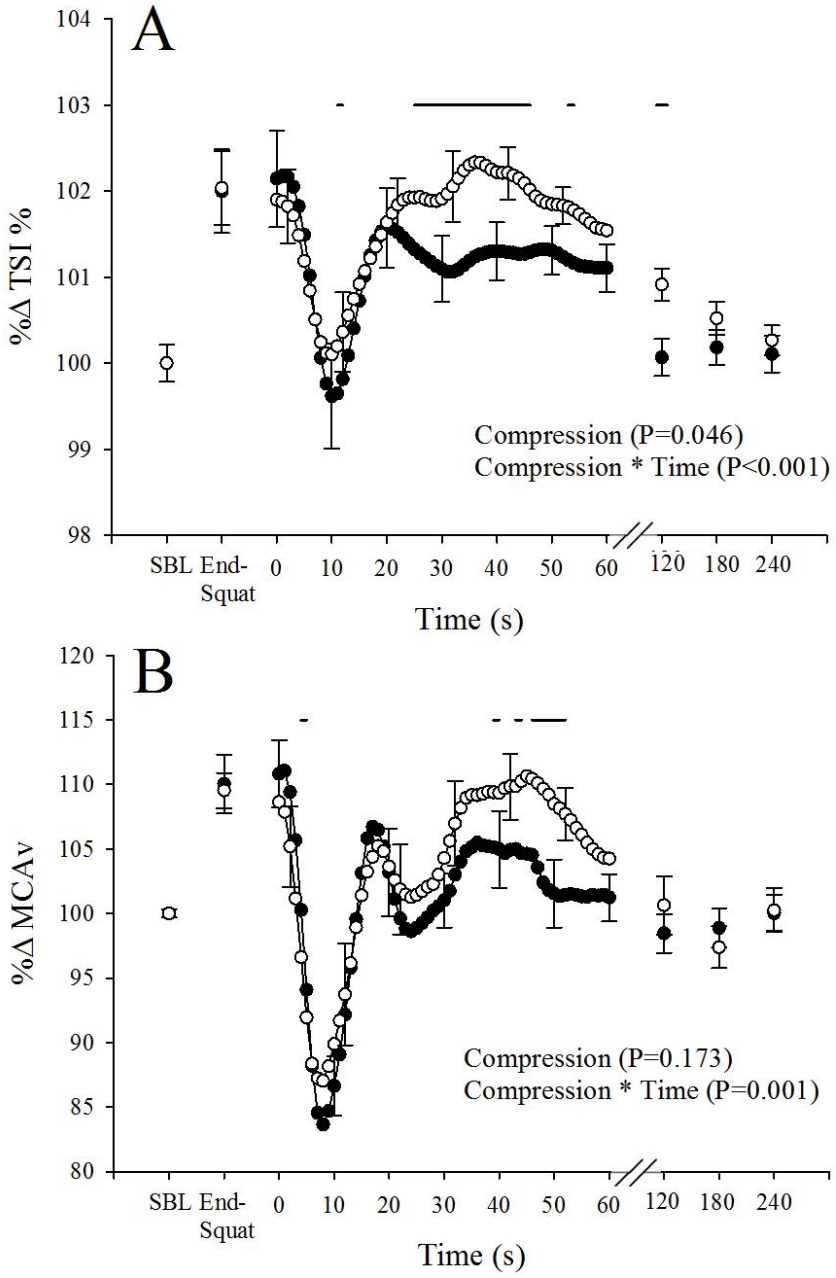

Figure 15. *TSI%* and *MCAv* in response to the *SS* transition
TSI% (A) and *MCAv* (B) in response to the *SS* transition for both no-compression (filled circles) and compression (unfilled circles) conditions, presented as percent change from standing baseline (*SBL*). All data points between 0 and 60 seconds represent means ± SEM interpolated at 1 Hz from all subjects, with time = 0 representing the onset of the stand. Data points at *SBL*, *End-Squat*, 120, 180 and 240 seconds are averages taken from the last 10 seconds of that time period. Intermittent compression was active from 0 to 120 seconds. The solid bar above each plot shows time points that are significantly different between compression and no-compression conditions as assessed by a 2-Way RM ANOVA with Holm-Sidak post-hoc testing.

4.3.4 Local hemodynamic response to the thigh cuff release maneuver

The dynamic, second-by-second analysis of the local response to the *TCR* maneuver revealed an increase in blood flow through the *SFA* with intermittent compression. This increase in blood flow through the *SFA* was apparent throughout the peak flow response from 6 to 15 seconds post-*TCR* (Figure 16A). There were no differences in *SFAv* between compression and no-compression conditions (Figure 16B).

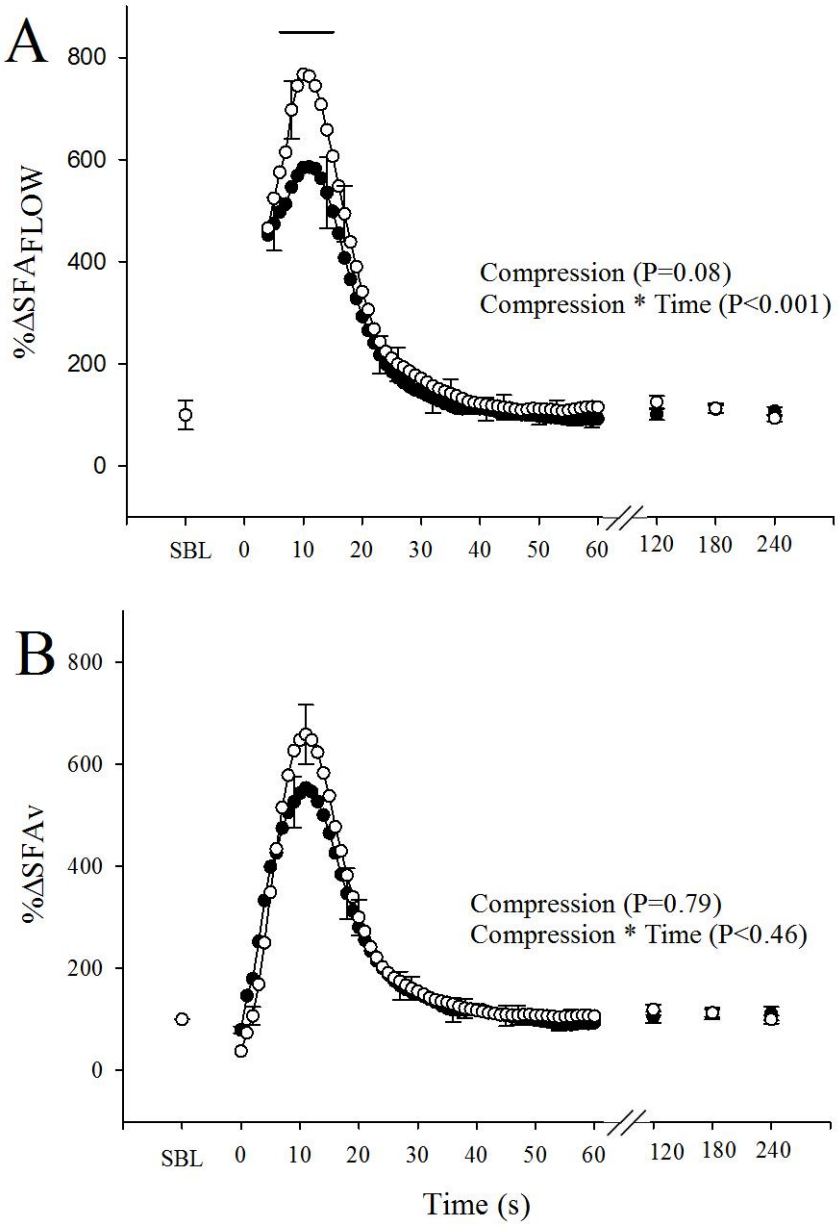

Figure 16. *SFA* flow and velocity in response to *TCR*

SFA blood flow (A) and velocity (B) in response to *TCR* for both no-compression (filled circles) and compression (unfilled circles) conditions, expressed as percent change from standing baseline (*SBL*). All data points between 0 and 60 seconds represent means ± SEM interpolated at 1 Hz from all subjects, with time = 0 representing occlusion release (SFA_{FLOW} data available from 5s onward). Intermittent compression was active from 0 to 120 seconds. Data points at *SBL*, 120, 180 and 240 seconds are averages taken from the last 10 seconds of each time period. The solid bar above each plot shows time points that are significantly different between compression and no-compression conditions as assessed by a 2-Way RM ANOVA with Holm-Sidak post-hoc testing.

Similar to the *SFAv* response, the change *SFA* diameter was also not different between compression and no-compression conditions (Figure 17). Thus, the resulting increase in *SFA* flow with intermittent compression likely involved small contributions from the non-significant differences in *SFAv* and diameter.

