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An examination of vascular responses to acute isometric handgrip exercise and a complementary ischemic-reperfusion cuff protocol

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Abstract

Isometric handgrip (IHG) training lowers resting arterial blood pressure (BP) in older, normotensive adults and it has been proposed that repeated exposure to oxidative stress, via IHG-induced ischemia-reperfusion (IR), could be an underlying mechanism. The objectives were to quantify the IHG neurovascular stimulus, determine if this IHG protocol elicits systemic vascular effects, and compare the response of the IHG stimulus to an IR cuff protocol. Vascular reactivity, assessed via flow-mediated dilation (FMD) and strain-gauge plethysmography (RVF), was determined prior-to and immediately-post an IHG bout in the exercised arm, non-exercised arm, and after IR in the reperfused arm. No significant differences were found in FMD or RVF after each of the three bouts. These findings suggest that vascular reactivity remains unchanged in either limb, both in response to an acute IHG bout and a complementary IR cuff protocol, indicating oxidative stress may not be a part of the acute IHG stimulus.

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ACE	Angiotensin-converting enzyme
ACh	Acetylcholine
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
ANP	Atrial natriuretic peptide
ANS	Autonomic nervous system
ATP	Adenosine triphosphate
AUC	Area under curve
AVP	Arginine vasopressin
BA	Brachial artery
BP	Blood pressure
BPM	Beats per minute
BH_4	Tetrahydrobiopterin
Ca^{2+}	Calcium
cAMP	Cyclic adenosine monophosphate
CC	Central command
CCC	Cardiovascular control centre
cGMP	Cyclic guanine 3,5 monophosphate
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
E	Epinephrine
ECG	Electrocardiogram
eNOS	Endothelial nitric oxide synthase
ET-1	Endothelin-1
FMD	Flow-mediated dilation
GPx	Glutathione peroxidase
H^+	Hydrogen
H_2O_2	Hydrogen peroxide
HT	Hypertension
HR	Heart rate
IHG	Isometric handgrip
IR	Ischemia-reperfusion
K^+	Potassium
MBF	Mean blood flow
MLC	Myosin light chain
mmHg	Millimeters of mercury
MVC	Maximum voluntary contraction
Na^+	Sodium
NADPH	Nicotinamide adenine dinucleotide phosphate

NE	Norepinephrine
$N\dot{O}$	Nitric oxide
$\dot{O}_2$	Superoxide radical
$O\dot{H}$	Hydroxyl radical
$ONOO^-$	Peroxynitrite
$PaCO_2$	Partial pressure of carbon dioxide
PEH	Post-exercise hypotension
PKA	Protein kinase A
PNS	Parasympathetic nervous system
RAAS	Renin-angiotensin-aldosterone system
ROS	Reactive oxygen species
RVF	Resistance vessel function
SBP	Systolic blood pressure
SD	Standard deviation
SNS	Sympathetic nervous system
SOD	Superoxide dismutase
SR	Shear rate
SV	Stroke volume
TPR	Total peripheral resistance
Q	Cardiac output

Chapter 1: Introduction & Literature Review

1.1 Hypertension

1.1.1 Introduction

Cardiovascular disease (CVD) is recognized as the leading cause of death worldwide (WHO, 2013). CVD is characterized by disorders of the heart and blood vessels and includes coronary heart disease, cerebrovascular disease, elevated blood pressure, peripheral arterial disease, rheumatic heart disease, congenital heart disease and heart failure (WHO, 2013). Empirically, it is estimated that CVD was responsible for 17.3 million deaths worldwide in 2008 (WHO, 2013). Further, it is projected that this rate will grow to almost 23.6 million deaths per year by 2030 (WHO, 2013). In Canada, CVD is a leading cause of mortality in both males and females (Statistics Canada, 2009). Economically, total costs relating to CVD in Canada were estimated to be $20.9 billion in 2005, with projections to $28.3 billion by 2020 (Theriault et al., 2010). Given the widespread prevalence and projected increases, coupled with the economic burden of this disease, the prevention and control of CVD is very important.

Although CVD is the leading cause of mortality worldwide (WHO, 2013) it is considered a preventable disease through the modulation of a number of behavioural risk factors. These behavioural risk factors include diet quality, physical activity status, stress, tobacco use, alcohol consumption, body composition, blood pressure, and blood cholesterol (PHAC, 2009). It has been estimated that these behavioural risk factors are responsible for approximately 80% of CVD (WHO, 2013). Additionally, there are non-modifiable risk factors, including age, gender, family history, ethnicity, and medical history that also contribute to the development of CVD (PHAC, 2009). While the

relative contributions of each of these factors is difficult to quantify, it appears that hypertension (HT), or chronically sustained elevations of systolic and/or diastolic arterial blood pressure (BP), accounts for approximately 7.5 million deaths worldwide each year and is a leading modifiable risk factor for CVD (WHO, 2013).

1.1.2 Arterial Blood Pressure Regulation

Prior to discussing the pathophysiology of HT, it is important to understand basic arterial BP control in a healthy physiological state. Efficient function of the cardiovascular system requires the maintenance of adequate arterial blood pressure (BP) during the cardiac excitation-relaxation cycle to allow for adequate perfusion of bodily tissues (Herd, 1970). Arterial BP can be sub-divided into systolic (SBP) and diastolic (DBP) components, which represent the different phases of cardiac contraction. SBP occurs during the period of cardiac contraction and represents the pressure that the blood exerts on the artery walls during ventricular systole (Herd, 1970). In contrast, DBP occurs during the period between cardiac contractions and represents the pressure exerted by the blood on vessel walls during cardiac relaxation and gives an indication of peripheral resistance (Herd, 1970). In individuals with normal resting arterial BP, measurements of pressure during systole average <120 mmHg, while measurements during diastole average 80 mmHg (Chobanian et al., 2003).

Arterial BP is derived from the product of cardiac output (Q) and total peripheral resistance (TPR). Q is the product of the rate of contraction of the heart (heart rate; HR) and the volume of blood pumped per contraction (stroke volume; SV). TPR is the sum of the resistance to blood flow provided by the peripheral vasculature within the entire systemic circulation (Washburn, 1921). TPR is greatly influenced by changes in the

radius of the peripheral vasculature through vasoconstriction or vasodilation. As such, modulation of BP may occur through changes in Q, via HR and/or SV, and/or through changes of the TPR within the system. These pressures are tightly controlled by a number of innate pathways including neural, hormonal, vascular, and genetic BP control mechanisms.

Innate Blood Pressure Control Mechanisms

Regulation of arterial BP is controlled through the coordinated efforts of neural, hormonal, vascular, and genetic control mechanisms. These mechanisms function via modulation of Q via HR and/or SV, as well as TPR, through alterations in vasodilation and vasoconstriction of the vascular smooth muscle.

Neural Blood Pressure Control

Neurological control of BP functions predominantly through the integration of the sympathetic (SNS) and parasympathetic nervous systems (PNS) within the autonomic nervous system (ANS). In brief, sympathetic activation augments resistance to blood flow, which raises arterial BP via increased TPR (Dampney et al., 2002), and also acts to increase HR and force of myocardial contraction, which lead to increases in both Q and BP (Dampney et al., 2002). In opposing fashion to the SNS, PNS stimulation leads to a significant decrease in HR and a small decrease in cardiac contractility (Dampney et al., 2002). The net neural effect on the cardiovascular system is dependent on the balance of stimulation between these two systems.

Coordination of the SNS and PNS is controlled by a brain region known as central command (CC), which sends information to the cardiovascular control center (CCC)

(Dampney et al., 2002; Eldridge et al., 1985). The CCC can be classified as an integration center as it receives both ascending and descending information when coordinating a response. CC is able to modulate the balance of the ANS through innervation of the CCC in response to the added stress of exercise (Victor et al., 1995). Additional sensory information is provided through signals received from arterial baroreceptors, chemoreceptors, and muscle afferent receptors.

Arterial baroreceptors contribute to the regulation of arterial BP through afferent feedback to the CCC (Lafranchi & Somers, 2002). These receptors, located in the heart, pulmonary vessels, and throughout the arterial walls of the carotid artery, indirectly monitor changes in arterial BP. As baroreceptors do not sense pressure, but the conformational change in the vessel wall caused by pressure changes, they are classified as stretch receptors (Angell-James, 1971). Afferent feedback allows the CCC to modulate the relative contributions of the SNS and PNS to maintain arterial BP around a homeostatic set point (Lafranchi & Somers, 2002). An increase in arterial BP triggers an increase in the frequency of impulses sent from the baroreceptors to the CCC resulting in increased PNS and decreased SNS activation (Lafranchi & Somers, 2002). Likewise, a decrease in arterial BP triggers a decrease in the frequency of impulse generation from the baroreceptors to the CCC resulting in increased SNS activation (Lafranchi & Somers, 2002).

The composition of metabolites within the circulating blood serves as a further stimulus to arterial BP regulation mechanisms. Chemoreceptors, located within the carotid and aortic bodies, sense changes in the partial pressure of oxygen (PaO_2) and carbon dioxide ($PaCO_2$), as well as hydrogen ion (H^+) concentrations, within the arterial

blood (Prabhakar, 2000). These receptors send afferent feedback to the CCC to induce SNS activation in response to reductions in PaO_2, increases in H^+ concentration, and increases in $PaCO_2$ (Prabhakar, 2000; Dampney et al., 2002). The resulting response consists of vasoconstriction of the vascular beds, increasing TPR, coupled with increases in ventilation in an effort to increase arterial BP.

Muscular afferent receptors function to regulate arterial BP in similar manner to the receptors described above. Type III afferent receptors, or mechanoreceptors, sense stretch and deformation due to pressure, whereas type IV afferents, or metaboreceptors, sense changes in the muscular milieu (Leshnower et al., 2001). More specifically, type IV receptors are stimulated by chemicals and metabolic by-products of muscle contraction such as lactic acid, dipronated phosphate, potassium (K^+), bradykinin, serotonin, and adenosine (Leshnower et al., 2001). Information provided by these receptors is processed in the CCC and the response is an alteration of the balance of the SNS and PNS within the ANS, leading to changes in HR and arterial BP (Leshnower et al., 2001; Pawelczyk et al., 1997).

Hormonal Blood Pressure Control

Certain hormones of the human body have a profound impact on the regulation of an individual's arterial BP. The most influential BP modulating hormones include the catecholamines epinephrine (E) and norepinephrine (NE), acetylcholine (ACh), the renin-angiotensin-aldosterone system (RAAS), arginine vasopressin (AVP), and atrial natriuretic peptide (ANP). The coordinated efforts of these hormones, along with

vascular, neural, and genetic mechanisms, function to defend homeostatic arterial BP levels.

The sympathetic nervous system is the distributor of the catecholamines E and NE, which elicit various cardiovascular responses based on which receptor they bind to and its location within the body. SNS nerve fibers form a synaptic junction with vascular beds within a close proximity to effectively distribute these catecholamines. When the SNS is stimulated it triggers the release of E and NE from the adrenal glands (Mannelli et al., 1990). E is released directly into the systemic circulation, whereas NE is released predominantly at the synaptic junction between the SNS nerve fiber and the vascular beds, though a small amount does also reach systemic circulation (Mannelli et al., 1990). E and NE regulate arterial BP through modulating both Q and TPR depending on specific receptor binding (Mannelli et al., 1990).

There are two main types of receptors bound by E and NE, the α-adrenergic receptors and the β-adrenergic receptors (Ruffolo et al., 1991). These are further sub-divided based on location and mechanism of action. α-adrenergic receptors are divided into α1-receptors, located within most sympathetic target organs, except the heart, and α2-receptors, located within the membranes of the NE nerve endings within the synaptic junction (Mannelli et al., 1990). β-adrenergic receptors are divided into β1-receptors, located within the heart, kidneys, liver, and adipose tissues, and β2-receptors, located within most sympathetic target organs (Mannelli et al., 1990). E's binding to the α-adrenergic receptors elicits a vasoconstrictory response, whereas it elicits a vasodilatory response when bound to β-adrenergic receptors (Mannelli et al., 1990). NE binds to post-

synaptic α-adrenergic receptors within the synaptic junction to elicit a vasoconstrictory response in the target vascular beds (Ruffolo et al., 1991). Modification of the state of smooth muscle contraction in the target vasculature regulates arterial BP through changes in TPR.

The catecholamines also influence Q through changes in HR. This pathway is mediated through second messenger cellular cascades initiated by cyclic adenosine monophosphate (cAMP) (Opie, 2004). cAMP is produced through the stimulation of β-adrenergic receptors by E within the cardiac cells, which then initiates cAMP-dependent protein kinase A (PKA) (Krammer, 1988). PKA increases HR by accelerating the rate of impulse generation at the sino-atrial (SA) node, as well as increasing diastolic filling time, which increases arterial BP (Larsson, 2010).

The PNS can regulate arterial BP through distribution of ACh, a neurotransmitter released from PNS nerve fibers (Dale et al., 1936). ACh regulates arterial BP through binding to muscarinic receptors within the cardiac cell membrane and inhibiting adenyl cyclase, which then inhibits elevated intracellular cAMP concentrations (Brodde & Michel, 1999). A decrease in intracellular cAMP reduces calcium channel phosphorylation, reducing the number of open calcium channels and inhibiting calcium influx (Brodde & Michel, 1999). As sarcoplasmic reticulum (SR) calcium release is dependent upon the influx of extracellular calcium, a process termed calcium-induced calcium release (CICR), and SR calcium release provokes cardiac contraction, a reduced influx depresses cardiac contractility, and therefore Q, leading to reduced arterial BP (Fabiato, 1983).

ACh can also reduce arterial BP through decreases in HR. The release of ACh can hyperpolarize the SA node, which increases the time required to reach the threshold potential for excitation (Brodde & Michel, 1999). As such, there is a greater amount of time between cardiac contractions, lowering HR, therefore lowering arterial BP.

The most important long-term arterial BP regulation system involves the integration of the kidneys and adrenal glands, as well as a number of hormones and enzymes, within the RAAS. The efficiency of this system stems from the effects it exerts on both total blood fluid volume and vascular tone. The RAAS is initiated when renin, a glycoprotein enzyme, is released via exocytosis from the kidneys (Vander, 1967). Renin is then able to facilitate the conversion of angiotensinogen, a pro-hormone secreted by the liver, to angiotensin I (Nguyen et al., 2002). Angiotensin I is considered an inactive form of this protein and is further modified by the angiotensin-converting enzyme (ACE) into angiotensin II, considered an active form (Donoghue et al., 2000). Angiotensin II is a potent vasoconstrictor and, as such, will have a direct effect on arterial BP through increases in TPR. Angiotensin II also triggers the release of aldosterone from the adrenal cortex (Carey & Siragy, 2003). Aldosterone is a hormone that promotes fluid retention through the conservation of sodium, which increases total blood fluid volume and, therefore, arterial BP (Leutscher & Johnson, 1954).