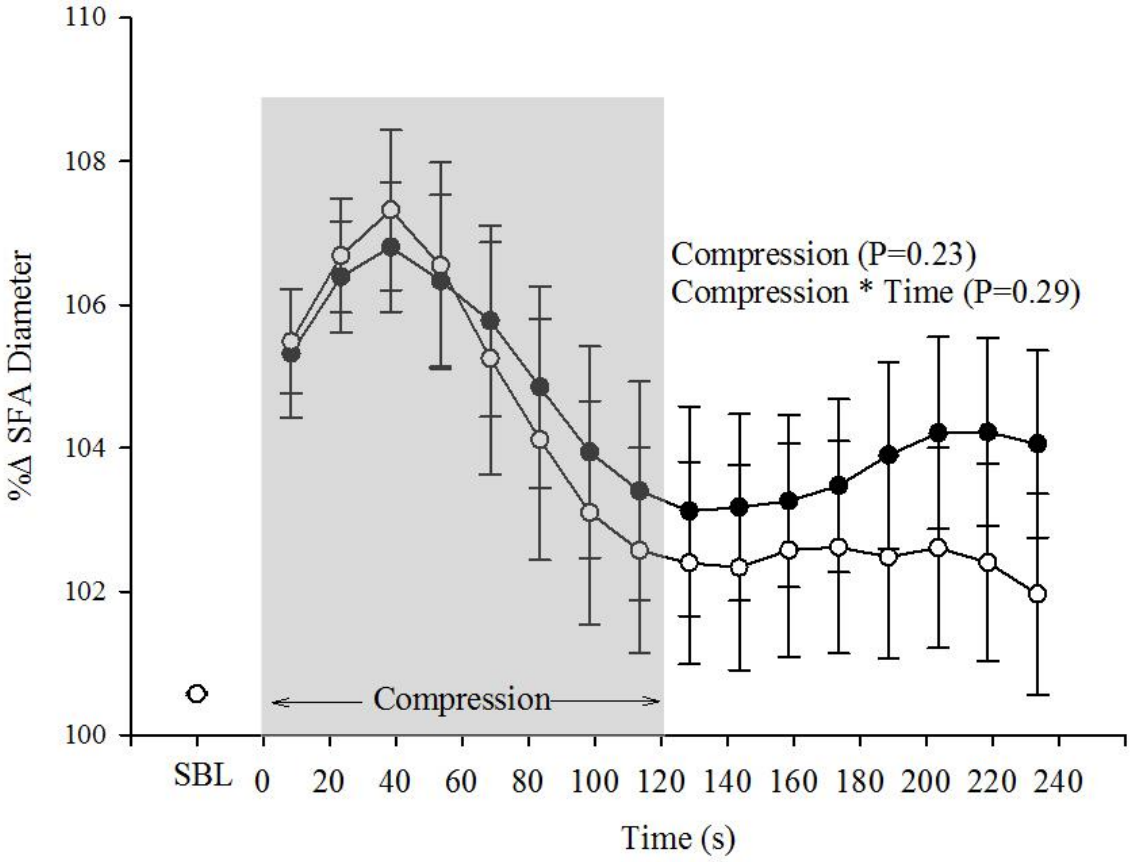

Figure 17. *SFA* diameter is response to *TCR*
SFA diameter in response to *TCR* transition for both no-compression (filled circles) and compression (unfilled circles) conditions, expressed as percent change from standing baseline. Each data point is a 15-second average ± SEM taken from 5s post-*TCR* to the 4-minute mark, with time = 0 representing occlusion release. The area shaded in grey represents when the compression cuffs were active in the compression condition. The (*) represents time periods where diameters are significantly different with compression as assessed by a 2-Way RM ANOVA with Holm-Sidak post-hoc testing.

4.3.5 Central and systemic hemodynamic response to the thigh cuff release maneuver

The dynamic assessment of the central hemodynamic response to *TCR* revealed that intermittent compression attenuated the *HR* response and exaggerated the *SV* response. *HR* was decreased from 11 seconds post-*TCR* until the end of the first minute. *HR* remained lower during the last 10 seconds of the second minute, and the effects of compression persisted until the end of the third minute (Figure 18A). Intermittent compression elevated *SV* from 6 seconds until the end of the first minute post-*TCR*, and remained elevated at the end of the second minute (Figure 18B). This relatively greater increase in *SV* with intermittent compression resulted in an exaggerated $\dot{Q}$ peak from 6 to 16 seconds following *TCR*. $\dot{Q}$ remained elevated at non-continuous time points between 20 and 39 seconds (Figure 18C). The initial decrease in *TPR* following the release of the thigh cuffs was transiently exaggerated with compression between 11 and 12 seconds. *TPR* was similar throughout the recovery until the end of the first minute and at the ends of minutes-2, -3 and -4 (Figure 18D). The magnitude of the initial *MAP* drop was attenuated with compression from 6 to 10 seconds post-*TCR*. *MAP* was also elevated from the no-compression condition from 19 seconds to the end of the first minute (seconds-23 and -24 trended towards an elevation, P=0.099 and P=0.096, respectively) (Figure 18E). The attenuated drop in diastolic blood pressure shortly after *TCR* in the compression condition resulted in only the no-compression condition meeting the criteria for *IOH*.

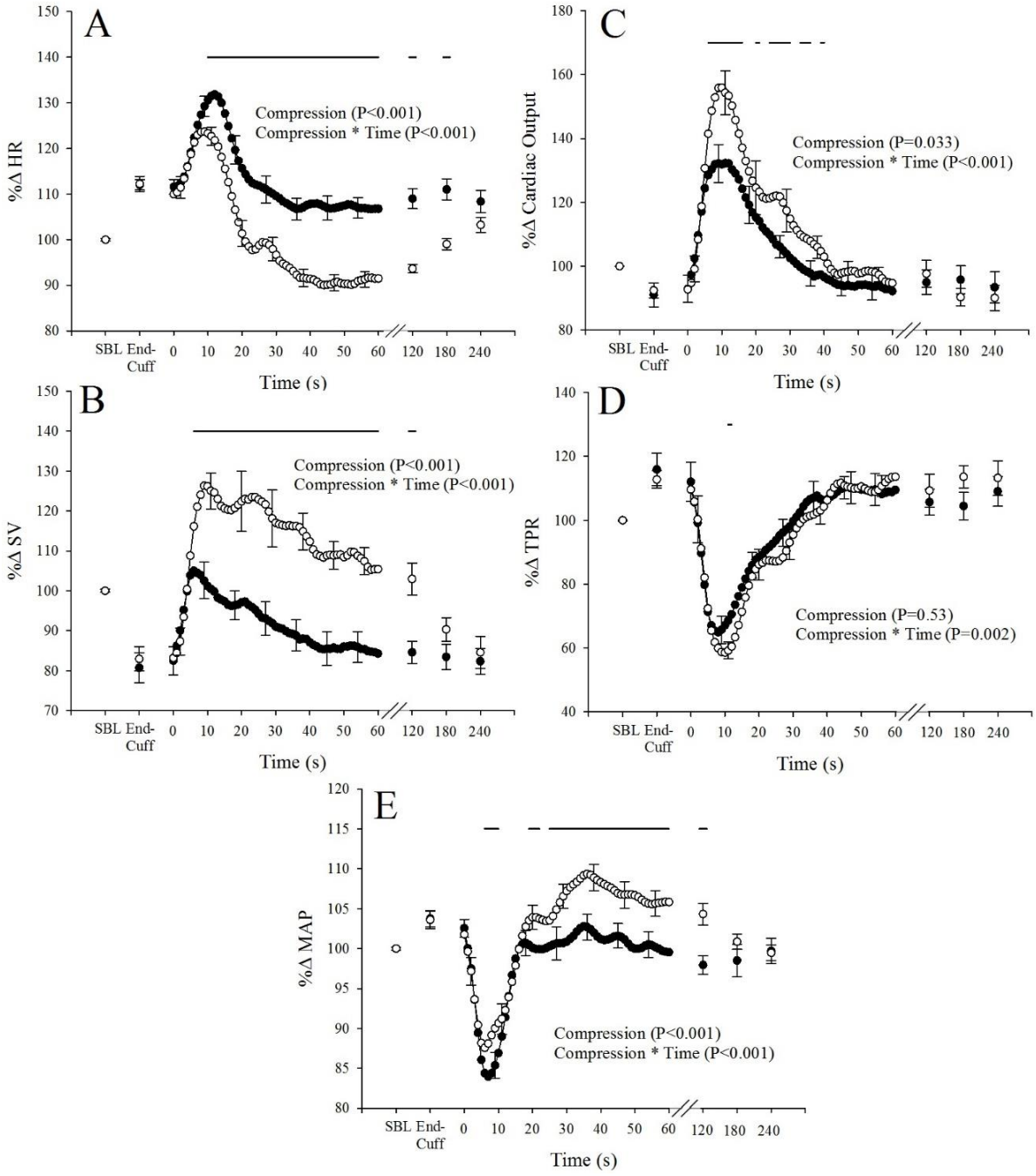

Figure 18. Average central and systemic hemodynamic responses to *TCR*
HR (A), *SV* (B), $\dot{Q}$ (C), *TPR* (D) and *MAP* (E) responses to *TCR* for no-compression (filled circles) and compression (unfilled circles) conditions, expressed as percent change from standing baseline (*SBL*). All data points between 0 and 60 seconds represent means ± SEM interpolated at 1 Hz from all subjects, with time = 0 representing occlusion release. Intermittent compression was active from 0 to 120 seconds. Data points at *SBL*, *End-Cuff*, 120, 180 and 240 seconds are averages taken from the last 10 seconds of that time period. The solid bar above each plot shows time points that are significantly different between compression and no-compression conditions as assessed by a 2-Way RM ANOVA with Holm-Sidak post-hoc testing.