An increase in arterial BP can induce stretching of the arterial walls within the heart. Cardiac atrial cells release ANP in response to this stretch in an effort to reduce arterial BP. ANP can decrease arterial BP via two different mechanisms. First, ANP prevents vascular vasoconstriction through the inhibition of actin-myosin binding, which dilates smooth muscle and reduces TPR (Potter et al., 2009). Second, by promoting

diuresis and sodium excretion, ANP reduces total blood fluid volume, which can also decrease arterial BP (deBold et al., 1981).

AVP, also referred to as anti-diuretic hormone, is released from the pituitary gland in response to a decrease in arterial BP (Henderson & Byron, 2007). AVP increases arterial BP via two mechanisms, which are similar, yet opposite, to that of ANP. AVP can induce vasoconstriction of smooth muscle, increasing TPR and therefore BP (Holmes et al., 2004). Further, AVP also influences fluid balance regulation. Through binding to the adenylyl cyclase-coupled vasopressin receptor in the kidney, AVP reduces urinary water excretion by promoting water reabsorption through the collecting duct (Holmes et al., 2003; Nielsen et al., 1995). Increased water reabsorption elevates blood fluid volume, which increases arterial BP.

Local Blood Pressure Control

Arterial BP can also be regulated by a number of locally released vasoactive substances, which are formed in response to changes in the metabolic needs of the body's organs and tissues (Haddy & Scott, 1968). Their primary mechanism of action is modulating the diameter of smooth muscle, through vasoconstriction and vasodilation, modifying TPR and effectively altering arterial BP. The most common of these substances include potassium (K^+), adenine derivatives, and endothelium-derived substances such as nitric oxide ($N\dot{O}$) and endothelin-1 (ET-1).

K^+ release into the interstitial space around arterioles is a byproduct of muscular depolarization, and thus increased muscular activity can lead to an accumulation of interstitial K^+ (Haddy et al., 2006). An increase in the concentration of K^+ stimulates the

sodium-potassium (Na^+/K^+) pump within the membrane of the smooth muscle (Haddy et al., 2006). As the Na^+/K^+ pump is electrogenic its increased stimulation results in hyperpolarization of the smooth muscle cells (Anderson, 1976; Haddy et al., 2006). Hyperpolarization of these cells increases the both the stimulus strength and time required for depolarization, effectively reducing the rate and magnitude of Ca^{2+} influx. The influx of extracellular Ca^{2+} is the main mechanism responsible for smooth muscle vasoconstriction (Karaki et al., 1997). As such, decreased Ca^{2+} influx reduces smooth muscle vasoconstriction allowing for increased tissue blood flow, reduced TPR, and decreased arterial BP. Increased tissue blood flow provides added blood supply to meet the increased metabolic demands of the working muscle tissue.

Derivatives of adenine, including adenosine triphosphate (ATP) and the byproducts of its catabolism, adenosine diphosphate (ADP), adenosine monophosphate (AMP), and adenosine itself (depending on the location of action) are considered vasodilatory substances (Haddy & Scott, 1968). ATP is a key substrate involved in muscular contraction. As such, a decrease in the concentration of ATP, and an increase in the concentration of the byproducts of its catabolism, is an indication of increased muscle metabolism. An increase in these metabolites induces a vasodilatory response in the vascular beds supplying the working tissues to meet the increased metabolic demand (Haddy & Scott, 1968).

The most potent vasoactive substance, $N\dot{O}$, also evokes a vasodilatory smooth muscle response (Steinburg et al., 1994). The increased metabolic demands of working muscle during exercise are met through increased blood flow to these tissues. Elevations in the rate and magnitude of blood flow increase shear stress, the force of the flowing

blood against the blood vessel walls, on the vascular endothelium lining the blood vessels supplying the muscles. Shear stress of the vascular walls is a potent stimulator of NȮ production (Corson et al., 1996). Likewise, chemical stimuli (e.g. ACh and bradykinin) are also stimulators of endothelial NȮ production (Fleming & Busse, 2003). NȮ is synthesized from the amino acid L-arginine via the NȮ synthase enzyme (eNOS) and diffuses out of the vascular endothelium (Moncada & Higgs, 1993). Once diffused, NȮ interacts with the vascular smooth muscle cells activating guanylyl cyclase and causing an accumulation of cyclic guanine 3,5 monophosphate (cGMP) (Umans & Levi, 1995). Accumulation of cGMP triggers the activation of cGMP-dependent protein kinase, which increases myosin light chain (MLC) phosphatase activity (Umans & Levi, 1995; Webb, 2003). Increased MLC phosphatase activity inhibits smooth muscle contraction via inhibition of the binding of actin and myosin (Umans & Levi, 1995; Webb, 2003). As such, the response is smooth muscle vasodilation, increased blood flow, and decreased TPR and arterial BP.

Vasodilatory substances such as K^+, adenosine derivatives, and NȮ are counter-balanced by locally released vasoconstrictive substances. ET-1 is released by the endothelium in response to chemical (angiotensin II, catecholamine, cytokine, hypoxic, growth factor) or mechanical (shear stress) stimulation (Touyz & Shiffrin, 2003). ET-1 induces vasoconstriction when bound to the ETa receptor through the initiation of a signaling cascade leading to the release of sequestered Ca^{2+} and the opening of Ca^{2+} membrane channels (Pollock et al., 1995). As intracellular Ca^{2+} determines the contractile state of smooth muscle, an increase in intracellular Ca^{2+} can induce vasoconstriction though increased smooth muscle contraction (Karaki et al., 1997).

Increased vasoconstriction increases TPR and, therefore, arterial BP. ET-1 can also regulate the contractile state of the smooth muscle via a negative feedback loop. When ET-1 binds to ETb receptors it stimulates the release of NȮ, the potent vasodilator described above (Pollock et al., 1995).

1.1.3 Measurement of Blood Pressure

The desire to measure the pressure of blood in the human body has led to the evolution of a number of measurement techniques. On the grandest scale these techniques can be divided into invasive or non-invasive measures. As one might surmise, invasive measures of arterial BP involve the insertion of a catheter into the artery to allow for direct, continuous recording of arterial BP (Perloff et al., 1993). Although this is considered the gold standard of arterial BP measurement the nature of this technique makes it impractical for use in clinical settings (Perloff et al., 1993). A number of non-invasive techniques, such as auscultatory sphygmomanometry, automated oscillometry, ultrasonography, the finger cuff method of Penaz, and applanation tonometry have been developed for non-invasive arterial BP measurement.

Sphygmomanometry and Auscultation

Measurement of arterial BP using sphygmomanometry and auscultation is considered the gold standard of non-invasive arterial BP measurement in clinical practice. This method involves placement of an inflatable cuff around the arm, above the elbow, to be used for brachial artery occlusion. This cuff is manually inflated to a pressure above SBP to fully occlude the artery in preparation for auscultation (Beevers et al., 2001). During brachial artery auscultation the pressure of the cuff is manually released at a

gradual rate of approximately 2-3 mmHg per second (Beevers et al., 2001). As the pressure of the cuff is released the observer will note a number of Korotkoff sounds. The first Korotkoff sound occurs when the pressure in the cuff drops below systolic arterial pressure and blood begins to flow through the artery (Korotkoff, 1905). The cuff pressure at this first Korotkoff sound is representative of SBP (Korotkoff, 1905). As the pressure of the cuff is continually decreased the observer will hear a series of 4 more Korotkoff sounds with the last one followed by a state of silence, which signifies DBP has been passed (Korotkoff, 1905).

Though this method of arterial BP measurement is considered the non-invasive gold standard there are notable limitations and disadvantages. The link between the pressure measurement of the cuff and the instance of the various Korotkoff sounds is the observer taking the measurement. As such, measurements may be influenced by terminal digit preference, observer prejudice, and/or observer bias (Beevers et al., 2001). Additionally, the Korotkoff method has been noted to give SBP and DBP values that are lower than direct intra-arterial measures (Holland & Humerfelt, 1964). These variances have been reported to be as great as 25 mmHg in certain cases (Breit & O'Rourke, 1974). Lastly, the auscultatory gap, which is a disappearance of the Korotkoff sounds between SBP and DBP only to return as deflation continues, may affect measures using this method (Pickering et al., 2005). This phenomenon tends to occur in older individuals with wide pulse pressures, but can often be eliminated by elevating the arm overhead for 30 seconds and returning it to its normal position for measurement (Pickering et al., 2005). This technique reduces vascular volume within the arm to allow for a greater

inflow of blood and improved Korotkoff sounds during measurement (Pickering et al., 2005).

Automatic Oscillometry

The automated oscillometric technique, developed by Etienne-Jules Marey in 1876, measures oscillations in pressure within a sphygmomanometer cuff during gradual deflation (Langewouters et al., 1998). The amplitude of these oscillations first increases and then decreases during gradual deflation and the peak amplitude corresponds with mean arterial pressure (Mauck et al., 1980; Langewouters et al., 1998). As these oscillations begin well above SBP, and continue below DBP, these values can only be indirectly estimated via an empirically derived algorithm (Pickering et al., 2005; Langewouters et al., 1998). This method is advantageous because placement of a transducer is not required, allowing for greater variability in cuff placement, and it is less susceptible to external noise (Pickering et al., 2005). The most prominent disadvantage of this method is that the oscillations may be influenced by factors other than arterial BP, such as arterial stiffness (Pickering et al., 2005). Further, the algorithm used to derive SBP and DBP is determined by the manufacturer and programmed into the device (Pickering et al., 2005).

Ultrasonography

Measurement of arterial BP using ultrasonography requires a sphygmomanometer cuff, used to occlude arterial blood flow, and an ultrasound transmitter and receiver, used to detect arterial wall movement. The cuff is initially inflated above SBP to occlude arterial blood flow while the ultrasound device is placed over the occluded artery. As the

cuff is steadily deflated there is a noticeable movement of the arterial wall once the pressure of the cuff meets SBP which causes a Doppler phase shift in the reflected ultrasound (Pickering et al., 2005). Just as the onset of arterial wall movement signifies SBP the point at which this movement begins to diminish signifies DBP (Pickering et al., 2005). Determination of arterial BP using ultrasonography is particularly useful for individuals with faint Korotkoff sounds and in infants and children (Pickering et al., 2005; Elseed et al., 1973).

Penaz/Wesseling Method

This method, developed by Penaz and Wesseling, involves the placement of a small pressure cuff around the finger. Arterial size is determined, and pulsation within the finger is detected, by an infrared transmission plethysmograph located under the cuff (Langewouters et al., 1998). The changes in arterial diameter detected by the plethysmograph feeds a servo-loop, which modulates cuff pressure to hold the artery in a constant-diameter partially opened state (Pickering et al., 2005). The arterial size set-point value of the servo-system was provided by the Physiocal criteria of Wesseling (Wesseling et al., 1995). Oscillations in arterial pressure are measured via an electric gauge and recorded as an indirect measure of the intra-atrial pressure waveform (Langewouters et al., 1998; Pickering et al., 2005). Further analysis by a pattern recognition program provides both systolic and diastolic pressure data per heart beat (Langewouters et al., 1998). This method has been shown to result in measures that are lower than direct intra-arterial measures and this has been attributed to differences in the pressure gradients of the hand versus the brachial artery (Imholz et al., 1991).

Tonometry

Measurement of arterial BP using tonometry is based upon a principle that states when an artery is partially compressed or splinted against a bone, the resulting pulsations are proportional to the intra-arterial pressure changes (Pickering et al., 2005). This technique continuously measures changes in arterial pressure through the cardiac cycle to determine the pulse pressure waveform. During resting conditions the average SBP and DBP measurements determined using tonometry correspond well with intra-arterial measures (Sato et al., 1993). This method is not without its limitations, most notably the position sensitivity of the probe over the artery. As proper signal transduction requires the probe to be situated directly over the center of the artery this technique is very position dependent (Pickering et al., 2005). Further, excessive hold-down forces may skew pressure waveforms, motion artifacts may infiltrate the data output, and the calibration of the tonometer requires the use of an external arterial BP measurement device (Wang et al., 2004).

1.1.4 Pathophysiology of Hypertension

HT is a prominent risk factor for CVD, which is currently a leading cause of morbidity and mortality in Canadians of both sexes (Statistics Canada, 2009). HT in adults is defined as having a resting SBP above 139 mmHg, and/or a resting DBP above 89 mmHg (Chobanian et al., 2003), and afflicts over 4.6 million Canadians (Statistics Canada, 2009). This is in contrast to normal resting arterial BP, which as stated previously in **1.1.2 Arterial Blood Pressure Regulation** is identified as <120/80 mmHg (Chobanian et al., 2003). HT can be further subdivided into stages defined by ranges of

resting arterial BP. Stage 1 HT is defined as a resting arterial BP within the range of 140-159/90-99 mmHg, whereas stage 2 HT encompasses resting arterial BP above 160/100 mmHg (Chobanian et al., 2003). Genetic and environmental factors, along with alterations in sex hormones with increasing age, have been implicated in the development of HT (Coylewright, 2008). Although significant improvements in screening, prevention, and treatment have been made, HT remains a major health problem for Canadians.

Types of Hypertension and their Characteristics

At the most general level HT can to divided into 2 categories: primary or secondary HT. Primary HT is the most common and denotes HT of an unknown cause (Carretero & Oparil, 2000), whereas secondary HT is far less common and signifies HT due to a known pathophysiology (Chobanian et al., 2003). Other HT sub-types include isolated systolic, resistant, malignant, and white-coat HT.

Primary Hypertension

Primary (essential, idiopathic) HT is characterized by elevations in resting arterial BP due to an unknown cause (Messerli et al., 2007; Carretero & Oparil, 2000). This type of HT is the most common, constituting approximately 95% of all cases (Carretero & Oparil, 2000), and numerous lifestyle factors may accumulate to initiate this sustained rise in BP. These factors, termed hypertensinogenic, are defined as substances or situations that tends to increase resting arterial BP and include aging, obesity, insulin resistance, excessive sodium intake, excessive alcohol intake, a sedentary lifestyle, excessive stress, low potassium intake, and/or low calcium intake (Sever & Poulter, 1989; INTERSALT, 1988). Further, these factors may interact with individually

inherited genetic traits resulting in modifications of resting arterial BP, which may lead to the onset of primary HT (Carretero & Oparil, 2000).

Secondary Hypertension

Secondary HT is defined as an elevated resting arterial BP due to a recognized underlying pathophysiology of the renal system, endocrine system, vascular system, lungs, and/or central nervous system (Chobanian et al., 2003). As the underlying pathophysiology causing secondary HT is identifiable, this condition is often referred to as curable or reversible (Danielson & Dammstrom, 1981; Lewin et al., 1985). Although much less common than primary HT, prevalence estimates of secondary HT range from approximately 5 to 10% (Berglund et al., 1976; Omura et al., 2004). Notable causes of secondary HT involve endocrine disorders (Sica, 2008; Chiong et al., 2008), renovascular disorders (Ram et al., 1995; Chiong et al., 2008), adrenal disorders (Chiong et al., 2008), neurological disorders (Chiong et al., 2008), pharmacological side effects (Chiong et al., 2008), and/or aortic diseases (Bhat et al, 2001; Chiong et al., 2008). One must also consider the possible co-existence of multiple factors, as well as interactions between factors, when identifying the underlying pathophysiology of individuals with secondary HT.