4.3.6 Cerebral hemodynamic response to the thigh cuff release maneuver

The *TSI%* response trended toward a main effect of compression (P=0.084), and showed a significant compression by time interaction (P<0.001). *TSI%* was elevated with compression from 30 to 35 seconds, and again from 40 to 56 seconds post-*TCR* (Figure 19A). *TSI%* remained elevated at the end of the second minute, and trended toward an elevation at the end of the third minute (P=0.087). Intermittent compression attenuated the initial decrease in *MCAv* from 6 – 8 seconds. *MCAv* was once again elevated from 15 to 38 seconds and intermittently between 41 and 60 seconds. Intermittent compression also resulted in an elevation in *MCAv* during the last 10 seconds of the second minute after the release of the thigh cuffs (Figure 19B).

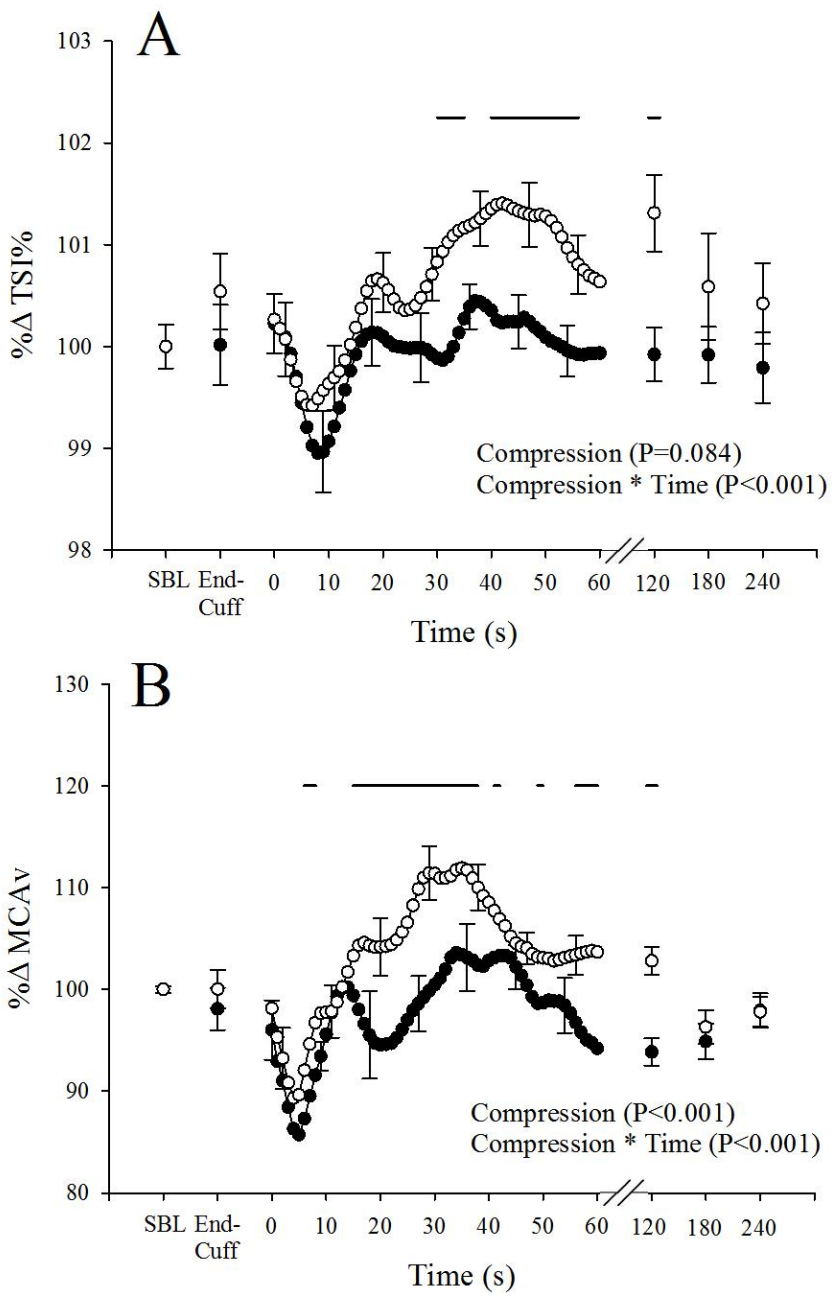

Figure 19. *TSI%* and *MCAv* response to *TCR*

TSI% (A) and *MCAv* (B) in response to *TCR* for both no-compression (filled circles) and compression (unfilled circles) conditions, presented as percent change from standing baseline (*SBL*). All data points between 0 and 60 seconds represent means ± SEM interpolated at 1 Hz from all subjects, with time = 0 representing occlusion release. Data points at *SBL*, *End-Cuff*, 120, 180 and 240 seconds are averages taken from the last 10 seconds of that time period. Intermittent compression was active from 0 to 120 seconds. The solid bar above each plot shows time points that are significantly different between compression and no-compression conditions as assessed by a 2-Way RM ANOVA with Holm-Sidak post-hoc testing.

Contrary to the *SS* transition, the application of intermittent compression after *TCR* resulted in both a main effect of compression, and a compression by time interaction for $ETCO_2$. $ETCO_2$ was higher with intermittent compression from 5 seconds post-*TCR* onwards until the end of the first minute. $ETCO_2$ remained elevated at the end of the second and fourth minutes, and trended toward an elevation at the end of the third minute (P=0.063). The $ETCO_2$ response to *TCR* for 3 subjects is shown in Figure 20.

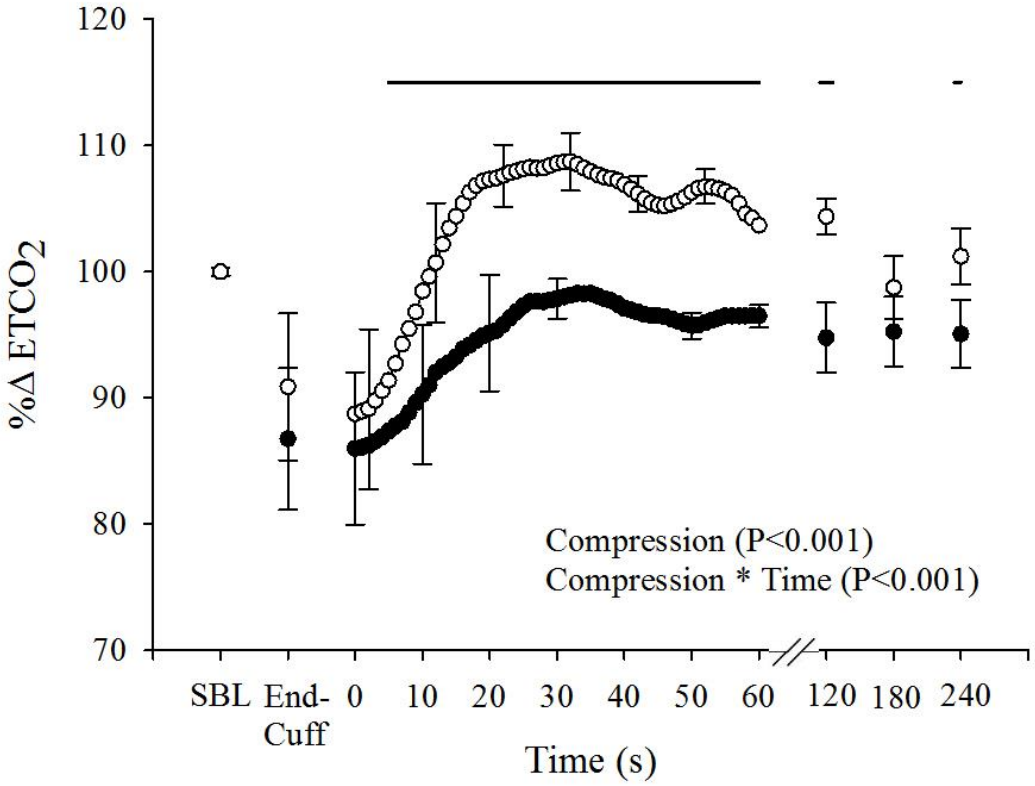

Figure 20. $ETCO_2$ in response to *TCR*
$ETCO_2$ in responses for 3 subjects to *TCR* for both no-compression (filled circles) and compression (unfilled circles) conditions, presented as percent change from standing baseline (*SBL*). All data points between 0 and 60 seconds represent means ± SEM interpolated at 1 Hz from all subjects, with time = 0 representing occlusion release. Data points at *SBL*, *End-Cuff*, 120, 180 and 240 seconds are averages taken from the last 10 seconds of that time period. Intermittent compression was active from 0 to 120 seconds. The solid bar above each plot shows time points that are significantly different between compression and no-compression conditions as assessed by a 2-Way RM ANOVA with Holm-Sidak post-hoc testing.