Hypertension Sub-Types

Isolated Systolic Hypertension

Isolated systolic HT has been generally defined as having a SBP of ≥140 mmHg, with a DBP of ≤ 90 mmHg. Early literature suggests that this type of HT predominantly affects the elderly, but more recent evidence indicates that isolated systolic HT is also the

predominant form of HT in adolescents and young adults (Franklin et al., 2001; Chiolero et al., 2007). This is consistent with the statement that isolated systolic HT is the most prevalent form of primary HT when considering all cases (Izzo et al., 2000).

There is clear evidence to support the notion that controlling isolated systolic HT leads to reductions in mortality, incidence of cardiovascular events, and stroke (Garland et al., 1983; Curb et al., 1985). Further, SBP has been shown to be more important than DBP in predicting cardiovascular morbidity and mortality (Rhutan et al., 1989; Kannel, 1974). The pathophysiology of isolated systolic HT can be divided into both structural and functional changes. Structurally, isolated systolic HT may be caused by a decrease in arterial compliance through stiffening of the arteries leading to increased peripheral resistance and elevated resting arterial BP (Kannel et al., 1971; Beltran et al., 2001). Isolated systolic HT is also associated with increases in SNS activity (Grassi et al., 1999), which may lead to structural remodeling, further increasing peripheral resistance and/or altering vascular distensibility (Heagerty, 1997).

Resistant Hypertension

Resistant HT is characterized by a persistent elevation in resting arterial BP, with failure to reach goal targets (e.g., arterial BP controlled to within the normal range), despite the concurrent use of at least 3 anti-hypertensive agents (See: **1.1.5 Treatment and Prevention of Elevated Blood Pressure** below for details regarding pharmacological treatment of HT) of different classes (Chobanian et al., 2003; Calhoun et al., 2008). Further, persons with controlled resting arterial BP via 4 or more anti-hypertensive agents are also considered resistant to treatment (Calhoun et al., 2008). Due

to factors such as poor adherence and under-treatment it is difficult to ascertain the precise prevalence of resistant HT independent of uncontrolled HT. Nonetheless, large, diverse, well-designed control trials provide evidence that this type of HT is fairly common (Cushman et al., 2002).

The pathophysiology of resistant HT is multifaceted and may involve contributions from various sources. More specifically, lifestyle factors such as obesity, alcohol intake, and sodium consumption along with secondary causes including obstructive sleep apnea, pheochromocytoma, primary aldosteronism, Cushing's syndrome, renal artery stenosis, renal parenchymal disease, hyperparathyroidism, aortic coarctation, and intracranial tumors have been implicated in the pathophysiology of resistant HT (Calhoun et al., 2008). A common thread between these factors is an increase in SNS activity, possibly due to excess aldosterone levels (Wray & Supiano, 2010; Gaddam et al., 2008), which has been proposed as a primary underlying mechanism of resistant HT (Calhoun et al., 2008; Tsioufis et al., 2011).

Malignant Hypertension

Malignant HT is defined by a sudden, large rise in arterial BP combined with a malignant vascular injury (Fleming, 2000). Pathological changes underlying malignant HT include endothelial injury, arteriolar modifications, and glomerular ischemia (Ruggenenti & Remuzzi, 1996). More specifically, kidneys often show cortical and sub-capsular hemorrhaging, while arteries may show proliferative endarteritis, necrosis, and nucleoid changes, and interlobular arteries may narrow due to fibrin, intimal hyperplasia,

and collagen deposits (Ruggenenti & Remuzzi, 1996). Taken together these factors may decrease renal tissue perfusion and lead to local ischemia.

Presentation of this disorder can occur independently, but it often complicates concomitant primary or secondary HT (Kitiyakara & Guzman, 1998). Although uncommon, this type of HT affects approximately 1% of those individuals already experiencing HT and is more prevalent in younger adults (Kitiyakara & Guzman, 1998). Further, the incidence of malignant HT is twice as high in males as compared to females (Kitiyakara & Guzman, 1998). Early investigations of malignant hypertension indicated poor prognosis and low patient survival rates, often due to uraemia (Kincaid-Smith et al., 1958; Leishman, 1959), but advancements in pharmacological treatments have significantly improved patient outcomes (Gudbrandsson et al., 1979; Gonzalez et al., 2010).

White Coat Hypertension

White coat HT is a unique transient situation in which individuals experience increased resting arterial BP readings in a clinical setting while maintaining arterial BP levels within normal ranges during regular daily living (Verdecchia et al., 2001). This white coat effect can be determined by comparing the average resting arterial BP measurements taken in a clinical setting to average daytime ambulatory BP readings. There is still much debate in the literature regarding potential cardiovascular risks associated with white coat HT. Long-term follow up studies suggest an increased incidence of cardiovascular events and/or disease progression in individuals with white coat HT relative to their normotensive peers (Gustavsen et al., 2003; Mancia et al., 2009),

while other investigations dismiss any significant risks associated with white coat HT (Grosse et al., 1993; Cohen & Townsend, 2010). Nonetheless, it appears that individuals who exhibit white coat HT may be more disposed to developing sustained HT with age (Mancia et al., 2009).

1.1.5 Treatment and Prevention of Elevated Blood Pressure

The primary goal of reducing resting arterial BP is to decrease morbidity and mortality related to cardiovascular and renal disease (Chobanian et al., 2003). Primary prevention and treatment recommendations for HT can be divided into two general areas: lifestyle modifications and pharmacological therapies. As most individuals with HT experience reductions of DBP concomitant to reductions in SBP the primary focus of these therapies is reaching target SBP levels (Chobanian et al., 2003). The treatment goal for individuals with HT, but without co-morbidities such as heart failure, diabetes, or chronic kidney disease, is to lower resting arterial BP below 140/90 mmHg (Chobanian et al., 2003). Further, for individuals with prehypertension (BP 120-129/80-89 mmHg), and no other co-morbidities, it is recommended that resting arterial BP should be reduced to within the normal range (<120/80 mmHg) through lifestyle modifications (described below) to prevent a progressive rise in resting arterial BP (Chobanian et al., 2003).

Lifestyle Modifications

As mentioned above, lifestyle modifications (dietary and physical activity) are cornerstones of prevention and treatment programs for those with, or at risk for, HT. Changes in the composition of an individual's diet and/or physical activity patterns are the first and most common suggestions for HT management (Chobanian et al., 2003;

Pescatello et al., 2004). Modifications that focus on HT management through consumption of healthier foods and regular physical activity have been shown to be effective in lower the resting arterial BP of multiple populations (Appel et al., 1997; Roberts et al., 2002; Blumenthal et al., 2010). The hypotensive effects of improved dietary food composition can be augmented through decreased alcohol intake, limited sodium consumption, and the maintenance of a healthy body weight (Chobanian et al., 2003). Further, adhering to the guidelines put forth in the Dietary Approaches to Stop Hypertension (DASH), which combines dietary modifications and physical activity, may provide added hypotensive benefits (Appel et al., 1997; Chobanian et al., 2003).

The DASH guidelines emphasize the consumption of fruits, vegetables, and low-fat dairy products while also including whole grains, fish, poultry, and nuts while limiting consumption of red meat, sweets and sugary beverages, total fat, saturated fat, and cholesterol (Appel et al., 1997). Though all individuals do not respond to the DASH diet to the same degree, responses are often equivalent to those observed through pharmacological treatment (Chobanian et al., 2003). The DASH diet itself has been shown to significantly reduce SBP, but there is support for additive effects of restricted sodium consumption, which may provide a more potent hypotensive response (Vollmer et al., 2001; Sacks et al., 2001). Though DASH non-responders may not experience the desired decreases in resting arterial BP, they may experience indirect health benefits, such as improved body composition, by adhering to healthier dietary food intakes.

As mentioned above, increased physical activity is an important component of the lifestyle modifications recommended for the prevention and treatment HT. The specific effects of chronic exercise training, as well as those of acute exercise, on resting arterial

BP, are discussed in detail below in the section entitled: **Exercise and Arterial Blood Pressure**.

Pharmacological Treatment

When lifestyle modifications and exercise interventions fail to elicit the desired decreases in resting arterial BP pharmacological treatment is recommended. There are different classes of pharmacologic substances that can be used alone or in combination to treat elevated resting arterial BP. The most common classes of pharmacological substances used to treat elevated BP include: diuretics, which increase water excretion from the body through their actions on the renal tubule, ACE inhibitors, which block the conversion of angiotensin I to angiotensin II, angiotensin receptor blockers, which block the activation of angiotensin II receptors, β-blockers, which block the binding of catecholamines to beta receptors in the heart and smooth muscle, and calcium channel blockers, which inhibit the movement of Ca^{2+} across the membrane of the smooth muscle. The site of action of each of these substances may differ, but the desired outcome is the same: decreased resting BP through modulation of Q or TPR in an effort to decrease the morbidity and mortality associated with HT.

Exercise and Arterial Blood Pressure

Physical activity modification is often used concurrently with dietary changes in treating and preventing HT. Physical activity can be sub-divided into aerobic-based exercise modalities, such as walking and cycling, and resistance-based exercise modalities, such as lifting weights. Further, resistance training can be divided into dynamic resistance or static (isometric) resistance exercise, as there is growing evidence

for the efficacy of isometric training in resting arterial BP regulation (McGowan et al., 2006; McGowan et al., 2007a; McGowan et al., 2007b; Taylor et al., 2003; Millar et al., 2009; Badrov et al., 2013a; Badrov et al., 2013b). It is important to note that quantification of exercise includes both an intensity and duration component. Specific exercise recommendations for hypertensive individuals have been developed by the American College of Sports Medicine (ACSM) and include 30-to-60 minutes of moderate (40 to 60% of an individual's HR reserve) aerobic activity performed 3 to 7 days per week (Thompson, 2010). Though these guidelines focus on aerobic exercise, resistance training is recommended as an adjunct that can be performed 2 to 3 times per week (Pescatello et al., 2004).

Chronic Aerobic Exercise

Literature regarding the effects of aerobic based exercise training protocols on resting BP is plentiful and consistently reports decreases of 5-7 mmHg in resting SBP and DBP (Halbert et al., 1997; Cade et al., 1984; Kiyonaga et al., 1985). To better understand the dose-response relationship of aerobic training and BP reduction we can examine the frequency and intensity of the exercise used in these investigations. A meta-analysis of aerobic-based interventions on BP modulation reveals a frequency range of 2.5 to 7 exercise sessions per week in the published literature (Cleroux et al., 1999). Though there may be a positive correlation between exercise frequency and resting arterial BP reduction it has been noted that approximately 75% of the hypotensive benefits of aerobic exercise garnered on a 7-session per week protocol can be achieved in 3 sessions per week (Nelson et al., 1986). With regards to exercise intensity, although multiple meta-analyses suggest exercise intensity does not influence the magnitude of post-training

resting arterial BP changes, others provide evidence to suggest that moderate, rather than high, intensity exercise may elicit the greater hypotensive response (Pescatello et al., 2004; Cleroux et al., 1999). Perhaps the most notable interpretation is that the hypotensive effects of aerobic exercise do not depend on increases in maximal aerobic capacity, but the effects do correlate well with an individual's level of activity with a higher activity level evoking a more hypotensive response (Cleroux et al., 1999).

The underlying mechanisms driving the hypotensive training response to aerobic exercise remain unclear. It is currently believed that resting arterial BP alterations due to aerobic training are more likely a derivative of changes in TPR rather than Q (Pescatello et al., 2005). Moreover, structural, neural and/or hormonal modifications may also be underlying mechanisms. Structural changes, including vascular remodeling and angiogenesis, have been suggested as causal mechanisms for the resting arterial BP lowering effects of aerobic exercise training (Pescatello et al., 2005). Aerobic exercise leads to a reduction in pre-capillary vascular resistance (Sexton & Laughlan, 1994), which may decrease TPR and, therefore, resting arterial BP (Pescatello et al., 2005). There is also indirect evidence to suggest that chronic aerobic exercise training may reduce SNS activity (Pescatello et al., 2005), and thus resting arterial BP. Aerobic training may also modulate resting arterial BP through alterations in vascular responsiveness. A collection of animal studies has shown that aerobic exercise training reduces vascular responsiveness to the vasoconstrictors NE and ET-1 (Chen et al., 1994; Wiegman et al., 1981; Laughlin et al., 2001). Genetic interactions have been estimated to account for approximately 17% of the reduction in resting arterial BP following aerobic exercise training although precise mechanisms remain unclear (Rice et al., 2002).

Acute Aerobic Exercise

During an acute bout of aerobic exercise arterial BP is modulated predominantly to meet the increased demands of the working muscles. Initially there is an increase in SBP to reach the necessary tissue perfusion required to maintain the increase in muscular metabolism (MacDonald, 2002). The observed arterial BP increase occurs due to increases in both HR and Q via reductions in PNS activation and increases in SNS activation (MacDonald, 2002). As described previously, the vasculature supplying the working muscles progressively vasodilates to provide adequate tissue perfusion, which effectively reduces TPR. After the initial peak in SBP there is a gradual reduction to normalized levels of approximately 140 – 160 mmHg as the contractile state of the vasculature, and TPR, becomes stabilized (McArdle et al., 2001).

There are also characteristic arterial BP changes during the periods post-exercise, most notably being post-exercise hypotension (PEH). Immediately following an aerobic exercise bout there is a decrease in arterial BP (Kenney & Seals, 1993). This phenomenon has been observed up to 22h post-exercise and is proportional to pre-exercise resting arterial BP (Rondon et al., 2002; Kenney & Seals, 1993), such that individuals with greater pre-exercise resting arterial BP will generally see a larger PEH response (Kenney & Seals, 1993). The exact mechanisms underlying PEH remain unclear, though there have been two main hypotheses proposed. The first hypothesis is that acute aerobic exercise decreases SNS outflow, though how this occurs remains unclear (Floras et al., 1989; Halliwill et al., 1996). The second hypothesis involves alterations in vascular responsiveness via decreased transduction of SNS outflow and/or

increased release of vasodilator substances due to increases blood flow and/or muscular work (Pescatello et al., 2005).

Chronic Resistance Exercise

Unlike aerobic training, research regarding the benefits of resistance training programs for resting arterial BP control is much less clear. An early meta-analysis of studies conducted between 1966 and 1998 indicated an overall decrease of approximately 3 mmHg for both SBP and DBP due to resistance training (Kelly & Kelly, 2000). Similar results were observed in a meta-analysis spanning 1996 to 2003 with average resting arterial BP reductions of 3.2 mmHg systolic and 3.5 mmHg diastolic (Cornelissen & Fagard, 2005), and in its updated version, which expanded the data set to 2012 (Cornelissen & Smart, 2013). Although reductions of this magnitude seem insignificant it should be noted that decreasing SBP by merely 3 mmHg yields significant reductions in cardiovascular morbidity and mortality (Verdecchia, 2000). As such, in 1990 the ACSM recognized resistance training as a beneficial component of a comprehensive fitness program for healthy adults.