4.4 Discussion

The main findings of the present study were that intermittent external compression of the lower legs attenuated the orthostatic-induced reductions in *MAP*, *CBF* velocity and indices of cerebral oxygenation, while simultaneously enhancing blood flow to the legs. These findings support the hypothesis that the local and central influences of intermittent compression translate into improved cerebral hemodynamics during orthostatic stress.

The use of compression technology to counter orthostatic stress

The application of lower body external compression as a countermeasure to orthostatic stress has been limited to static compression garments, ranging in coverage from the lower-legs, all the way up to the abdomen (Lucas et al., 2012; Platts et al., 2009; Podoleanu et al., 2006; Protheroe et al., 2011; Smit et al., 2004; Stenger et al., 2013). Static compression technology presents the possibility that by mechanically impeding peripheral pooling in dependent vessels of the lower body, more blood will be available to central circulation. However, the efficacy of static compression to augment systemic, and more importantly cerebral hemodynamics, has been questioned (Lucas et al., 2012; Protheroe et al., 2011). During combined 60° head-up tilt and lower-body negative pressure, Protheroe et al. found no differences in blood pressure, *SV*, $\dot{Q}$, or *MCAv* with ankle-to-knee graduated compression stockings applying low level pressures between 29 and 35 mmHg (Protheroe et al., 2011). In addition, Lucas et al. showed that although the *MAP* nadir was delayed by 14% following a supine-to-stand transition with commercially available compression stockings, the magnitude of the initial *MAP* and *MCAv* nadirs were not different (Lucas et al., 2012). On the contrary, in patients with pure autonomic failure, Denq et al. showed

that compression garments encompassing the calves, thighs and lower abdomen were capable of significantly improving blood pressure, peripheral resistance and orthostatic symptoms, when compared to leg compression alone during 80° head-up tilt (Denq et al., 1997). In a similar patient population, Smit et al. showed that 40 mmHg static compression of the legs and abdomen attenuated the drop in blood pressure during 40 - 60° head-up tilt, which was the result of increased and *SV* (+14%) and $\dot{Q}$ (+13%), and not *TPR* (+1%) (Smit et al., 2004). These improvements in blood pressure have since been supported with only lower- and upper-leg static compression in a healthy population (Helmi, Lima, Gommers, Bakker, & van Bommel, 2013). Although Denq, Smit, and Helmi revealed augmented central hemodynamics, none of these studies assessed the impact of static compression on cerebral hemodynamics.

As opposed to static compression garments, intermittent compression presents the possibility of improving the local and systemic hemodynamic state during orthostatic stress by imparting energy into the circulation and actively promoting blood flow back to the heart. In the present study, intermittent compression was used to simulate the action of the muscle pump by transiently reducing venous pressure and generating an increased arteriovenous pressure gradient (van Bemmelen et al., 1994). Timing the compression cycle with the local diastolic phase of each cardiac cycle was chosen to optimize blood flow *into* and *out of* the leg. It was reasoned that a rapid inflation-deflation cycle timed to local diastole would improve venous outflow while mitigating the possibility of impeding arterial inflow. Additionally, it was reasoned that low level compression (~65 mmHg) would be capable of compressing venous vessels and not arterial vessels, which operate under much high pressures at that level of the circulatory system. Research coming out of our lab has revealed that this specific compression profile accentuated the muscle pump during standing plantar flexion exercise, resulting in both increased venous outflow and

arterial inflow (Zuj et al., 2017). Although the relative contributions of other potential influencing factors cannot be explained with these results, previous studies investigating intermittent compression indicate that both an altered arteriovenous pressure gradient, as well as local vasodilation likely play prominent roles in the increase in *SFA* flow (Delis, Azizi, Stevens, Wolfe, & Nicolaides, 2000; Lurie, Awaya, Kistner, & Eklof, 2003; Prince et al., 2017; van Bemmelen et al., 1994).

Hemodynamic effects of intermittent compression during the SS transition

As can be seen in Figures 12 and 13, the increase in *SFA* flow upon standing from the squat position is primarily a function of increased *SFAv*. However, it should be noted that 3-minutes of squatting did result in a ~6% increase in *SFA* diameter within 15 - 30 seconds of standing, which then further increased to +8% shortly thereafter in the compression condition (Figure 13). This increase in conduit artery diameter is contrary to that commonly seen with hand-grip exercise (Carlson, Kirby, Voyles, & Dinenno, 2008; Walker et al., 2007) and prolonged squatting (Tschakovsky et al., 2011). Nevertheless, the 6% increase in *SFA* diameter had minimal contribution to *SFA* flow, as *SFAv* increased by over 1000% when compared to the standing baseline values (Figure 12A and B). Furthermore, the increase in *SFA* flow caused by compression coincided with that of *SFAv*, both occurring 19 seconds post-stand and continuing throughout the duration of the first minute of standing. One possible explanation for the increase in *SFA* diameter from 45 to 105 seconds post-stand with intermittent compression is an increase in shear stress shortly after attaining the standing position. Contrary to the *TCR* condition, intermittent compression increased *SFAv* 10 seconds post-stand and remained elevated semi-continuously for the duration of the first minute (Figure 12B). In the brachial artery, transient and substantial

elevations in shear rate have been shown to induce maximum dilation in 40 seconds in adults (Evanoff, Kelly, Steinberger, & Dengel, 2015). Thus, consistent with our understanding of flow-mediated dilation, it is possible that the increase in shear stress caused by intermittent compression contributes to the release of autocoids (e.g., nitric oxide) from the endothelium, triggering the exaggerated vasodilatory response seen from 45 to 105 seconds post-stand.

In alignment with hypothesis (2), intermittent compression attenuated the initial drop in *MAP* and maintained an elevated blood pressure throughout the first minute of standing post-*SS* transition. As can be seen in Figure 14, the resulting increase in *MAP* is a consequence of increased $\dot{Q}$, as *TPR* is consistently lower with intermittent compression. The pattern of elevation in blood flow through the heart seen with intermittent compression closely matches that seen in the *SFA*. This finding is expected, as only the aorta and common femoral artery (2 large conduit arteries) separate the heart and *SFA*. It is also apparent that intermittent compression stimulated baroreflex activity. Almost immediately following the *SS* transition, intermittent compression increased *SV* throughout the duration of the first minute, which was followed shortly thereafter by a decrease in *HR*. The attenuated *HR* response with intermittent compression was overshadowed by the increase in *SV*, as $\dot{Q}$ is consistently elevated. These findings were consistent with the *TCR* maneuver, as well.

Figure 15 reveals that the augmented local, central and systemic hemodynamics with intermittent compression are translated into the cerebral circulation during the *SS* transition. The influence of intermittent compression is apparent during the initial orthostatic hypotensive state, as is seen in the attenuation of the *TSI%* and *MCAv* nadirs, as well as throughout the first minute of recovery. Additionally, the influence of compression on *TSI%* persists to the end of the second minute of standing (Figure 15A). Interestingly, the *MCAv* and *TSI%* responses to both the *SS*

transition and *TCR* maneuver are comparable in both the timing of the nadirs and patterns of recovery, suggesting an influence of cerebral blood flow on cortical tissue oxygenation detected by NIRS. Although *TSI%* has been considered a reflection of cerebral tissue oxygenation, the translation of arterial blood flow to NIRS derived indices of tissue oxygenation is not always clear, in part due to that status of tissue metabolism (Ferradal et al., 2017; Lin et al., 2013). However, it has been assumed that ~30% of NIR light absorption in cerebral tissue is due to arterial blood (Boushel et al., 2001). The similarities in *MCAv* and *TSI%* during both the *SS* transition and *TCR* maneuver in the present study support the influence of arterial blood flow on tissue oxygenation detected by NIRS.

Hemodynamic effects of intermittent compression during the TCR maneuver

Similar to the *SS* transition, the increase in *SFA* flow was driven by a large increase in *SFAv* in both the compression and no compression conditions (Figure 16A and B). Intermittent compression accentuated the initial increase in *SFA* flow but had little to no impact on *SFA* flow or velocity beyond 15 seconds post-*TCR*. The increase in *SFA* diameter was similar to the *SS* transition (+7%) but contrary to the *SS* transition, *SFA* diameter was not affected by intermittent compression (Figure 17). The lack of effect of compression on *SFAv* (and thus, shear stress) and *SFA* diameter, supports the notion the elevated shear stress influenced the increase in *SFA* diameter seen with intermittent compression in the *SS* test.

Intermittent compression attenuated the initial drop in *MAP* and maintained an elevated blood pressure throughout most of the first minute of standing and persisted to the end of the second minute (Figure 18E). This increase in blood pressure was driven by an increase in *SV* and

$\dot{Q}$, as the *HR* response was attenuated and the *TPR* reduction was exaggerated with intermittent compression (Figure 18 A, B, C and D). It is also interesting to note the *HR* remained lower with intermittent compression even 1 minute after the compression system was deactivated, suggesting an influence of compression that persisted even beyond its activity. Contrary to the *SS* transition, the pattern of $\dot{Q}$ elevation with intermittent compression does not closely resemble the pattern of elevation seen in *SFA* blood flow. While intermittent compression increased $\dot{Q}$ relatively consistently for the first ~40s post-*TCR*, the effect of intermittent compression on *SFA* blood flow is not detectable after ~15 seconds. One possible explanation for the apparent discrepancy between $\dot{Q}$ and *SFA* flow is the influence of retrograde arterial blood flow imparted by intermittent compression during the *TCR* test. The absence of significant muscle work during 3 minutes of thigh occlusion likely results in a different biochemical environment within the muscles of the legs when compared to 3 minutes of squatting, which is perhaps influential in these discrepant findings between to the orthostatic stress tests. Recent work coming out of our lab has shown that intermittent compression augments the blood flow velocity profile through the popliteal artery during quiet standing, resulting in retrograde blood flow velocity during compression, followed by an increase in anterograde velocity during the remainder of diastole (Prince et al., 2017). Thus, perhaps the influence of retrograde blood flow velocity caused by intermittent compression resulted in the discrepancy in $\dot{Q}$ and *SFA* flow from ~15 – 40 seconds post-*TCR*.

Consistent with the *SS* transition, the local and central hemodynamic effects of intermittent compression post-*TCR* were translated into the cerebral circulation. The magnitude of the initial decrease in *MCAv* was attenuated with compression, and both *MCAv* and TSI% were elevated throughout most of the first minute and at the end of the second minute post-*TCR* (Figures 19A and B). Contrary to the *SS* transition, intermittent compression exaggerated the increase in

$ETCO_2$ following the release of thigh cuff occlusion, which was likely influential in the observed increases in *MCAv* and *TSI*% (Poulin, Liang, & Robbins, 1996) (Figure 20).

Accounting for the improvement in central hemodynamics with intermittent compression

The consistent elevation of *MAP* throughout the first minute of the *SS* transition and *TCR* suggests a shifting of the baroreflex operating point. During exercise, muscle performance is directly related to its perfusion pressure, and elevations in *MAP* support appropriate muscle functioning, while assuring the brain and heart receive a consistent flow of blood (McCord & Kaufman, 2010). There is ample evidence suggesting that this rightward and upward shift of the baroreflex response slope is primarily influenced by central command and the exercise pressor reflex (Joyner, 2005; Rowell & O'Leary, 2013). The absence of conscious movement following both orthostatic stresses in the present study negates the impact of central command as a potential contributor to the observed increase in *MAP* with intermittent compression. Additionally, because intermittent compression was applied immediately following the *SS* transition and *TCR* maneuver, a similar local biochemical environment would be expected prior to the initiation of compression, indicating that the metaboreflex response is likely similar in both compression and no compression conditions. Therefore, it can be deduced that the elevation in *MAP* is, at least in part, influenced by thinly myelinated type III mechanoreceptor stimulation triggered by repeated squeezing of the muscles of the lower leg. In support of this theory, Williamson et al. have shown that static external compression of the legs causes increases in *MAP* and diastolic pressure dependent on the applied pressure. The *MAP* response was abolished with the administration of epidural anesthesia, indicating the role of pressure-sensitive afferents located within the muscle in the regulation of blood pressure (Williamson, Mitchell, Olesen, Raven, & Secher, 1994). Furthermore, Bell and

White showed that a standardized level of compression (300 mmHg) around the calf muscles during post-exercise circulatory occlusion increased *MAP* and the magnitude of elevation was dependent on prior exercise intensity (Bell & White, 2005). These results suggest that metaboreceptors are sensitized by metabolite accumulation within the muscle. In relation to the present study, it is possible that the accumulation of metabolites within the muscle during 3-minutes of squatting and 3-minutes of bilateral thigh occlusion sensitized type III mechanoreceptors located in the calf and soleus muscles, which were then by further stimulated by intermittent compression. The compounding effect of intermittent compression on the exercise pressor reflex could possibly explain the elevation in *MAP* seen throughout the first minute of both orthostatic stress tests. Additionally, it is possible that reflexive muscle contraction opposing the force of compression could further stimulate the muscle pressor reflex through metaboreceptor activation. However, because EMG data was not collected, this cannot be confirmed.

Accounting for improvement cerebral hemodynamics with intermittent compression

Although an augmented baroreflex response curve could account for the consistently elevated *MAP* and thus cerebral perfusion pressure, our current understanding of cerebral autoregulation would suggest that cerebrovascular compensation would counteract a paralleled increase in *CBF* (Tzeng & Ainslie, 2014; Willie et al., 2014). Thus, the increase in *MAP* alone cannot be solely responsible for the increases in *MCAv* seen with intermittent compression during both orthostatic stress tests. One possible explanation for the increase in *MCAv* is the independent influence of $\dot{Q}$ on *CBF* (Meng, Hou, Chui, Han, & Gelb, 2015; van Lieshout et al., 2001). Consistent with the findings of the present study, van Lieshout et al. showed that moving from supine to standing caused a decrease in $\dot{Q}$ that was paralleled by decreases in *MCAv*, indices of

cerebral oxygenation and $PaCO_2$. Engaging in lower body muscle tensing after 5 minutes of standing increased $\dot{Q}$, *MCAv*, indices of cerebral oxygenation and $PaCO_2$ without altering *MAP*, suggesting an independent relationship between $\dot{Q}$ and *CBF* (van Lieshout et al., 2001). In addition, the recent findings of Nagaya et al. indicate that passive lower leg muscle movement can alter cerebral hemodynamics independent of blood pressure. Nagaya et al. showed that both active and passive ankle exercise elicited similar increases in cerebral oxyhemoglobin concentrations, although the increase occurred despite unchanged *MAP* and *HR* with passive exercise (Nagaya et al., 2015). Similar findings have been reported by Matteis et al. and Doering et al., both showing significant increases in both *MCAv* and cerebral oxyhemoglobin with passive exercise in the absence of concomitant changes in *MAP* and *HR* (Doering et al., 1998; Matteis et al., 2001). Due to unaltered cerebral perfusion pressure in all of these studies, the resulting increase in *MCAv* and indices of cerebral oxygenation must be the result of reduced cerebrovascular resistance, likely driven by sympathetic withdrawal incurred by increases in central blood volume (Meng et al., 2015). However, because $\dot{Q}$ measures were only reported in the study by van Lieshout et al., we can only infer that the active and passive exercise implemented in the other studies elevated $\dot{Q}$ as well.

Contrary to the *SS* transition, intermittent compression increased $ETCO_2$ for most of the first 2 minutes of standing post-*TCR*, which could also contribute to the increase in *MCAv* (Figure 20). The sensitivity of the cerebrovasculature in response to changes in $ETCO_2$ is robust and rapid (Poulin et al., 1996). $ETCO_2$ is often used as a surrogate for arterial CO_2 because its non-invasive nature and strong correlation with arterial CO_2 in a wide-variety of testing conditions (Brothers, Ganio, Hubing, Hastings, & Crandall, 2011; McNulty, Roy, Torjman, & Seltzer, 1990; Yanes & Reckelhoff, 2011). Thus, the ~4% increase in $ETCO_2$ over the first minute with compression

compared to the ~5% decrease seen in the control condition, likely reflects an augmented haematological state, and influences the cerebrovascular response. The elevation in $ETCO_2$ with *TCR* is a relatively consistent finding in the literature (Hildebrandt & Mason, 1979; Panerai et al., 2015). One explanation for the increase in $ETCO_2$ with the release of thigh cuffs is the recirculation of blood accumulated in the muscle during circulatory occlusion (Hildebrandt & Mason, 1979). The exaggerated $ETCO_2$ response with intermittent compression in the present study might be a result of increased venous outflow, which would promote greater recirculation of accumulated metabolites and carbon dioxide in the muscle. Additionally, pending the venous-arterial CO_2 pressure difference across the lungs remains relatively stable, the elevation in $\dot{Q}$ seen with compression would result in an increase in the rate of expired CO_2 (Mahutte, Jaffe, Sassoon, & Wong, 1991). However, similar increases in *MCAv* with intermittent compression during the *SS* trial occurred despite unchanged $ETCO_2$, discrediting the role of $ETCO_2$ as the primary contributor to the elevation of *MCAv* during orthostatic stress.

4.5 Limitations

Measurements of *MCAv* with transcranial Doppler ultrasound only reflect changes in *MCA* flow if the vessel diameter remains unchanged throughout perturbations brought upon by the present orthostatic challenges. Arguments have been made regarding vessel diameter constancy during dynamic changes in blood pressure and $ETCO_2$ (Ainslie & Hoiland, 2014; Coverdale, Gati, Opalevych, Perrotta, & Shoemaker, 2014; Giller, Bowman, Mootz, & Kirppner, 1993; Giller, 2003; Kontos, 1989). Due to the dynamic nature of the present study, assessing intra- or extra-cranial artery diameter was not realistic. Using high resolution 3T magnetic resonance imaging, Coverdale et al. reported a ~0.4% change in *MCA* diameter for every 1 Torr change in $ETCO_2$ (Coverdale et al., 2014). During craniotomies, Giller et al. measured *MCA* diameter changes

during moderate fluctuations in blood pressure brought upon by nitroprusside infusion, and reported *MCA* diameters to change ~0.1% for every 1 mmHg change in blood pressure (Giller et al., 1993). Given the relationships between $ETCO_2$ and blood pressure with *MCA* diameter proposed by Coverdale and Giller, the resultant *MCA* diameter changes brought upon by intermittent compression in present study would only very modestly affect *MCA* diameter.