Acute Resistance Exercise

An acute bout of resistance exercise induces a sudden, drastic increase in both SBP and DBP of up to 400/200 mmHg, respectively (MacDougall et al., 1985). This response is due to the high intramuscular pressures induced by resistance exercise, which compresses the vasculature supply blood to the working muscles (Mayo et al., 1999). This compression increases TPR and may cause blood flow occlusion to the working muscle (Mayo et al., 1999). In an effort to prevent ischemia and increase muscular

perfusion the activity of the SNS is increased (HR & cardiac contractility), increasing arterial BP (MacDougall et al., 1985; Mayo et al., 1999). The arterial BP response to acute resistance exercise is proportional to the muscle mass activated and muscular work performed (MacDougall et al., 1985). Unlike aerobic exercise, the occurrence of PEH with resistance training remains equivocal. If PEH is observed after resistance exercise it may simply be due to reperfusion of previously working muscles experiencing increased intramuscular compression (MacDougall et al., 1985; MacDonald, 2002).

Chronic Isometric Hand-Grip Exercise

The role of isometric hand-grip (IHG) exercise in the modulation of resting arterial BP has received increasing attention over the past decade. Recently endorsed by the American Heart Association as an alternative BP-lowering treatment, this exercise modality is a novel form of static-resistance training where individuals perform multiple, timed, sustained contractions on a programmed handgrip dynamometer at a set percentage of their maximum voluntary effort (Brook et al., 2013). IHG training has shown to markedly reduce both resting SBP and DBP in men and women with and without HT, including those currently on medication(s) to treat their HT, and those who regularly exercise (McGowan et al., 2006; McGowan et al., 2007a; McGowan et al., 2007b; Millar et al., 2007, Millar et al., 2008; Peters et al., 2006; Taylor et al., 2003; Wiley et al., 1992; Ray & Carrasco, 2000; Badrov et al., 2013a; Badrov et al., 2013b). Collectively, these studies employed IHG training protocols of a wide range of frequencies (3 to 5 sessions per week), durations (45-second to 2-minute contractions), intensities (30 to 50% of MVC) and intervention lengths (5- to 10-weeks). Recent meta-analyses concluded that resting arterial BP reductions with IHG training appear similar

to, if not greater than, those elicited by aerobic exercise training (Kelley & Kelley, 2010; Cornelissen & Fagard, 2005; Cornelissen & Smart, 2013).

Older females with normal resting arterial BP (<120/80 mmHg) appear to respond to IHG with greater post-training (8 weeks) resting arterial BP reductions than age-matched males (Millar et al., 2008). Further, preliminary results from our research laboratory (Physical Activity and Cardiovascular Research Laboratory, Department of Kinesiology, University of Windsor) suggest that in contrast to the older cohort, sex differences do not exist in response to IHG training in young, healthy normotensive men and women (Hanik et al., 2012). Unknown is whether older normotensive males and females respond differently, via neurovascular responses such as HR, BP, smooth muscle function, and/or HR variability, to the acute exercise stimulus (e.g., an single, acute bout of IHG exercise). Thus, identifying possible differences in the neurovascular response to IHG exercise may elucidate the mechanism underlying the observed variation in response between sexes in older populations.

The underlying mechanisms regulating chronic resting arterial BP responses to IHG exercise remain equivocal. Furthermore, it is still unclear whether IHG training elicits a systemic vascular effect, as the untrained limbs are often not analyzed. McGowan and colleagues (2007b) have shown that IHG training in medicated hypertensive individuals does not change endothelial-dependent vasodilation in the untrained limb, but further study is warranted. Nonetheless, current hypothesized mechanisms of post-IHG training resting arterial BP reductions include improvements in ANS function, improved NȮ-dependent vascular function (McGowan et al., 2006; McGowan et al., 2007a; McGowan et al., 2007b; Taylor et al., 2003; Millar et al., 2009;

Badrov et al., 2013), and/or reductions in oxidative stress (Peters et al., 2006). It is conceivable that the mechanisms are multi-faceted, and may differ among individual subjects and populations (e.g., old versus young, medicated versus un-medicated HT).

Acute Isometric Hand-Grip Exercise

The underlying mechanisms regulating the cardiovascular effects of a single, acute bout of IHG exercise may or may not be similar to that observed during chronic IHG exercise. Observations immediately after an IHG bout indicate no changes in HR and an increase in arterial BP due to vascular compression in the working arm, which increases TPR (Araujo et al., 2011). Further, elevated arterial BP returned to near resting values after the first minute of post-exercise rest, possibly indicating the progression of PEH (Araujo et al., 2011). PEH has been observed 1 minute after a single IHG contraction at 35% MVC in young healthy subjects (Stewart et al., 2007). Likewise, PEH was also observed 5 minutes post IHG exercise (four 2-minute contractions at 30% MVC with 1 minute rest intervals) in older individuals (Millar et al., 2009). There is also indirect evidence of improved cardiac autonomic modulation, possibly due to enhanced vagal activation, after an acute bout of IHG exercise (Millar et al., 2009). The mechanisms regulating acute arterial BP responses to IHG exercise may provide insight into chronic adaptations and/or have clinical implications themselves.

1.2 Reactive Oxygen Species, Oxidative Stress and Arterial Blood Pressure

Reactive oxygen species (ROS), also known as oxidative free radicals, have been implicated in the pathophysiology of HT and have garnered increasing investigation (Touyz, 2004; Datla & Griendling, 2010). ROS refer to atoms, molecules or ions that

have unpaired electrons in their outer valence shells (Chen et al., 2012). This characteristic makes ROS highly reactive within biological systems, allowing them to react with adjacent molecules such as proteins, lipids, carbohydrates and nucleic acids (Chen et al., 2012). ROS are produced through biochemical reactions that reduce the state of O_2 (Hancock et al., 2001). The reduction of O_2 produces a number of ROS, the most common of these including superoxide radicals ($\dot{O}_2$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($O\dot{H}$), and peroxynitrite ($ONOO^-$) (Hancock et al., 2001).

Endogenous sources of ROS production include: the mitochondrial electron transport chain, xanthine oxidase, cyclooxygenase, lipoxygenase, $N\dot{O}$ synthase, heme oxygenases, peroxidases, hemoproteins such as heme and hematin, and NADPH oxidases (Pennathur & Heinecke, 2007; Kojda et al., 1999). The most important of these ROS sources is suggested to be the NADPH oxidase enzyme that, using electrons supplied by NADPH, catalyzes the reaction of O_2 to form $\dot{O}_2$ (Hancock et al., 2001). The reduction of O_2 by one electron produces $\dot{O}_2$, which is highly reactive due to the unpaired electron in the outer valence shell (Sies, 1997; Chen et al., 2012). Likewise, O_2 can be reduced to water (H_2O), but the intermediate steps include the formation of H_2O_2 and $\dot{O}H$ (Sies, 1997). These molecules can induce pathophysiological changes to lipids, proteins and DNA, by altering the structure and function of these cells and tissues (Bandyopadhyay et al., 1999).

Evidence suggests that ROS are not only negative by-products of substrate metabolism, but that they may also play a role in cellular signaling (Hancock et al., 2001). The differing roles of ROS, as signaling molecules or destructive metabolic by-products, may depend on the concentration of ROS present (Thannickal et al., 2000).

ROS are continuously produced at relatively low rates during rest, but their synthesis markedly increases during exercise (Reid & Durham, 2002; Bejma & Ji, 1999). At relatively low concentrations ROS function as signaling molecules, which maintain vascular integrity through modulation of smooth muscle contraction and endothelial function (Touyz et al., 2003). In contrast, chronic elevations in ROS production have been linked to endothelial dysfunction, lipid peroxidation, smooth muscle cell growth, inflammation, monocyte invasion, increased contractility and increased extracellular matrix protein deposition (Diep et al., 2002; Taniyama & Griendling, 2003).

The term "oxidative stress" refers to a state of imbalance between the production of ROS and the removal and/or stabilization of these substances by cellular antioxidant defense systems (Rodriguez-Manas et al., 2009). An antioxidant refers to any molecule capable of neutralizing or stabilizing a free radical before they can damage cells and tissues (Chen et al., 2012). These substances can be produced endogenously within the body, or consumed through exogenous dietary sources. The most prominent endogenous antioxidants include superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (Paravicini & Touyz, 2008). SOD catalyzes the dismutation of $\dot{O}_2$ into H_2O_2 (Chen et al., 2012). H_2O_2 can then be converted into H_2O and O_2 via GPx or catalase. GPx consumes reduced glutathione and convert H_2O_2 into H_2O, while catalase facilitates the conversion of H_2O_2 into H_2O and O_2 (Chen et al., 2012; Paravicini & Touyz, 2008). The balance between oxidants and antioxidants may be key in the pathophysiology of many chronic diseases, and accordingly, oxidative stress has been implicated in HT as well as atherosclerosis, vascular disease, diabetes, and aging (Harman, 1956; Pannathur & Heinecke, 2007; Touyz, 2004; Higashi et al., 2002).

Effects of Exercise on Reactive Oxygen Species and Blood Pressure Regulation

As alluded to above, oxidative stress is thought to play an important role in arterial BP regulation and the underlying pathophysiology of HT (Nakazono et al., 1991; Ferroni et al., 2006; Harrison et al., 2007), and its reduction has been implicated in the resting arterial BP reductions observed with IHG training (Peters et al., 2006).

When the endogenous antioxidant capacity becomes saturated continued production of ROS induces a state of oxidative stress. ROS produced in the neural, renal, and vascular systems are implicated in the pathophysiological signaling that leads to the development of HT (Datla & Griendling, 2010). Further, increased bioavailability of ROS can promote endothelial dysfunction through reductions in NȮ bioavailability (Pennathur & Heinecke, 2007), upregulation of the RAAS (Diep et al., 2002), increases in systemic inflammation (Diep et al., 2002), and vascular remodeling (Shiffrin, 2004). These outcomes can individually and collectively contribute to the sustained elevations in resting arterial BP associated with HT.

The relative importance of NȮ to the health and function of endothelium, and its reduced bioavailability due to ROS, has prompted increasing investigation into the implications for HT development. The ROS $\dot{O}_2$ is able to react with NȮ to produce the highly reactive $ONOO^-$ (Hancock et al., 2001). As the rate of reaction between $\dot{O}_2$ and NȮ is far greater than the rate of reaction between $\dot{O}_2$ and SOD, an endogenous antioxidant capable of neutralizing its activity, $\dot{O}_2$ will more readily react with NȮ (Chen et al., 2012). As NȮ serves an important role in the health and contractile state of the smooth muscle (Sarkar et al., 1996; Moncada et al., 1991), a decrease in its

bioavailability may produce negative consequences to vascular health, impaired vasodilation, and increased resting arterial BP.

ROS may further influence the bioavailability of NȮ through its actions on the critical eNOS cofactor tetrahydrobiopterin (BH_4). When reactive $\dot{O}_2$ is produced from vascular NAD(P)H oxidase enzymes it oxidizes BH_4 and reduces the bioavailability of this critical cofactor (Landmesser et al., 2003). In the presence of reduced amounts of BH_4, and increased amounts of oxidized BH_4, the stimulation of eNOS produces large amounts of $\dot{O}_2$ (Bevers et al., 2006; Landmesser et al., 2003). The negative consequences of this cycle, namely chronic reductions in NȮ and increases in $\dot{O}_2$, may be critical in the progression and underlying pathophysiology of HT.

Reactive Oxygen Species Production and Acute Exercise

In general, acute exercise leads to increased O_2 consumption and an increase in the production of ROS, such as $\dot{O}_2$, from oxidative metabolism (Bejma & Ji, 1999). With respect to aerobic exercise, transient increases in ROS production and consequent oxidative stress, as indicated by an increased ratio of oxidized glutathione-to-total whole-blood glutathione ratio (GSSG:TGSH), have been observed following an acute bout of aerobic exercise (Bloomer et al., 2005). Participants cycled at 70% of VO_2max for 30 minutes and blood was drawn for analysis pre, post, 1h-post, 6h-post, and 24h-post exercise. The GSSG:TGSH ratio was increased immediately following exercise, but returned to baseline at 1h-post exercise. The authors hypothesized that an acute increase in oxidative modification of TGSH occurs immediately post-exercise, increasing production of GSSG, but the rapid action of glutathione reductase catalyzes the

conversion of GSSG to reduced glutathione (Bloomer et al., 2005). It is possible that the observed oxidative stress in this study is due to increased $\dot{O}_2$ bioavailability. To neutralize reactive $\dot{O}_2$ it can be converted to H_2O_2 by the enzyme SOD (Gohil et al., 1988). H_2O_2 formed by this reaction is then neutralized by the actions of the enzyme glutathione peroxidase, which converts H_2O_2 to H_2O, but in the process produces GSSG (Gohil et al., 1988). The observation of increased oxidative stress after acute aerobic exercise of differing intensities (i.e.: exhaustive, prolonged) and modalities (i.e.: running, cycling) has been repeatedly shown in the literature (Alessio et al., 2000; Sastre et al., 1992; Gohil et al., 1988).

Acute resistant exercise also augments ROS production, possibly leading to oxidative stress and the associated negative consequences. A circuit of resistance exercise (10 exercises, 9 repetitions, at 75% of 1 repetition maximum (RM)) acutely increased plasma antioxidant concentrations (α-tocopherol, γ-tocopherol, β-carotene, lycopene), but this was not sufficient to prevent augmented lipid peroxidation immediately post-exercise (Ramel et al., 2004). Likewise, a resistance training circuit (2 circuits, 8 exercises, 3 sets of 10 repetitions at 100% of 10 RM) significantly increased the concentration of malonyldialdehyde (MDA) immediately post-exercise. MDA is a biomarker of free radical interaction with the lipids of cell membranes. The observation that acute resistance exercise elicits significant increases in the concentration of MDA has been shown repeatedly (Hoffman et al., 2007; Heunks et al., 1999). There is also evidence of significant increases in xanthine oxidases, which can generate $\dot{O}_2$, immediately post-resistance exercise (Heunks et al., 1999). As resistance exercise does

not tax aerobic metabolism heavily future study should attempt to elucidate possible differences between acute aerobic and resistance ROS production.

This observation is not unique to aerobic or resistance exercise modalities and has also been observed following an acute bout of IHG. For example, in work conducted by Alessio et al. (2000), participants performed 45 second contractions at 50% MVC with 45 second rest periods until the accumulated time was equal to the time it took to complete a VO_2max test (approximately 15 minutes) (Alessio et al., 2000). Increases in lipid hydroperoxides, an indicator of increased oxidative stress, were observed immediately post-exercise (Alessio et al., 2000). These findings may be due to increased post-exercise bioavailability of $\dot{O}_2$ generated via: metabolite-induced production, exercise-induced temperature rise, mechanical-stress induced inflammation and/or ischemia-reperfusion (IR) induced production (Alessio et al., 2000; Holecek et al., 2004). $\dot{O}_2$, if not neutralized, can be reduced or dismutated to H_2O_2, which can be further reduced by iron to produce $\dot{O}H$, a free radical capable of peroxidation and lipid hydroperoxide production (Girotti, 1998).