The assessment of beat-to-beat *SV* with aortic Doppler ultrasound during an *SS* transition is a challenging process. However, potential sources of error arising from changes in thorax angle, such as experienced during *HUT* testing (van Lieshout, Toska, et al., 2003), were eliminated by ensuring all subjects maintained an erect, vertical back during the squat and transition into the standing position. Thus, tilting of the ascending aorta caused by altering the gravitational force vector upon the heart would not pose a confounding influence. Additionally, by ensuring that subjects maintained upper body positioning throughout the squat, transition and stand, continuous Doppler measurements could be made throughout the entire *SS* transition. However, interaction between the *TCD* and aortic Doppler signal resulted in aortic Doppler measurements being removed in 5 of the 14 subjects.

$ETCO_2$ was recorded in only 3 of the 14 subjects. Additionally, $ETCO_2$ was measured using a nasal cannula and different CO_2 monitor (DATEX-OHMEDA 5200 CO_2 Monitor, Mundelein, IL, USA) in 12 subjects. It was realized that this system had a maximum detection of ~4.3% CO_2 (well within physiological range), and for this reason was not included in the analysis. However, even with this minimal detection range, $ETCO_2$ responses to both orthostatic stress tests matched the results detected with the more reliable Ametek CO_2 device that was used on the additional 3 subjects.

4.6 Conclusions, Applications and Future Perspectives

In summary, intermittent compression of the lower leg during *SS* transitions and *TCR* increased *CBF* velocity and indices of cerebral oxygenation. This finding provides promise for the use of active compression as a therapeutic tool for individuals vulnerable to orthostatic stress. Future research should investigate the effects of intermittent compression during other conditions that compromise *CBF* both transiently and chronically.

CHAPTER 5: GENERAL DISCUSSION

The ability to combat orthostatic stress though non-pharmacological means has been investigated with great scrutiny for decades (Convertino et al., 2005; Denq et al., 1997; Figueroa et al., 2010; Krediet et al., 2005, 2007; Lucas et al., 2012; Nagaya et al., 2015). Such countermeasures have been limited primarily to physical counter-pressure maneuvers and static compression garments, however, the feasibility and efficacy of these measures to provide an advantageous impact on cerebral hemodynamics has been lacking (Krediet et al., 2005, 2007; Lucas et al., 2012; Protheroe et al., 2011). This thesis included two studies that enabled us to reliably assess the efficacy of an alternative and novel countermeasure to orthostatic stress. The use of intermittent external compression timed to correspond with the body's cardiovascular system proved effective in mitigating decreases in both blood pressure and cerebral blood flow velocity during the preliminary stages of orthostatic stress. Thus, there is potential for the use of intermittent compression of the lower legs to counteract symptoms associated with orthostatic stress.

5.1 Summary of Findings

To effectively understand the complex interrelationships between central and cerebral hemodynamics during the immediate responses to orthostatic stress, one must be able to monitor beat-to-beat fluctuations in $\dot{Q}$. However, the assessment of SV and $\dot{Q}$ using the Modelflow method has proven unreliable in various conditions that share similarities to those experienced transiently during orthostatic stress (Dyson et al., 2010; Hughson et al., 2017; Shibasaki et al., 2011). The ostensible accuracy of Modelflow estimates during non-steady state conditions has remained an issue in cardiovascular research since the advent of Finometer® devices, despite countless comparative studies with more direct methods (Dyson et al., 2010; Harms et al., 1999; Hughson

et al., 2017; Shibasaki et al., 2011; van Lieshout, Toska, et al., 2003). A major advantage of Modelflow is its ability to provide continuous beat-to-beat estimations of *SV* in a wide variety of conditions, however, the reliability of these beat-to-beat *SV* estimations has yet to be validated during major changes in physiological state. Chapter 3 provided evidence that the Modelflow method does not accurately track changes in *SV* during dramatic and rapid decreases in blood pressure and *TPR*. It was shown that when compared to $SV_{U/S}$, Modelflow underestimated *SV* by up to 25% during the first 3 – 11 seconds of the *SS* transition and by up to 16% during the first 3 – 7 seconds of *TCR*. Thus, the findings of chapter 3 highlight that beat-to-beat estimates of *SV* from the Modelflow method during major changes in physiological state need be interpreted with a high degree of caution.

We proceeded to investigate the efficacy of a novel intermittent compression device as a countermeasure to orthostatic stress. The study discussed in Chapter 4 adopted the same orthostatic stress tests employed in Chapter 3 to test the efficacy of intermittent compression during conditions in which cerebral blood flow is compromised. Chapter 4 demonstrated that intermittent compression of the lower legs timed to the local diastolic phase of each cardiac cycle sufficiently counteracted orthostatis-induced reductions in blood pressure, cerebral blood flow velocity and indices of cerebral oxygenation, while simultaneously promoting blood flow to the legs. As opposed to static compression garments (Lucas et al., 2012; Protheroe et al., 2011), the findings of Chapter 4 support the hypothesis that intermittent compression provides potential as an effective means to counteract symptoms associated with orthostatic stress.

5.2 Future Applications

Intermittent compression technology provides an alternative to the largely ineffective static compression garments to be used as a therapeutic tool to combat symptoms of orthostatic

stress. Future work should continue to investigate the efficacy of this technology to mitigate cerebral hypoperfusion brought upon by various insults to the cardiovascular system. Such conditions could include orthostatic hypotension, post-exercise hypotension, post-long duration spaceflight, anti-G straining induced cerebral hypoperfusion and exhaustive exercise.

Additionally, the efficacy of intermittent compression should be assessed on patient populations who are chronically vulnerable to the symptoms of cerebral hypoperfusion. The present work was conducted on healthy, young individuals, none of whom had diagnosed orthostatic hypotension or autonomic disorders. It is possible, and maybe even likely, that the improvements in blood pressure and cerebral blood flow would be accentuated in individuals vulnerable to orthostatic stress due to their greater room for improvement.

Finally, the applicability of intermittent pneumatic compression devices, such as the one used in the present study, requires advancements in the present technology. Pneumatic compression devices capable of providing the required pressure likely cannot be made portable due to the reservoir of air required to pressurize the garment. Alternative technologies should be explored that will enable intermittent compression garments to be light, effective and worn comfortably.

5.3 Thesis Limitations

The primary limitation in Chapter 3 is the absence of the gold standard method to non-invasively assess *SV* fluctuations in a beat-by-beat manner during orthostatic stress. Although aortic Doppler ultrasound is a more direct method to assess *SV* and has fewer limitations within the present conditions, it also has a degree of error. To control for potential sources of error with aortic Doppler ultrasound we ensured subjects maintained consistent upper body positioning throughout the squat, transition, and stand to mitigate the potential shifting of the heart during

changes in posture. Additionally, during pilot testing we decided to supplement the *SS* test with the *TCR* maneuver. Standing *TCR* was chosen largely because it was capable of inducing significant drops in blood pressure without requiring subject movement, ensuring that the ultrasound insonation angle could be held constant. Similarities in Modelflow bias, which was calculated as the difference between SV_{MF} and $SV_{U/S}$ in Chapter 3, indicate that ensuring a constant vertical upper body position during the *SS* transition controlled for this proposed source of error and resulted in accurate $SV_{U/S}$ estimates during the first 20 seconds following the *SS* transition.

The assessment *MCAv* as an index of *CBF* in Chapter 4 poses an issue if the *MCA* diameter changes during the orthostatic stress tests. Arguments have been made regarding vessel diameter constancy during dynamic changes in blood pressure and $ETCO_2$ (Ainslie & Hoiland, 2014; Coverdale et al., 2014; Giller et al., 1993; Giller, 2003; Kontos, 1989; Verbree et al., 2014). Recently, using high resolution magnetic resonance imaging techniques, Coverdale et al. reported an ~8% increase in *MCA* diameter during a 9 mmHg elevation in $ETCO_2$, which resulted in an underestimation of *CBF* from *MCAv* measurements. They also reported that there was a ~0.4% change in diameter per 1 Torr change in $ETCO_2$ (Coverdale et al., 2014). In the study described in Chapter 4, there was a difference in $ETCO_2$ caused by intermittent compression in the *TCR* trial. Post-*TCR*, $ETCO_2$ increased up to ~37 mmHg in the no compression condition, and up to ~40 mmHg in the compression condition (maximum values within the first four minutes post-*TCR*). Given the relationship between *MCA* diameter and $ETCO_2$ reported by Coverdale et al., this discrepancy in $ETCO_2$ would equate to a ~1% difference in *MCA* diameter, which is unlikely to greatly influence *MCA* flow. In a subsequent study, Coverdale et al. reported that the maximum increase in *MCA* diameter was not reached until 4 minutes after $ETCO_2$ was elevated by 15 mmHg from pre-hypercapnic values (Coverdale, Lalande, Perrotta, & Shoemaker, 2015). These results

indicate that the small ~3 mmHg $ETCO_2$ discrepancy detected during the *TCR* trial would likely not influence *MCA* diameter during the 4 minutes of standing post-*TCR*. Additionally, any undetected changes in *MCA* diameter would only act exaggerate the increase in *CBF* seen with intermittent compression. There is limited reliable information available regarding *MCA* diameter sensitivity to changes in blood pressure. During surgical craniotomies, Giller et al. measured *MCA* diameter during fluctuations in *MAP* brought upon by nitroprusside infusion and reported a ~0.14% change in *MCA* diameter per 1 mmHg change in blood pressure (Giller et al., 1993). In the present study, the maximum *MAP* discrepancy brought upon by compression was ~7 mmHg, which would equate to a potential *MCA* diameter difference of ~1%. Presently there is limited information regarding the combined influences of $ETCO_2$ and blood pressure on *MCA* diameter, however, it is unlikely that the differences in $ETCO_2$ and *MAP* brought upon by compression had a major impact of *MCA* diameter given the study conditions.