Work by McGowan et al. (2006) supports the observation of increased oxidative stress following an acute IHG bout. Investigators observed post-IHG bout reductions in $N\dot{O}$-dependent vasodilation in the brachial artery of the exercised limb (an indirect indication of increased oxidative stress) immediately following a bout of IHG (4x2-minute unilateral contractions at 30% MVC with 4-minutes rest between) in an older population of medicated hypertensives, which is suggestive of reduced local $N\dot{O}$ bioavailability. As $\dot{O}_2$ reacts rapidly with $N\dot{O}$ it is plausible that acute IHG exercise

augments $\dot{O}_2$ production, reducing N$\dot{O}$ bioavailability, inhibiting N$\dot{O}$-dependent vasodilation.

ROS Production and Chronic Exercise

Most importantly, while a single bout of aerobic or IHG exercise acutely increases ROS production and oxidative stress, this effect is transient, and chronic exposure to either stimulus leads to adaptation and an increased basal antioxidant capacity (Peters et al., 2006; Miyazaki et al., 2001).

Although the acute effects of augmented ROS production due to aerobic exercise may be interpreted as detrimental to health, they appear to serve a beneficial long-term purpose. For example, chronic aerobic exercise training (i.e., repetitive exposure to acute bouts) leads to lower levels of oxidative damage after an intense exercise bout due to upregulation of antioxidant enzymes such as SOD, GPx, and glutamylcysteine synthase (Salminen & Vihko, 1983). Likewise, increased SOD activity in response to aerobic training has been shown repeatedly in animal studies of various durations, intensities, and lengths (Higuchi et al., 1985; Powers et al., 1994; Leeuwenburgh et al., 1997). If chronic aerobic exercise increases the endogenous antioxidant capacity of the system it could neutralize greater concentrations of ROS, such as $\dot{O}_2$, preventing the negative effects of oxidative stress (i.e.: reduced N$\dot{O}$ bioavailability) from negatively effecting arterial BP.

As with aerobic training, ROS produced during acute IHG bouts may also confer long-term benefits. It has been shown that, following 6 weeks of IHG training (3 sessions per week, four total 45s contractions at 50% MVC, bilaterally, with 1 minute recovery between intervals), there is a marked increase in the resting TGSH:GSSG, an

indication of increased antioxidant capacity (Peters et al., 2006). This work was supported by McGowan and colleagues who observed, in the group of participants with hypertension described above, an improvement in NȮ-dependent function of the brachial artery at rest (McGowan et al., 2006; McGowan et al., 2007a; McGowan et al., 2007b), which is suggestive of an increased basal antioxidant capacity. Further, there is evidence to suggest that IHG training results in improved basal NȮ-dependent resistance vessel function (McGowan et al., 2007a; Badrov et al., 2013). Increased antioxidant capacity through repeated exposures to the IHG stimulus may provide a greater capacity to neutralize future increases in free radical production, reduce the incidence and severity of oxidative stress, improve basal NȮ-dependent dilation supporting improved resistance vessel function, leading to resting arterial BP reduction. It is important to note, however, that post-bout attenuation in vascular function (FMD) was unchanged with 8-weeks of IHG training, suggesting that although basal function is improved with chronic exposure to the IHG stimulus, there is a lack of adaptation in stress-induced ROS antioxidant buffering capacity (McGowan et al., 2007a).

The observed improvements in NȮ-dependent vasodilation may be due to increased basal antioxidant concentrations. As stated previously, $\dot{O}_2$ reacts rapidly with NȮ reducing its bioavailability and inhibiting NȮ-dependent vasodilation. Increased basal antioxidant action would better ameliorate the effects of elevated ROS production preserving NȮ for its role in resistance vessel function and arterial BP regulation. The IHG studies to date have been underpowered to detect sex-based differences in the vascular responses to acute IHG and/or IHG training, including oxidative stress and antioxidant capacity, if they do exist.

1.3 Ischemia-Reperfusion

As mentioned in the above section entitled: **Reactive Oxygen Species Production and Acute Exercise,** IR is mechanism of ROS production. IR refers to the sequence of events whereby the blood supply to cells and tissues is restricted for a period of time, inhibiting the supply of nutrients required for cellular metabolism, followed by reintroduction of the blood supply. IR can occur due to trauma, such as during myocardial infarction or stroke (Dorweiler et al., 2007), but also due to high intramuscular pressures experienced during some exercise modalities, such as IHG exercise at 30% MVC or greater (Sadamoto et al., 1983; Sakakibara & Honda, 1990). This response is due to the high intramuscular pressures induced by resistance exercise, which compresses the vasculature supplying blood to the working muscles, increasing TPR, which may cause occlusion of blood flow (Mayo et al., 1999). Occlusion of blood flow is known to augment the production of ROS, which can induce a state of oxidative stress (Kloner et al., 1989). A constant onslaught of oxidative stress would plausibly lead to a multitude of negative effects, but as the above described chronic exercise training suggests, there may be a long-term benefit to repeated acute exposures.

Exposure to ischemic conditions can increase antioxidant concentrations, which may evoke an adaptive response and protection during subsequent exposure to oxidative stress (Hoshida et al., 1993; Powers et al., 1998). An adaptive response refers to the ability of an organism to better defend against the damaging effects of a toxic agent after previous exposure to a lesser dose (Crawford & Davies, 1994). Exposure of various cell cultures to oxidative stress evokes an adaptive response which renders them much more resistant to subsequently higher doses (Spitz et al., 1987; Laval, 1988; Lu et al., 1993).

Further, it has also been shown that aerobic exercise training (running for 1h/day with increasing speed and gradient over the first 10-weeks to 35m/min at 15% incline, 5 days/week, for 18 weeks), which, as mentioned previously, acutely augments ROS production, can lead to adaptations that decrease post-exercise lipid peroxidation relative to sedentary controls (Alessio & Goldfarb, 1988). Lipid peroxidation is a frequently measured marker of increased ROS production and oxidative stress. Decreased post-exercise lipid peroxidation indicates a greater basal antioxidant capacity, capable of neutralizing augmented ROS production. Lastly, running on a treadmill for 60 minutes per day at 5m/min at 15% incline, 5 days per week, over a 10-12 week training period has been shown to restore diminished BH_4 in older rats, improving $N\dot{O}$-dependent vasodilation within the arterioles (Sindler et al., 2009). Consistent evidence provides support for the notion that transient exposure to oxidative stress, be it through exercise and/or IR, may evoke an adaptive response leading to an increased basal antioxidant capacity.

IHG Exercise and Ischemia Reperfusion

Acute exposure to increased ROS via IR may be the mechanism underlying the observed positive adaptations due to IHG exercise that improve basal $N\dot{O}$-dependent dilation and resistance vessel function while also lowering resting arterial BP. IHG performed at ≥30% MVC progressively reduces forearm blood flow until total occlusion occurs at about 70 % MVC (Sakakibara & Honda, 1990). As blood flow to the working tissues is decreased proportional to force during IHG exercise (Sakakibara & Honda, 1990; Sejersted et al., 1984), and reactive hyperemia is increased during the recovery phase proportional to exercise intensity (Kagaya & Homma, 1997), it is possible that IR

is an underlying mechanism of the resting arterial BP reductions observed following 8 weeks of thrice weekly, 30% MVC IHG training. Increased antioxidant capacity may allow for greater neutralization of $\dot{O}_2$ leading to a greater bioavailability of N$\dot{O}$, improving resistance vessel function and N$\dot{O}$-dependent vasodilation. If the resting arterial BP reductions from IHG exercise could be replicated using a simple IR protocol, one could quantify the stimulus and isolate IR as a causal mechanism. Furthermore, sex-specific differences in the resting arterial BP response to IR may provide insight into the observed sex differences in the resting arterial BP response to IHG training in older populations.

Thesis Purpose & Hypotheses

The purpose of this study was to: 1) quantify the IHG stimulus in terms of the neurovascular responses and determine if a sex difference exists in these responses, 2) determine if the IHG protocol has systemic vascular effects and to determine if any systemic response sex differences exist, and 3) compare the response of the traditional IHG stimulus to a complementary IR cuff protocol within the exercised limb.

Due to elevations in oxidative stress, it was hypothesized that N$\dot{O}$-dependent vasodilation would be transiently reduced in the conduit (brachial) and resistance vessels of the trained limb immediately following an acute bout of IHG exercise (McGowan et al., 2006; McGowan et al., 2007a). Further, females were expected to experience a greater neurovascular response to acute IHG exercise (through greater reductions in N$\dot{O}$-dependent vasodilation and reduced resistance vessel function). It was hypothesized that an acute bout of IHG exercise would produce a systemic vascular response, and that females would experience a greater response than males. It was also hypothesized that

the IR cuff protocol would produce similar neurovascular responses to that of an acute bout of IHG exercise in the exercised limb. As with IHG exercise, it was hypothesized that females would experience a greater vascular response. With respect to the latter, sex-specific differences in the arterial BP response to IR may elucidate the observed sex differences in the resting arterial BP response to IHG training.

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Chapter 2: Sex differences in the neurovascular response to acute isometric handgrip exercise and a complementary ischemic-reperfusion cuff protocol

2.1 Introduction

Hypertension (HT), a condition characterized by sustained elevations of resting systolic (SBP) and/or diastolic blood pressure (DBP), increases the risk of developing cardiovascular disease (CVD) and afflicts over 4.6 million Canadians (Statistics Canada, 2009). Currently, CVD is a leading cause of morbidity and mortality in Canadians of both sexes (Statistics Canada, 2009). Genetic and environmental factors, along with alterations in sex hormones with increasing age, have been implicated in the development of HT (Coylewright, 2008). Although significant improvements in screening, prevention, and treatment have been made, HT remains a major health problem for Canadians as evident by the statistics presented above. Thus, primary prevention strategies targeting older individuals, a key population at an increased risk for HT development, are very important.

Isometric handgrip (IHG) training is a novel and simple form of isometric resistance exercise where participants perform multiple, timed, constant contractions (squeezes) on a programmed handgrip at a set percentage of their maximal voluntary contraction (MVC), where each contraction is separated by a timed rest period. This form of training, recently endorsed by the American Heart Association as a method to reduce BP (Brook et al., 2013), reduces both resting SBP and DBP in men and women with and without HT, including those currently on medication(s) to treat their HT, and those who regularly exercise (McGowan et al., 2006; McGowan et al., 2007a; McGowan et al., 2007b; Millar et al., 2007, Millar et al., 2008; Peters et al., 2006; Taylor et al., 2003; Wiley et al., 1992; Ray & Carrasco, 2000; Badrov et al., 2013a; Badrov et al., 2013b). Collectively, these studies use IHG training protocols of varying frequencies (3 to 5

sessions per week), durations (45-second to 2-minute contractions), intensities (30 to 50% of MVC) and intervention lengths (5- to 10-weeks). The most commonly employed and studied bilateral IHG protocol consists of four, two-minute contractions at 30% MVC, performed with alternating hands with 1-minute rest between. The most common unilateral IHG protocol consists of four, two-minute contractions at 30% MVC, with 4-minutes rest between. Recent meta-analyses have concluded that resting arterial BP reductions with this form of training appear similar to, if not greater than, those observed with aerobic exercise training (Kelley & Kelley, 2010; Cornelissen & Fagard, 2005).

Older females with normal resting arterial BP appear to respond to IHG with greater post-training reductions in resting arterial BP than age-matched males (Millar et al., 2008). Further, preliminary results from our lab suggest that this is not upheld in a younger cohort (Hanik et al., 2012), such that both sexes respond to IHG training with similar magnitudes of resting arterial BP reduction. A small body of evidence exists to suggest that an acute bout of IHG (four, two-minute contractions at 30% MVC, with 2-minutes rest between) elicits an increase in arterial BP due to vascular compression in the working arm increasing TPR, but no changes in HR (Araujo et al., 2011). Further, elevated arterial BP returned to near rest after the first minute of post-exercise rest, possibly indicating the progression to PEH (Araujo et al., 2011). PEH has been observed 1 minute after a single 2-minute IHG contraction at 35% MVC in young healthy subjects (Stewart et al., 2007). Likewise, PEH was also observed 5 minutes post-IHG bout (four 2-minute contractions at 30% MVC with 1 minute rest intervals) in older individuals (Millar et al., 2009). The arterial BP changes appear concomitant to improved cardiac autonomic modulation, possibly due to enhanced vagal activation after an acute bout of

IHG exercise (Millar et al., 2009). However to date the studies have been underpowered to detect sex differences, if they do exist. As such, whether older normotensive males and females respond differently (i.e.: neurovascular responses such as HR, arterial BP, conduit & resistance vessel function, or HR variability) to an acute bout of IHG exercise, potentially contributing to the differences in post-training response, remains unknown.

The mechanisms underlying the acute and chronic arterial BP responses to IHG exercise remain equivocal. Hypothesized mechanisms of post-IHG training resting arterial BP reductions include improvements in autonomic nervous system function (as assessed via direct sympathetic outflow to muscle (MSNA), or indirectly via HR variability), improved NȮ-dependent blood vessel (endothelial) function (McGowan et al., 2006; McGowan et al., 2007a; McGowan et al., 2007b; Taylor et al., 2003; Millar et al., 2009; Badrov et al., 2013a), and reductions in oxidative stress (Peters et al., 2006). The mechanisms underlying the effects of an acute bout of IHG exercise may, or may not, be similar. It is conceivable that the mechanisms are multi-faceted, and may differ among individuals (e.g., genetics) and populations (e.g., normotensive versus unmediated hypertensive versus medicated hypertensive; men versus women). Quantifying the neurovascular stimulus will provide valuable insight into the mechanisms of IHG-induced arterial BP adaptations.

Exercise (i.e., isometric or aerobic) leads to increased O_2 consumption and an increase in the production of ROS from oxidative metabolism (Bejma & Ji, 1999). With respect to isometric exercise, increased oxidative stress has been observed following an acute IHG bout, and has been attributed to increased post-exercise bioavailability of $\dot{O}_2$ via: 1) metabolite-induced production, 2) exercise-induced temperature rise and

mechanical-stress induced inflammation, and/or 3) ischemia-reperfusion (IR) induced production (Alessio et al., 2000; Holecek et al., 2004). This transient increase in oxidative stress post-IHG is not unique to an isometric exercise modality, but also occurs after acute bouts of aerobic exercise (Bloomer et al., 2005). Importantly, while a single bout of IHG exercise acutely increased ROS production and oxidative stress, this effect is transient, and chronic exposure to the IHG stimulus leads to adaptation and an increased basal antioxidant capacity (Peters et al., 2006). As with the work mentioned previously, samples sizes were too small to detect any sex differences in post-training improvements in NȮ-dependent function and/or oxidative stress.

The positive adaptation to acute ROS exposure has also been shown in aerobic exercise studies (Miyazaki et al., 2001). With respect to aerobic exercise, transient increases in ROS production and consequent oxidative stress, as indicated by an increased ratio of oxidized glutathione-to-total whole-blood glutathione ratio (GSSG:TGSH), have been observed following an acute bout of aerobic exercise (Bloomer et al., 2005). Participants cycled at 70% of VO_2max for 30 minutes and blood was drawn for analysis pre, post, 1h-post, 6h-post, and 24h-post exercise. The GSSG:TGSH ratio was increased immediately following exercise, but returned to baseline at 1h-post exercise. The authors hypothesized that an acute increase in oxidative modification of TGSH occurs immediately post-exercise, increasing production of GSSG, but the rapid action of glutathione reductase catalyzed the conversion of GSSG to reduced glutathione (Bloomer et al., 2005). It is possible that the observed oxidative stress in this study was due to increased $\dot{O}_2$ bioavailability. To neutralize reactive $\dot{O}_2$ it can be converted to H_2O_2 by the enzyme SOD (Gohil et al., 1988). H_2O_2 formed by this

reaction is then neutralized by the actions of the enzyme glutathione peroxidase, which converts H_2O_2 to H_2O, but in the process produces GSSG (Gohil et al., 1988). The observation of increased oxidative stress after acute aerobic exercise has been repeatedly shown in the literature (Alessio et al., 2000; Sastre et al., 1992; Gohil et al., 1988).

Although the acute effects of augmented ROS production due to aerobic exercise appear to be detrimental to health they may serve a very beneficial long-term purpose. Exposure to chronic aerobic exercise training leads to lower levels of oxidative damage after an intense exercise bout due to upregulation of antioxidant enzymes such as SOD, GPx, and glutamylcysteine synthase (Salminen & Vihko, 1983). Likewise, increased SOD activity in response to aerobic training has been shown repeatedly in animal studies of various durations, intensities, and lengths (Higuchi et al., 1985; Powers et al., 1994; Leeuwenburgh et al., 1997). If chronic aerobic exercise augments endogenous antioxidant production the system could neutralize greater concentrations of ROS, such as $\dot{O}_2$, preventing the negative effects of oxidative stress, such as reduced $N\dot{O}$ bioavailability, on resting arterial BP regulation.

Work by McGowan et al. (2006) indirectly supports the observation of improved antioxidant capacity with IHG training. Acutely, investigators observed post-IHG bout reductions in $N\dot{O}$-dependent vasodilation in the brachial artery of the exercised limb (an indirect indicator of increased oxidative stress) in an older population of medicated hypertensives prior to an 8-week IHG training intervention. Although this acute response was unchanged with 8-weeks of IHG training, the overall resting $N\dot{O}$-dependent function of the brachial artery was improved (McGowan et al., 2006; McGowan et al., 2007a; McGowan et al., 2007b). Further, there is evidence to suggest that IHG training results in

improved basal NȮ-dependent resistance vessel function (McGowan et al., 2007a; Badrov et al., 2013a). Increased antioxidant capacity through a repeated exposure to the IHG stimulus may provide a greater capacity to neutralize future increases in free radical production, reduce the incidence and severity of oxidative stress, improve basal NȮ-dependent dilation supporting improved resistance vessel function, and lead to reductions in total peripheral resistance and resting arterial BP .

IR with IHG increases ROS production, as mentioned above. It has been shown that exposure to ischemic conditions can increase antioxidant concentrations, which may lead to better protection during subsequent exposure to oxidative stress (Hoshida et al., 1993; Powers et al., 1998). As blood flow to the working tissues is decreased proportional to force during IHG exercise (Sejersted et al., 1984), and reactive hyperemia is increased during the recovery phase proportional to exercise intensity (Kagaya & Homma, 1997), it is possible that IR is an underlying mechanism of resting arterial BP modulation due to IHG exercise. If the resting arterial BP reductions from IHG exercise could be replicated using a simple IR cuff protocol (i.e., creating IR via arterial occlusion using an inflatable cuff), one could quantify the stimulus and isolate IR as a potential causal mechanism. Further, if there is a sex difference in the vascular response to IR it may provide insight into the observed sex differences in the resting arterial BP response to IHG exercise in older individuals (Millar et al., 2008).

Expansion of currently available HT prevention therapies is needed, particularly in older individuals at risk of developing HT. IHG training is a novel, simple, and time-efficient form of exercise that is effective in reducing resting arterial BP in individuals with and without high arterial BP, particularly in older women. The inherent sex

differences in response to the IHG stimulus are under-investigated. Furthermore, the mechanisms responsible for the observed resting arterial BP reductions and/or existing sex-differences in these mechanisms, such as basal antioxidant modulation, are not well known. The rationale for conducting the present study is based upon these experimental findings. It is hoped that the results of this research will provide further support for the use of IHG training as a modulator of resting arterial BP, as well as shed light on possible associated mechanisms and sex differences.

2.2 Purpose & Hypotheses

The purpose of this study was to: 1) quantify the IHG stimulus in terms of the neurovascular responses and determine if a sex difference exists in these responses, 2) determine if the IHG protocol has systemic vascular effects and to determine if any systemic response sex differences exist, and 3) compare the response of the traditional IHG stimulus to a complementary IR cuff protocol within the exercised limb.

Due to elevations in oxidative stress, it was hypothesized that NȮ-dependent vasodilation would be transiently reduced in the conduit (brachial) and resistance vessels of the trained limb immediately following an acute bout of IHG exercise (McGowan et al., 2006; McGowan et al., 2007a). Further, females were expected to experience a greater neurovascular response to acute IHG exercise (through greater reductions in NȮ-dependent vasodilation and reduced resistance vessel function). It was hypothesized that an acute bout of IHG exercise would produce a systemic vascular response, and that females would experience a greater response than males. It was also hypothesized that the IR cuff protocol would produce similar neurovascular responses to that of an acute bout of IHG exercise in the exercised limb. As with IHG exercise, it was hypothesized

that females would experience a greater vascular response. With respect to the latter, sex-specific differences in the arterial BP response to IR may elucidate the observed sex differences in the resting arterial BP response to IHG training.

2.3 Methods

Study Participants

A total of eight non-smoking, recreationally active (2-3x / week), healthy men (n=1); 63 years, and women (n=7); 65 ± 4 years, with normal arterial BP (man: 103 ± 13 SBP/68 ± 7 DBP; women: 100 ± 10 SBP/60 ± 5 DBP) were recruited from Essex county and enrolled in the protocol. Exclusion criteria included any arterial BP or vascular altering medications, recent hospitalization (<3 months), use of hormone replacement therapy, diabetes (by history, use of oral hypoglycemic agents or a fasting blood glucose value >7.0 mmol/L), autonomic neuropathy, frequent premature ventricular heart beats or a paced heart rhythm at rest, CVD and/or heart failure. The University of Windsor Research Ethics Board approved the study described herein (REB # 12-207).

Sample size was calculated with consideration of the neurovascular responses following IHG exercise (Millar et al., 2009; McGowan et al, 2006; McGowan et al, 2007a). With an assigned alpha of 0.05, a beta of 0.2 and an effect size of 0.4, sample sizes ranging from 6 to 16 were estimated.

Study Design

After inquiring about the study, potential participants who believed they met the inclusion criteria met with investigators at the Physical Activity and Cardiovascular

Research lab (PACR; HK 240, Department of Kinesiology, University of Windsor, ON, Canada) for an informational meeting and, if interested, to sign an informed letter of consent (Appendix A), a letter of information (Appendix B), followed by the administration of a medical questionnaire (Appendix C) to determine eligibility. Upon review of the medical questionnaire and the initial establishment of initial eligibility, participants had their resting arterial BP measured. Resting arterial BP was measured following 10-minutes of seated rest using the Dinamap brachial artery oscillometric device (Appendix D; Dinamap Carescape v100, Critikon, Tampa, Florida, USA). Specifically, arterial BP measurements involved the placement of a cuff around the right arm (upper portion), which was inflated to a pressure greater than systolic BP to occlude the brachial artery. Arterial BP was measured 4 times, with a 2-minute rest period between measures, and the last 3 values were then averaged (Stiller-Moldovan et al., 2012; Badrov et al., 2013a; Badrov et al., 2013b).

If resting arterial BP was within the eligible range (< 140/90 mmHg) individuals who were still interested were given a physical activity readiness medical examination (PAR-MedX) questionnaire if over the age of 70 years (Appendix E), or a PAR-Q if under the age of 70 years (Appendix F) and a letter of information for their primary healthcare provider (Appendix G), both of which were signed by their health care provider (HCP) and returned to the study investigators prior to the scheduling of the familiarization session.

Upon completion of all forms, individuals returned to the PACR lab for a familiarization session. First, participants had their resting arterial BP measured a second time in the same manner as described above. If average resting arterial BP values from

Visit 1 and Visit 2 were < 140/90 mmHg participants were deemed eligible to participate, and continued with the habituation session and the investigation. Participants then practiced all portions of the investigation including the IHG exercise protocol. Any further questions or concerns related to the study were answered at this time. This session also functioned to reduce participant anxiety as well as reduce possible white coat effects during subsequent BP readings (Verdecchia et al., 2001). Following the completion of the familiarization session, testing was then conducted on three separate days to evaluate neurovascular function (Figure 1; pre- to post-IHG exercise in the exercised and non-exercised arm, and pre- to post-IR cuff protocol in the cuffed arm).

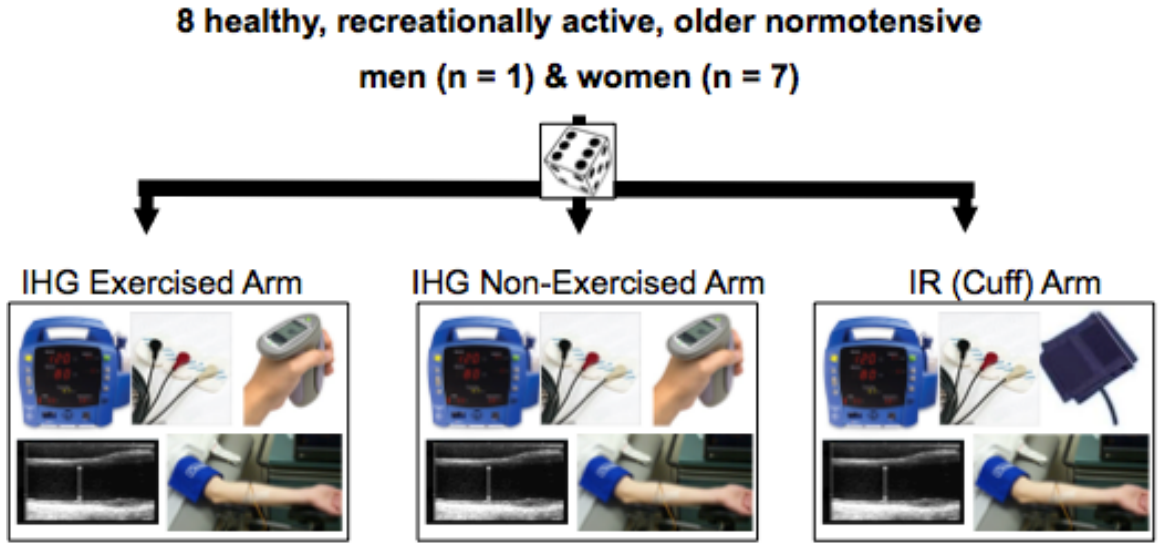

Figure 1. Vascular testing days

Testing Protocol

Testing days were conducted in random order, and were separated by at least 24 hours. To minimize the influence of external factors on the cardiovascular measurements, participants were asked to refrain from the consumption of alcohol or the participation in

vigorous exercise for 24 hours prior to each testing session, to avoid caffeine consumption for 12 hours prior, and following a 4 hour fast. All testing was conducted in a quiet, temperature-controlled room at the same time of day according to standardized published procedures (Stiller-Moldovan et al., 2012). Participants were asked to void their bladder prior to all testing sessions to minimize the effects of bladder distension on resting arterial BP (Fagius & Karhuvaara, 1989).

TESTING DAY 1: Assessment of neurovascular function (indirect assessment of oxidative stress) prior to and following an acute IHG bout in the exercised arm (Time: 2.5 hours)

After 10 minutes of seated rest, arterial BP was be measured 4 times in the right arm with a 2-minute rest period between measures, and the last 3 values were then averaged, as described above. Following arterial BP acquisition, participants remained in a seated position with their non-dominant arm adjusted to an angle of ~90° from their upper arm, while their arm was rested on a table located directly in front of the participant. A rapid inflating BP cuff was also placed around the non-dominant forearm (2-4 cm distal to the antecubital fosse). A 3-lead ECG was attached to record continuous HR data throughout the protocol (Appendix H; Powerlab ML 870/P, AD Instruments, Colorado Springs, Colorado, USA).

Neurovascular Measure #1: Brachial artery NȮ-dependent vasodilation was assessed using a flow-mediated dilation (FMD), a gold standard validated technique, in accordance with international guidelines (Harris et al., 2010). A linear array vascular probe (8L-RS; 13MHz), attached to a high-resolution Doppler ultrasound machine

(Appendix I; Vivid*i*, GE Healthcare, Pittsburgh, PA, USA) was used to image the brachial artery in the distal third of the upper arm. Ultrasound gel was applied to the area of interest and ultrasound parameters were set to optimize the clarity of the brachial artery image. The placement of the probe and anatomical landmarks were recorded for future imaging sessions to ensure identical placement of the probe during repeat testing. All recording was performed at an insonation angle of 60 degrees. Recording sample volume spanned the entire width of the artery, but was clear of the vessel walls. A rapid inflation/deflation cuff was inflated to 200mmHg and held at a constant pressure for 5 minutes to occlude blood flow, after which time the cuff was released and blood flow restored to the lower arm. In brief, brachial artery diameters (B Mode) and blood velocity (PW mode) were recorded continuously for 1 minute prior to cuff inflation and for 3 minutes following cuff release.

Participants then completed a single bout of IHG, comprising 4, 2-minute isometric contractions at 30% MVC (determined at the onset of exercise via electronic linear load cells contained within each IHG device) using the same hand with a 4-minute rest interval between on a programmed handgrip dynamometer (Appendix J; ZonaPLUS, Zona HEALTH, Boise, Idaho, USA). Upon completion of this bout, measures of FMD were repeated.

Participants then underwent 30 minutes of supine rest before measures of resistance vessel function were taken.

Neurovascular Measure #2: Venous strain-gauge plethysmography (Appendix K; Hokanson EC6 Strain Gauge and Photo Plethysmograph, D.E. Hokanson, Inc.,

Bellevue, Washington, USA) with reactive hyperemia was used to assess resistance vessel function (Badrov et al., 2013a). The non-dominant upper-arm was elevated above heart level and fitted with an inflatable cuff (Appendix L; Hokanson Cuff, D.E. Hokanson, Inc., Bellevue, Washington, USA). The largest part of the forearm was then fitted with a strain gauge (Hokanson EC6 Strain Gauge and Photo Plethysmograph, D.E. Hokanson, Inc., Bellevue, Washington, USA). The non-dominant wrist was then fitted with a small cuff, which was inflated 60 seconds prior to resting blood flow measurement to a pressure above SBP (200mmHg). Venous blood flow emptying was achieved by inflating the upper arm cuff to 60mmHg (a pressure below DBP) for intervals of 10 seconds, followed by 5 seconds of deflation. Three resting blood flow measurements were performed over 1 minute to calculate mean resting blood flow. Following resting blood flow measurements, forearm ischemia (occlusion of arterial blood flow to the forearm) was achieved by inflating the cuff on the upper arm to 200mmHg. After 5-minutes, the upper arm cuff was released and forearm blood flow was measured every 10 seconds for a total of 60 seconds. Following these measurements, the strain gauge and wrist cuff was removed from the participant, and his/her arm was lowered to his/her side.