The assessment of $ETCO_2$ in Chapter 4 only included 3 subjects. The effects of intermittent compression on $ETCO_2$ should be verified with a larger sample size. Additionally, the influence of elevated $ETCO_2$ on *MCAv* should be assessed to quantify its relative contribution to the increase in *CBF* with intermittent compression during orthostatic stress.

To determine the efficacy of intermittent compression as a countermeasure to orthostatic stress, it was chosen to investigate active changes in posture to simulate common experiences of day-to-day life. However, because compression was only applied for 2 minutes post-stand and *TCR*, we are not able to determine whether the influences of intermittent compression persist for extended periods of standing. In addition, the study only assessed the effects of compression on young, healthy individuals void of diagnosed orthostatic hypotension. A similar study should be

conducted on a patient population to verify the effectiveness of intermittent compression in conditions in which cerebral perfusion may be significantly compromised.

5.4 Thesis Conclusions

In conclusion, intermittent compression of the lower legs timed to the local diastolic phase of each cardiac cycle sufficiently attenuated decreases in both blood pressure and cerebral blood flow velocity during orthostatic stress. This research provides merit for the use of intermittent compression as a therapeutic tool for individuals vulnerable to the symptoms of orthostatic stress.

CHAPTER 6: REFERENCES

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APPENDICES

Appendix A *– Consent Form*

UNIVERSITY OF Waterloo

CONSENT OF PARTICIPANT

Study Title:
Hemodynamic effects of active compression as a countermeasure to orthostatic stress

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I have read the information presented in the information letter about the procedures and risks involved in this study. I have had the opportunity to ask any questions related to the study and have received satisfactory answers. I am aware that I may withdraw from the study without penalty at anytime by making the researchers aware of this decision. If I have any further questions about participation in this study I know that I may contact Richard Hughson, PhD, by phone at 519-888-4567, ext. 32516, or by e-mail at hughson@uwaterloo.ca.

This project has been reviewed and received ethics clearance through a University of Waterloo Research Ethics Committee (ORE# 21433). I was informed that I may contact the Director, Maureen Nummelin, PhD, at 519-888-4567, ext. 36005, or by e-mail at maureen.nummelin@uwaterloo.ca with any comments or concerns about my participation in this study.

With full knowledge I agree, on my own free will, to be a participant in the research project identified above. I am aware that by signing the consent form, I am not waiving my legal rights or releasing the investigator(s) or involved institution(s) from their legal and professional responsibilities.

Participant (print name)	Participant (signature)
Witness (print name)	Witness (signature)
Date	Location

Appendix B *– Health Status Form*

HEALTH STATUS FORM

Study: Hemodynamic effects of active compression as a countermeasure to orthostatic stress

Study ID# (to be filled out by researcher): _____

SELF REPORT CHECK LIST

Health Problems

Rheumatic Fever	( )	Bleeding disorders	( )
Diabetes	( )	Kidney and liver disease	( )
Heart Murmur	( )	Deep Vein Thrombosis	( )
High Blood Pressure	( )	Pulmonary Embolism	( )
Raynaud's disease	( )	Varicose Veins	( )
Congenital Heart Disease	( )	Disease of Arteries	( )
Heart Attack	( )	Peripheral vascular disease	( )
Heart Operation	( )	Back, Knee, Ankle Injuries	( )

Drug reactions, food allergies and allergies and sensitivities to gels or adhesives (specify):_____

_____

_____

List medications or vitamin supplements taken in last 3 months:

1._____ 3._____

2._____ 4._____

For females: Pregnant Nursing

List of symptoms

Irregular Heart Beat	( )	Fatigue	( )
Chest Pain	( )	Cough Up Blood	( )
Short of Breath	( )	Back Pain/Injury	( )
Persistent Cough	( )	Leg Pain-Injury	( )
Dizziness	( )		

Habits: Smoking: Never ( ) Ex-smoker ( ) Regular ( ) Average # cigarettes/day ( )

Exercise: Never ( ) Irregular ( ) Regular ( ) Specify:

_____

Researchers names: Travis Gibbons, Kathryn Zuj, PhD and/or Chekema Prince, PhD

Signature of Researcher:_____

Date:_____

The current study has been identified as requiring medical clearance: Yes ( ) No (X)

Those with any current medical problem related to cardiovascular disease, kidney disease, chronic inflammatory disease, diabetes, neurological disorders, deep vein thrombosis, pulmonary embolism, or skin sensitivity (i.e., psoriasis), peripheral vascular disease, or Raynaud's disease will be excluded from participation in this study. Additionally, women who are pregnant will also be excluded from the study.

Appendix C *– PAR-Q & YOU*

Physical Activity Readiness Questionnaire - PAR-Q (revised 2002)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check YES or NO.

YES	NO		
☐	☐	1.	**Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?**
☐	☐	2.	**Do you feel pain in your chest when you do physical activity?**
☐	☐	3.	**In the past month, have you had chest pain when you were not doing physical activity?**
☐	☐	4.	**Do you lose your balance because of dizziness or do you ever lose consciousness?**
☐	☐	5.	**Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?**
☐	☐	6.	**Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?**
☐	☐	7.	**Do you know of any other reason why you should not do physical activity?**

If you answered YES to one or more questions

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

- You may be able to do any activity you want — as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
- Find out which community programs are safe and helpful for you.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active – begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever – wait until you feel better; or
- if you are or may be pregnant – talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

No changes permitted. You are encouraged to photocopy the PAR-Q but only if you use the entire form.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

NAME _____

SIGNATURE _____ DATE _____

SIGNATURE OF PARENT or GUARDIAN (for participants under the age of majority) _____ WITNESS _____

Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

CSEP | SCPE

Appendix D *– Information Letter*

INFORMATION LETTER

You have been invited to take part in a research study. This letter will outline the purpose of the project, describe the procedures that are required, tell you about potential risks and benefits to yourself, and discuss your rights and confidentiality issues. If you wish to participate, you will be asked to sign a consent form at the time of the study. Feel free to ask any questions you might have at any time.

Study Title:

Hemodynamic effects of active compression as a countermeasure to orthostatic stress

Researchers and Contact Information:

[1]**Richard Hughson, PhD**	phone: 519-888-4567 ext 32516	e-mail: hughson@uwaterloo.ca
[2]**Sean Peterson, PhD**	phone: 519-888-4567 ext 38722	e-mail: peterson@uwaterloo.ca

Student Investigator

[1]**Travis Gibbons**	phone: 226-606-0463	e-mail:t3gibbon@uwaterloo.ca

Collaborators

[1]**Kathryn Zuj, PhD**	phone: 519-888-4567 ext 38073	e-mail: kazuj@uwaterloo.ca
[2]**Chekema Prince, PhD**	phone: 519-888-4567 ext 38722	e-mail:cprince@uwaterloo.ca

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What is the purpose of this study?

This research is designed to determine the effect of compression of the lower legs during orthostatic stress (stress relating to an upright posture). Understanding the effects of compression may bring new insights about improving the response to stress upon standing. Such information may be used in future studies to create new tools and strategies to improve cardiovascular responses to challenges of daily living activities as well as recreational and leisure activities in various populations such as those with orthostatic hypotension, cardiovascular disease, peripheral vascular disease, workers with regular sustained periods of inactivity, athletes, and astronauts retuning from long-duration space flight. This research project was designed by Travis Gibbons and is part of his Master's thesis.

What will I be asked to do? How much time will it take?

As a participant, you will be asked to attend two laboratory sessions. On the first visit you will be asked to complete the consent form, health status form and PAR-Q questionnaire that will be reviewed by researcher Travis Gibbons. The PAR-Q questionnaire can be answered with simple 'yes' or 'no' answers and is used to get an idea of any risks you may be susceptible to if you become more physically active. Your height and weight will then be measured and you will be familiarized with the protocols and procedures to be used in this study. You may ask as many questions as you would like to clarify the information presented. Also on the first day you will be asked to complete three squat-stand tests and three thigh-cuff release tests while data is continuously recorded. Heart rate, blood pressure, cardiac output, artery and vein blood flow, muscle activity, exhaled carbon dioxide and brain blood flow will be continuously measured during the tests. The total time commitment will be 1.5 hours. All of these measurements used are non-invasive and all procedures will be conducted by trained researchers.

Approximately three to four weeks later, you will return to the lab for a secondary testing session. During this session, you will be asked to complete the same squat-stand and thigh-cuff release tests with the addition of external lower-leg compression applied immediately after the onset of standing and once the thigh-cuff pressure has been completely released. The squat-stand test and thigh-cuff release tests will be repeated four times each, with external lower-leg compression occurring on half of the trials. The total time commitment will be 2 hours.

Am I eligible?

This study focuses on 18-40 years old healthy men and women. You will be asked to complete a health status form to determine eligibility for the study. This one-page form will ask important information about your health, including past and current medical conditions and current medications. Individuals with any current medical problem related to cardiovascular disease, kidney disease, chronic inflammatory disease, diabetes, neurological disorders, deep vein thrombosis, pulmonary embolism, Raynaud's disease, allergies and/or sensitivities to gels or skin sensitivity conditions (i.e., psoriasis) will be excluded from participation in this study. Additionally, pregnant women and individuals with present injuries or chronic conditions that prevent them from completing a 3-minute squat will also be excluded from the study.

What are the procedures? Will there be any risk involved?

Upon your first visit to the laboratory, your height and weight will be measured and you will fill out the Physical Activity Readiness Questionnaire (PAR-Q) and the medical screening form. We will ask you complete three repetitions of a squat-stand test, which consists of a 3-minute squat followed by a quick transition into the standing position. Additionally, you will complete three thigh-cuff release tests, consisting of the application of external compression around each of your upper legs and the rapid release of pressure to induce a temporary state of low blood pressure. Prior to both the squat-stand and thigh-cuff release tests, we will familiarize you with the protocol to ensure you are comfortable with both of the procedures. Continuous data collection will occur throughout each of these tests. On the second day of testing you will be asked to wear a series of cuffs around your calves which will supply external compression. You will be asked to perform four squat-stand tests and four thigh-cuff release tests with and without external compression of your lower leg. At the onset of standing and thigh-cuff release, there is a risk that you may feel faint, dizzy or light-headed. For this reason, there will be mats placed on each of your sides to protect you in the case of a fall. In the rare circumstance that you begin to fall, there will be two spotters to assist you slowly to the matted ground to ensure a graceful transition into the lying down position. Also, there is a chance that the thigh-cuffs may irritate and/or pinch the skin when being pressurized. In this event we will rapidly release the pressure and re-apply the thigh cuffs in a more comfortable position. Heartrate, blood pressure, cardiac output, blood flow and exhaled carbon dioxide will be measured throughout the testing. All of these measurements are non-invasive and all procedures will be conducted by trained researchers. The non-disposable equipment will be sanitized between uses by alcohol wipes.

Squat-Stand Test

The squat-stand test consists of a deep 3-minute squat followed by a rapid transition into the upright, standing position. All squats will occur with your back being supported by a wall in order to maintain an upright position in your upper body. Upon instruction from the researcher, you will move into the standing position. You will remain standing for 2 minutes while researchers collect data.

Thigh-Cuff Release Test

The thigh-cuff release test consists of the application of two thigh blood pressure cuffs to the upper legs. While in the standing position, the cuffs will be rapidly inflated to 30 mmHg above your systolic blood pressure to prevent blood from flowing into your legs. After 3 minutes of lower-leg blood occlusion, the thigh cuffs will be rapidly depressurized allowing blood to flow into your legs. You will remain standing for an additional two minutes.

Compression system

On the second day of testing, you will be asked to wear a series of cuffs around your lower legs which will apply compression to your calves. Five cuffs (~6cm wide) will be wrapped around your lower legs. The cuffs will activate sequentially from ankle to knee applying a pressure less

than 100mmHg with all cuffs applying pressure and relaxing in less than one second. The pressure applied to your leg will not prevent blood flow to your leg and there are no risks associated with intermittent pressures of this level. With the cuff system activated, it will feel similar to having your leg massaged. ***Heart rate measurements***

Heart rate will be continuously monitored beat by beat by an electrocardiogram (ECG) through 3 spot electrodes on the skin surface. The disposable electrodes are normally placed in the upper and lower portions of the chest (two on the left side and one on the right side). The ECG electrodes will be placed by a researcher of the same sex. **In a very small group of individuals, a skin rash might occur due to 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 the area clean.

Blood pressure measurements

Arterial blood pressure will be measured by a small cuff placed on the middle finger and from a cuff placed around your upper arm. This device applies light pressure around the finger in order to determine the pressure in these arteries. Any discomfort should be minimal with this measurement system. **In case of discomfort, please notify the researcher immediately.**

Cardiac output measurements

Cardiac output is a measure of the amount of blood emitted from the heart per minute. It is continually estimated by the blood pressure recording device using a blood pressure wave tracing. The blood pressure wave form is acquired using the same finger cuff device used to measure arterial blood pressure. A second cuff around the upper arm is rapidly inflated, altering the blood pressure wave in a way that allows for the estimation of stroke volume. With the subject's height, weight, age and sex, the blood pressure device can use the blood pressure wave form to accurately estimate cardiac output from measures of stroke volume and heart rate.

Muscle activity measurements

Electromyography (EMG) will be used to monitor the electrical activity generated by active muscles in the lower leg. Disposable electrodes will be placed directly on the skin of the lower leg. **In a very small group of individuals, a skin rash might occur due to 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 the area clean.

Ultrasound measurements of blood flow

Ultrasound is a non-invasive technique that can be used to measure blood flow in arteries and veins. This method will be used to determine blood flow in the popliteal artery and vein (located behind your knee) and the superficial femoral artery (front inside of your thigh, half way between your hip and knee). Using this method, a probe will be placed over the skin with the ultrasound

directed toward the blood vessel and held in place by the researcher with the assistance of an elastic bandage. Ultrasound will also be used to measure brain blood flow where a small probe will be held by your right ear using a head band. For blood flow in the leg and the brain, the probe will be always adjusted to the minimum power level necessary to obtain a clear signal. At this level you should not notice any sensation. This method is widely used. **However, in the unlikely event that you should feel a sharp pain or burning, please inform the experimenter immediately.** In the very unlikely case that a skin burn should occur, it should be treated as any other burn by application of cold compress, first aid cream and sterile covering. The procedure involves the use of water soluble ultrasound gel. It can be removed simply by applying water and wiping the skin and/or hair with a paper towel or a cloth.

The arteries and veins of the leg will also be imaged by the use of echo Doppler ultrasound. This technique is entirely non-invasive and is similar to what is used in hospitals to investigate the heart and blood vessels or to look at a baby during pregnancy. The probe will be held against the skin in the back and front of the leg to image the diameter of the arteries and veins as well as obtain blood velocity tracings. Ultrasound monitoring requires the use of water-soluble, hypoallergenic gel between the probe and the surface of the skin. Ultrasound procedures will be conducted by a researcher delegated to do so by a physician under the Delegation of a Controlled Act.

Are there any special instructions I should follow?

We will request that you refrain from consuming alcohol, caffeinated beverages, and from engaging in vigorous exercise (that is, exercise where you are breathing so hard that you would be unable to carry on a conversation) **24 hours** prior to testing. We also request that you do not eat a large meal **within 2 hours** of testing. You should wear comfortable exercise clothing such as shorts, t-shirt, socks, and sneakers.

Will I benefit from this study?

There are no direct benefits to participation in the study. You will gain more knowledge about the effects of passive and active compression on cardiovascular hemodynamic responses, the research process, and appreciate the types of changes that can happen during these procedures. It is important to understand if compression can affect physiological responses to orthostatic stress by improving the return of blood back to the heart and increasing the delivery of blood to the brain. This study may have implications for future studies with different populations such as increasing performance by athletes, decreasing fatigue for workers that spend a lot of time standing or walking (soldiers, firefighters, police officers, delivery workers) and improving circulation for older people, people with cardiovascular diseases, or peripheral vascular disease, as well as astronauts returning from long-duration space flight. **If requested, you will be provided a feedback letter and a summary of the research findings at the completion of study.**

Will I be rewarded for volunteering my time?

There will be no remuneration for this study.

Can I withdraw from the study?

Your participation in this study will be voluntary. **You may withdraw from the study without penalty or any consequences** by making the researchers aware of your decision. For publication and presentation purposes, data collected from this study will be presented as averages of participants who completed the study. If for any reason you would like your data removed from the group average, please inform the researchers as soon as possible. In this situation, your data will be removed from the pooled averages and deleted. All data collection hard copies and linking documents will be shredded and discarded to ensure your anonymity will be preserved. Once the research has been released to publishing you will be unable to remove your data from the pooled averages. However, all documents connecting you to the data can be destroyed to ensure your identity cannot be linked to the published data.

How confidential and secure is my personal information?

Each participant will be identified by a special identification code known by the main investigator and the research assistants. After all of the identifying information has been removed, the data will be kept for 25-years in an encrypted format in a password-protected secure location, locked in the Cardiorespiratory and Vascular Dynamics Laboratory in BMH 2421 at the University of Waterloo. The results will be used for publications in conferences, papers, and reports and will be primarily presented as group averages. For all data presented, participants' names, study identification codes, and any other type of identifying information will be removed to maintain confidentiality.

Has this study received ethics clearance?

This project has been reviewed and received ethics clearance through a University of Waterloo Research Ethics Committee (ORE# 21433). You may contact the Director at the University, Maureen Nummelin, PhD, by phone at 519-888-4567, ext 36005, or by email at maureen.nummelin@uwaterloo.ca with any comments or concerns about your participation in this study.

We would like to remind you that if you have questions, you can contact us at any time. Our contact info is on the first page of this letter. Thank you for considering our study.