Participants then completed a single bout of IHG as described previously in **Neurovascular Measure 1**. Upon completion of this bout, measures of resistance vessel function were repeated.

Neurovascular Measures #1 and #2 occurred in random order on each testing day to minimize any potential order effects.

TESTING DAY 2: Assessment of neurovascular function (indirect assessment of oxidative stress) prior to and following an acute IHG bout in the non-exercised arm (Time: 2.5 hours)

Testing Day 2 mirrored Testing Day 1 with the following exception: neurovascular measures were performed in the non-exercised arm.

TESTING DAY 3: Assessment of neurovascular function (indirect assessment of oxidative stress) prior to and following an acute IR cuff protocol (Time: 2.5 hours)

Testing Day 3 procedures were identical to Testing Day 1 procedures with the exception of the acute IHG bout. The acute IHG bout was replaced by a single bout of the ischemic-reperfusion cuff protocol that mimics the IHG protocol. Participants had an inflatable cuff (Appendix L; Hokanson Cuff, D.E. Hokanson, Inc., Bellevue, Washington, USA) positioned on their non-dominant forearm 3cm distal to the olecranon process. A rapid inflation/deflation cuff was inflated to 200mmHg and held at a constant pressure for 2 minutes to occlude blood flow, after which time the cuff was released, and the participants then had a 4-minute rest period. This sequence was repeated 4 times in succession to complete the bout, mimicking the IHG exercise protocol.

Data Analysis

Brachial artery blood flow velocity. Mean blood flow velocity signals post-occlusion were recorded from the ultrasound (Vivid*i*, GE Healthcare, Pittsburgh, PA, USA) using customized software (Stoik Capturer, Moscow, Russia) directly onto a PC (Dell Optiplex 980, Toronto, Ontario, Canada) as Audio Video Interleave (AVI) files. AVI files were converted to Waveform Audio (WAV) files for processing. WAV files

were then processed using customized software to determine mean blood flow velocity for each QRS interval (Hemodynamic IQ, Windsor, ON, Canada). Mean blood flow velocity was then analyzed using rolling 3-frame average bins (Thijssen et al., 2011), within which the peak bin value was ascertained. Each frame corresponded to a single QRS interval. Resting velocities were recorded for 60-seconds pre-occlusion for later manual analysis (Vivid*i*, GE Healthcare, Pittsburgh, PA, USA). The average of a 20-frame segment was used to determine mean resting blood velocity. The coefficient of variation for blood velocity using our customized program versus traditional manual Doppler ultrasound measurements of mean blood velocity in our laboratory is 4.4%.

Brachial artery diameter. Vessel diameter was determined using automated edge-detection software (Artery Measurement System (AMS) v2.0, Gothenburg, Sweden). A Digital Imaging and Communications in Medicine (DICOM) image was saved at end-diastole for every QRS interval for the entirety of the resting (60 sec) and post-occlusion (180 sec) recordings and merged into two (resting, post-occlusion) stacked DICOM series using DICOM editing software (Sante DICOM Editor version 9.10.9200.16721, Santesoft, Athens, Greece). The stacked DICOM files were then analyzed using AMS software (Artery Measurement System (AMS) v2.0, Gothenburg, Sweden). This program allows a user to identify a region of interest along the portion of the vessel where the near and far walls are clearest. The program collects one diameter measure for each pixel column within the region of interest and provides a mean diameter for the entire frame (approximately 100 measurements). All brachial artery diameters were measured from leading edge to leading edge.

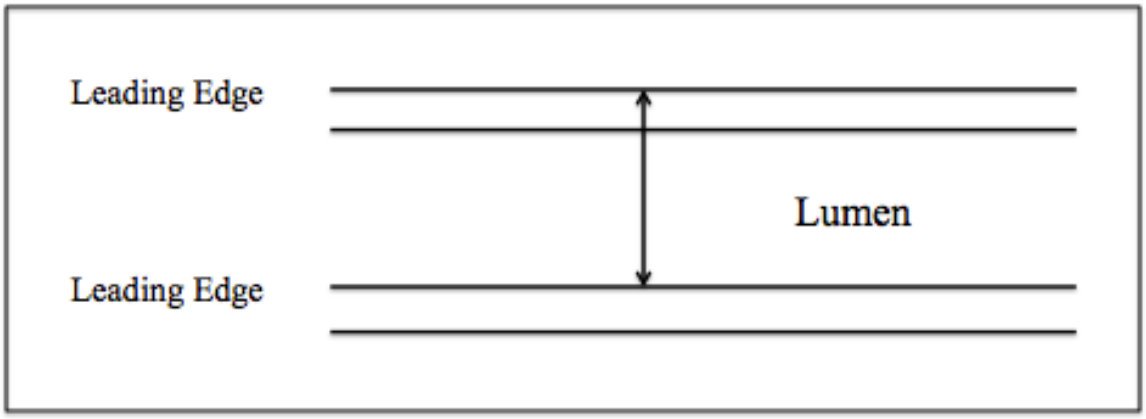

Figure 2. Assessment of BA diameter using AMS

Resting diameter measures were determined by averaging the 20-frame segment of diameters corresponding to the segment used to determine resting blood velocity measures. Brachial artery diameter measures post-release were analyzed using rolling 3-frame average bins. This allowed for diameter and velocity measurements to be frame-aligned at all time points both at rest and post-release (Thijssen et al., 2011).

Both inter- and intra-observer reproducibility were conducted on resting BA diameter measures as this had yet to be done in our laboratory. The inter-observer coefficient of variation was calculated using the formula: CV = SD of resting BA diameter from Investigator 1 & 2 / mean resting BA diameter from investigator 1 & 2 * 100, using measures taken on the CUFF testing day (pre-cuff). Inter-observer method error was calculated using the formula: ME = SD of the difference between investigator 1 & 2 / Grand Mean * 100, using measures on the CUFF testing day. The inter-observer correlation coefficient was calculated by correlating the resting BA diameter measures of investigator 1 with those from investigator 2. Intra-observer reproducibility was determined using pre-cuff resting BA diameter values from the CUFF testing day

compared to those of the EIHG testing day. The coefficient of variation was 2.01%, the method error was 3.16%, and the correlation coefficient was 0.97 for resting BA diameter inter-observer reproducibility (Appendix Q). For resting BA diameter intra-observer reproducibility, the coefficient of variation was 3.10%, the method error was 5.71%, and the correlation coefficient was 0.97 (Appendix Q).

Resting and peak blood flows. Resting blood flow was determined using the mean circumference of the artery at rest calculated using mean diameter values (cm) and resting mean blood velocity values (cm/s). Resting diameter and velocity values were determined using corresponding 20-frame bin segment means (Thijssen et al., 2011). The formula used to calculate resting blood velocity was PI * ((mean resting diameter / 2) ^ 2) * mean resting velocity * 60 and the final value was expressed as milliliters per minute (ml/min). Peak blood flow was determined using the peak circumference of the artery calculated using peak diameter values (cm) and peak mean blood velocity values (cm/s). Peak diameter and mean blood velocity values were determined using corresponding 3-frame bin segment means (Thijssen et al., 2011). The formula used to calculate peak blood velocity was PI * ((mean resting diameter / 2) ^ 2) * mean peak blood velocity * 60 and the final value was expressed as milliliters per minute (ml/min).

Flow mediated dilation. Absolute flow mediated dilation, expressed in millimeters, was calculated using the formula: FMD (mm) = Peak Diameter - Baseline Diameter, where peak diameter was the highest 3-frame bin post-occlusion and baseline diameter was the mean of 20 frames recorded pre-occlusion on the ultrasound (Vivid*i*, GE Healthcare, Pittsburgh, PA, USA). Relative flow mediated dilation was calculated using the formula: FMD (%) = [Peak Diameter - Baseline Diameter] / Baseline

Diameter*100, and reported as percentage change (Corretti et al., 2002). FMD was also normalized using shear rate area under the curve and reported as such. The formula used for normalization was FMD (%) / AUC(s^-1)*1000 (Pyke & Tschakovsky, 2007). It is important to note that normalized FMD to AUC should only be considered if there is at least a consistent, moderate correlation among measures across all research settings (Thijssen et al., 2011). A consistent, moderate correlation was not found within this study.

Shear rate area under the curve. Shear rate area under the curve (AUC) was calculated using velocity and diameter values derived as outlined above. Shear rate for each rolling 3-frame bin segment was calculated using the formula: 8*mean blood velocity / diameter (Harris et al., 2010). Shear rates were calculated from cuff release until peak diameter, signifying peak dilation. Shear rates were graphed over the time interval from cuff release to peak dilation, and AUC was calculated using a macro within the statistical software (SigmaPlot 11.0, Systat Software Inc, San Jose, California).

Resistance vessel function. Resistance vessel function was determined using venous strain-gauge plethysmography. All data were recorded using PowerLab hardware (ML870 PowerLab 8/30) onto LabChart 7 Pro v7.3.7 (ADInstruments, Colorado Springs, CO) on a PC (Dell Optiplex 980, Toronto, Ontario, Canada). The slope of the flow over the first three cardiac cycles for each baseline blood flow curve was determined for each of three measures, and these slopes were then averaged, to determine mean baseline blood flow. After 5-minutes of complete occlusion (200 mmHg), one measure of peak blood flow was recorded. The slope of the first 3 cardiac cycles of the first blood flow curve was recorded as the peak blood flow post-occlusion and used for analysis.

Resistance vessel function, expressed as percentage change, was then calculated using the formula: RVF = [Peak Flow - mean Baseline Flow] / mean Baseline Flow * 100. The intra-observer reproducibility of resistance vessel endothelial function in our laboratory is: 6.6 % (coefficient of variation), 2.2 % (method error), and 0.92 (correlation coefficient). The inter-observer reproducibility of resistance vessel endothelial function in our laboratory is: 4.3 % (coefficient of variation), 10.4 % (method error), and 0.93 (correlation coefficient) (Badrov et al., 2013a).

See Appendix O for raw data.

Statistical Analysis

Baseline data was analyzed using one-way analysis of variance (ANOVA) to determine if there were baseline differences between testing days. Outcome data were analyzed using two-way (Time X Exercise) ANOVA with repeated measures. Tukey post hoc procedures were used to evaluate specific differences between means where applicable (SigmaPlot, v.11.0). An alpha level of ≤ 0.05 was considered statistically significant. Data are presented as mean ± standard deviation (SD), unless otherwise specified. See Appendix P for detailed statistical analysis.

2.4 Results

Participant baseline characteristics are displayed in Table 1. During active recruitment over a period of 6 months, 10 males and 26 females expressed interest in participating in the study. Of those expressing interest, 6 males and 17 females scheduled an initial visit. Due to inclusion and/or exclusion criteria (medication use, activity levels, health status), 4 males and 6 females were deemed ineligible during the initial visit, and

one further male was deemed ineligible during the second visit. Further, 3 females voluntarily withdrew, and an additional female and 2 males did not attend their scheduled visits. Given a final participant total of 7 females, but only a single male, the male data was excluded from further statistical analyses. Primary barriers to participation included medication use, the opportunity cost of time away from work, and the duration of testing days. There were no reported changes in exercise, diet, and/or medication throughout the investigation among all participants. Further, there were no statistically significant differences in baseline SBP, DBP, MAP, HR, pre-exercise resting blood flow, pre-exercise peak blood flow, or brachial artery diameters on any of the testing days (all $p > 0.05$). However, baseline FMD and RVF were not strongly correlated between the CUFF and EIHG testing days indicating day-to-day variability within these measures.

Table 1. Participant characteristics

	Male	Female
N	1	7
Age (years)	63	65 ± 5
Height (cm)	180	161 ± 6
Weight (kg)	74	68 ± 8
BMI	23	26 ± 4
Resting SBP (mmHg)	103 ± 13	100 ± 10
Resting DBP (mmHg)	68 ± 7	60 ± 5
Resting MAP (mmHg)	80 ± 8	74 ± 5
Resting HR (BPM)	60 ± 5	69 ± 5

SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; HR, heart rate. Values are mean ± SD. All $p > 0.05$.

Resting Mean Blood Flow and Peak Mean Blood Flow

There were no significant differences in resting mean blood flow between exercise days either pre-, or post-, exercise intervention (Table 2). Likewise, there were no significant differences in peak mean blood flow between exercise days either pre, or post, exercise intervention (Table 2). Further, there were no significant differences in baseline of peak BA diameter measurements between testing days.

Table 2. Vascular reactivity following exercise intervention (n = 7)

Testing Day		CUFF	EIHG	NEIHG
Resting MBF (cm/s)	Pre	32.52 ± 20.52	34.10 ± 18.41	33.73 ± 15.11
	Post	31.31 ± 26.46	36.51 ± 12.04	25.19 ± 7.06
Resting BA Diameter (mm)	Pre	2.89 ± 0.34	2.94 ± 0.48	3.10 ± 0.31
	Post	2.92 ± 0.40	2.93 ± 0.31	3.05 ± 0.38
Resting SR (s^{-1})	Pre	22.64 ± 12.46	21.29 ± 4.11	18.86 ± 6.67
	Post	22.38 ± 18.67	26.75 ± 15.26	15.31 ± 4.07
Peak MBF (cm/s)	Pre	419.26 ± 168.53	464.05 ± 206.80	422.73 ± 100.02
	Post	380.47 ± 87.60	446.45 ± 135.66	470.94 ± 174.39
Peak BA Diameter (mm)	Pre	3.20 ± 0.40	3.47 ± 0.75	3.40 ± 0.31
	Post	3.12 ± 0.31	3.32 ± 0.55	3.28 ± 0.29
Peak SR (s^{-1})	Pre	239.34 ± 80.10	219.87 ± 52.35	207.62 ± 38.82
	Post	229.42 ± 40.67	234.78 ± 45.82	238.23 ± 55.49

CUFF, ischemic-reperfusion cuff testing day; EIHG, isometric handgrip testing day with vascular measures on the exercised arm; NEIHG, isometric handgrip testing day with vascular measures on the non-exercised arm; Pre, pre exercise/cuff intervention; Post, post exercise/cuff intervention; MBF, mean blood flow; BA, brachial artery; BF, blood flow; SR, shear rate. Values are mean ± SD. All $p > 0.05$.

Flow Mediated Dilation

There were no significant differences noted in relative FMD post-exercise in any of the three exercise bouts (Table 3). See Appendix R for FMD scatter plots by testing day. Normalization of relative FMD to shear rate AUC also showed no significant differences post-exercise for any of the three exercise bouts (Table 3). Additionally, there were no significant differences in shear rate AUC post-bout on any of the testing days.

Table 3. Flow mediated dilation following exercise interventions (n = 7)

Testing Day		CUFF	EIHG	NEIHG
Relative FMD (% change)	Pre	10.86 ± 6.94	13.76 ± 11.74	8.41 ± 7.01
	Post	8.89 ± 8.62	13.32 ± 13.4	7.74 ± 5.12
SR AUC (s^-1)	Pre	4316.96 ± 2759.36	4177.18 ± 2408.96	2494.96 ± 3027.78
	Post	3392.90 ± 2409.29	4416.80 ± 2359.98	3286.47 ± 1682.01
Normalized FMD (FMD:AUC)	Pre	3.25 ± 2.08	5.14 ± 4.65	7.08 ± 7.95
	Post	3.57 ± 3.05	4.01 ± 3.85	2.61 ± 2.18

CUFF, ischemic-reperfusion cuff testing day; EIHG, isometric handgrip testing day with vascular measures on the exercised arm; NEIHG, isometric handgrip testing day with vascular measures on the non-exercised arm; Pre, pre exercise/cuff intervention; Post, post exercise/cuff intervention; FMD, flow-mediated dilation; SR, shear rate; AUC, area under curve. Values are mean ± SD. All $p > 0.05$.

Resistance Vessel Function

There were no significant differences noted in resistance vessel function post-exercise in each of the three exercise interventions (Table 4). See Appendix R for RVF scatter plots by testing day.

Table 4. Resistance vessel function following exercise interventions (n = 6*)

Testing Day		CUFF	EIHG	NEIHG
RVF (% change)	Pre	1107.16 ± 742.27	447.30 ± 283.93	505.35 ± 326.12
	Post	753.44 ± 434.06	333.49 ± 228.80	859.0 ± 290.93

CUFF, ischemic-reperfusion cuff testing day; EIHG, isometric handgrip testing day with vascular measures on the exercised arm; NEIHG, isometric handgrip testing day with vascular measures on the non-exercised arm; Pre, pre exercise/cuff intervention; Post, post exercise/cuff intervention; RVF, resistance vessel function. Values are mean ± SD. All $p > 0.05$. * One participant was removed from statistical analysis as data was missing due to equipment malfunction.

2.5 Discussion

HT is a major modifiable risk factor for the development of CVD (WHO, 2013). Recently, the World Health Organization has identified HT as a global epidemic (WHO, 2013). IHG training is an effective means of lowering resting arterial BP in numerous populations (McGowan et al., 2006; McGowan et al., 2007a; McGowan et al., 2007b; Taylor et al., 2003; Millar et al., 2009; Badrov et al., 2013a). Interestingly, older females with normal resting arterial BP appear to respond to IHG with greater post-training reductions in resting arterial BP than age-matched males (Millar et al., 2008). The mechanism(s) underlying the reduction in resting arterial BP due to IHG training are

currently unknown. It has been proposed that transient exposure to oxidative stress, due to IR induced by the IHG contraction, may promote an adaptive response and an increased basal antioxidant capacity (Peters et al., 2006). This increased basal antioxidant capacity more readily neutralizes oxidative free radicals, increasing the bioavailability of NȮ, improving endothelial function and potentially lowering resting arterial BP. Due to poor recruitment of male participants, this study was unable to address possible sex differences described in the initial hypotheses. However, this is the first study conducted to explore IR as an underlying mechanism modulating resting arterial BP in response to IHG exercise. Further, this is the first study to compare acute IHG exercise to IR induced by a simple cuff.

2.5.1 Effects of an Acute Bout of IHG Exercise on Vascular Reactivity in the Exercised Arm

No significant differences were found in FMD, normalized FMD, or resistance vessel function (RVF), indicating that an acute bout of IHG did not significantly alter vascular reactivity in the exercised arm. However, there were consistent mean trends in the data. Mean vascular reactivity in the exercised arm was suppressed post-IHG exercise when assessed via FMD (13.76 ± 11.74% to 13.32 ± 13.40%), normalized FMD (5.14 ± 4.65 to 4.01 ± 3.85), and RVF (447.30 ± 283.93% to 333.49 ± 228.80%). The acute IHG exercise protocol used in this investigation was unilateral in the non-dominant arm, with 4-minutes rest between contractions. In contrast, the protocol used by Peters and colleagues (2006) was a bi-lateral protocol with 1-minute rest between contractions. As such, the added 3-minutes of rest after the final contraction before post-IHG testing may have functioned as a washout period reducing the possibility of noting any changes

in vascular reactivity. Alternatively, it is possible that the limited number of participants (n=7), and their degree of inter-individual variation, did not allow for adequate power to detect significant changes in vascular reactivity following an acute IHG bout.

The potential also exists that the acute bout of IHG exercise used in this study did not provide a stimulus strong enough to elicit an acute state of oxidative stress. If the basal antioxidant capacity of the participants was not exceeded it is conceivable that NȮ bioavailability may not have been decreased enough to elicit a significant change in acute vascular reactivity. However, if this were indeed true, it would diminish the possibility that modulations in basal antioxidant capacity due to repeated bouts of acute IHG exercise is a mechanism underlying the resting arterial BP response to IHG training. Nonetheless, further investigation with increased power is necessary.

2.5.2 Effects of an Acute Bout of IHG Exercise on Vascular Reactivity in the Non-Exercised Arm

Similar to that observed after an acute IHG bout in the exercised arm, no significant differences were found in FMD normalized FMD, or RVF in the non-exercised arm. However, while FMD (8.41 ± 7.01% to 7.74 ± 5.12%), and normalized FMD (7.08 ± 7.95 to 2.61 ± 2.18), both trended down in a similar direction to that of the exercised arm, RVF (505.35 ± 326.12% to 859.00 ± 290.93%) did not follow this trend

Peak MBF, an indirect index of RVF, also increased post IHG bout in the non-exercised arm mirroring the RVF measure. This provides further support for the observation that an acute IHG exercise bout did not decrease vascular reactivity in the RVF of the non-exercised arm. As described previously, the acute IHG exercise protocol

used in this investigation was unilateral in the non-dominant arm, with 4-minutes rest between contractions. The added 3-minutes of rest between the final contraction and vascular reactivity testing may function as a washout period, reducing the possibility of noting any changes.

Given that FMD is an indicator of vascular reactivity in the larger conduit arteries (Corretti et al., 2002), and RVF is an indicator of vascular reactivity in the smaller downstream vessels (Higashi & Yoshizumi, 2003), it is possible that the stimulus strength was lower in the non-exercised arm and thus did not effect the downstream resistance vessels as potently. If an acute bout of IHG exercise does indeed elicit localized IR and, in turn, localized oxidative stress, it is possible that these excess free radicals are neutralized before reaching the distal resistance vessel of the contralateral arm. However, this may indicate that an acute bout of IHG exercise does not elicit a systemic effect, at least not at the level of the resistance vessel, putting into question their role in the known resting arterial BP response to IHG exercise.

Furthermore, the role of arm dominance cannot be discounted when comparing the response to an acute IHG bout. As all exercise was conducted in the non-dominant arm, vascular reactivity on this testing day was measured in the dominant arm. It is possible that, due to favoured use over the lifespan, the dominant arm is relatively more trained than the non-dominant arm. As such, the stimulus provided by the acute IHG bout in the non-dominant arm may have not been strong enough to elicit any changes in vascular reactivity in the dominant arm. As touched on previously, the variability between the small number of participants (n=7) makes it difficult to draw clear

conclusions. Nonetheless, the difference in trends of vascular reactivity in the exercise and non-exercised arms does provide interesting theoretical implications.

2.5.3 Effects of an Acute Bout of Ischemic-Reperfusion on Vascular Reactivity in the Reperfused Arm

In response to a bout of IR mimicking the timing and duration of the IHG protocol there were no significant differences observed in vascular reactivity measured in the arm of reperfusion. Although not statistically significant, both FMD (10.86 ± 6.94 to 8.89 ± 8.62) and RVF (1107.16 ± 742.27% to 753.44 ± 434.06%) decreased post-IR as observed in the exercised arm after a bout of IHG. Interestingly, once FMD was normalized to shear rate area under the curve this trend was reversed (3.25 ± 2.08 to 3.57 ± 3.05). However, as previously stated, normalization of FMD to AUC should only be considered when there is at least a correlation across all research settings (Thijssen et al., 2011). Given that a consistent, moderate correlation did not exist between these measures in this study relative FMD would be a more applicable index of vascular reactivity (See Appendix P).

The IR protocol applied in this investigation used an inflation pressure of 200 mmHg, a pressure significantly above SBP, to ensure full occlusion of the lower arm. As such, this stimulus may be stronger than that of an acute IHG bout as IHG does not elicit ischemia progressively until 30% MVC and above (Sadamoto et al., 1983; Sakakibara & Honda, 1990). It is therefore conceivable that oxidative stress would be increased to a greater degree via the cuff than that of the IHG, leading to greater reductions in vascular reactivity after IR induced by a cuff. However, a lack of significance does not allow for

meaningful comparison. Although the direction of mean changes between the cuff and the acute IHG bout in the exercised arm are similar, a lack of statistical significance, likely due to a lack of power, does not allow for adequate comparison.

The position of the IR cuff used to mimic the acute IHG bout in this study was located on the forearm. The rationale behind this placement was that muscular contraction during a bout of IHG is primarily in the forearm, however contributions from the muscles of the upper arm cannot be ruled out. If the IR cuff was placed above the elbow, eliciting occlusion and reperfusion of a greater tissue volume as well as part of the brachial artery, it is likely that the stimulus strength would be higher, which may produce differing results. An interesting direction of future research would be the comparison of various IR cuff pressures and positions to an IHG exercise bout to determine relative equivalencies. This would allow for more direct IR stimulus comparisons.

2.6 Perspectives

A number of interesting and important concepts were explored within this project, including the acute effects of a bout of IHG exercise, the role of IR as a potential mechanism underlying resting arterial BP changes in response to IHG exercise, and the systemic effects of an acute bout of IHG exercise. Further, the difficulties in studying this specific population were identified for future investigations. Participant recruitment was of particular difficulty predominantly due to the medical status of most individuals within this population as well as the time commitment necessary to complete the study. Post hoc sample size analysis determined a sample size range of 5 to 217, depending on the variable, to reach statistical significance. Future studies should attempt to quantify IR

stimuli relative to IHG contraction strengths to determine relative equivalencies, compare an equivalent IR stimuli to the most common IHG exercise protocols in the training literature, including the most common bilateral protocol which employs minimal rest between contractions, investigate potential differences in vascular reactivity after an acute bout of IHG in the exercised and non-exercised arms to determine systemic effects, and investigate IR training to determine if resting arterial BP would decrease similar to that observed after IHG training. As the limited male participant recruitment of this study did not allow for a sex-based comparison, the response to an acute bout of IHG between sexes should also be investigated.

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Appendix A: Informed Consent

CONSENT TO PARTICIPATE IN RESEARCH

Title of Study: Gender differences in the neurovascular response to acute isometric handgrip exercise and a complimentary ischemic-reperfusion cuff protocol

You are asked to participate in a research study conducted by *Josh Seifarth, BHk*, & *Dr. Cheri McGowan*, PhD, from the Department of Kinesiology at the University of Windsor.

If you have any questions or concerns about the research, please feel to contact Josh Seifarth via telephone (519-253-3000 ext. 4979).

PURPOSE OF THE STUDY

Hypertension increases the risk of cardiovascular disease. Isometric handgrip exercise (constant squeeze) training is a type of exercise training that lowers blood pressure (BP) in people with and without high BP. The goal of this study is to see if men and women respond differently (i.e., blood pressure, heart rate, blood vessels) to a single bout of handgrip exercise. We will also see if inflating a cuff around your lower arm will have the same results as a single bout of handgrip exercise.

Familiarization Session (30 minutes):

In the familiarization session, you will be introduced to all the techniques that will be used in this study. To minimize anything that may influence results, we will ask you to refrain from the consumption of alcohol and participation in vigorous exercise for 24 hours prior to each testing session, and to avoid caffeine consumption for 12 hours prior. Further, we ask that you refrain from eating for 4 hours prior to all testing sessions. All testing will be conducted in a quiet, temperature-controlled room at the same time

of day. You will be asked to void their bladder prior to all testing sessions to minimize the effects of a full bladder on resting BP.

The following testing days will be conducted in random order, and the testing measures within each day will also be in random order.

Testing Day 1 (approximately 3 hours):

First, your resting blood pressure will be measured in your upper arm after 10 minutes of seated rest. Your blood pressure will be measured 4 times, with 2-minutes of rest between measures. This will repeated again at least 24-hours after Testing Day 1.

Next, while remaining seated, a blood pressure cuff will be placed on your non-dominant forearm. A technique called flow-mediated dilation will be used on your upper arm to measure the size of your artery and the speed of blood flowing through it. To do this a probe with ultrasound gel will be placed on your upper arm. The cuff on your forearm will be inflated to ~200mmHg for 5 minutes and then released. Next, you will engage in 4 bouts of handgrip exercise in your non-dominant arm comprising 2-minute squeezes with 4-minute breaks between each. Following the exercise, measures of artery size and blood flow will be repeated. You will then lie on your back for 30 minutes of rest.

After 30 minutes of rest, blood vessel function will be measured using a technique called venous strain-gauge plethysmography. In this procedure, your non-dominant arm will be elevated above heart level and a rubber strain gauge (similar to an elastic) will be placed around the largest part of your forearm. A cuff will be placed on your wrist and will be inflated to a pressure above systolic BP (generally close to 200mmHg). The cuff on your upper arm will be inflated for 10 seconds and then released for 5 seconds, and this will continue for 1 minute. Next, the upper arm cuff will be inflated to 200mmHg for 5 minutes and then released. Forearm blood flow will be measured every 10 seconds for 1 minute after the cuff is released. You will then engage in 4 bouts of handgrip exercise in your non-dominant arm comprising 2-minute squeezes with 4-minute breaks between each.

Following the exercise, the blood vessel function technique will be repeated as described above.

Testing Day 2 (approximately 3 hours):

Testing Day 2 will mirror Testing Day 1 with the following exception: testing measures will be performed in the non-exercised limb. You will exercise using the same arm as Testing Day 1 (non-dominant arm), but we will measure all of our variables in your non-exercising arm (dominant arm).

Testing Day 3 (approximately 3 hours):

Testing Day 3 Procedures are identical to Testing Day 1 procedures with the exception of the hand-grip bout. The hand-grip bout will be replaced by a single bout of cuff inflation and deflation. You will have an inflatable cuff positioned on your non-dominant forearm below the elbow. The cuff will be inflated above systolic BP and held at a constant pressure for 2 minutes, after which time the cuff will be released for 4 minutes. This sequence will be repeated 4 times in a row to complete the bout so to mimic what occurs during the hand grip exercise. This protocol should produce minimal discomfort as the duration and pressure is well below what has been found to cause even low discomfort.

POTENTIAL RISKS AND DISCOMFORTS

You may experience tendonitis in the tendons of the exercising arms with handgrip exercise however this risk is minimal if the exercise is properly performed. The blood pressure and blood flow measurement procedures are non-invasive but you may experience numbness and/or tingling in the limb with the cuff(s) while we are taking our measurements. The gel used to image your blood vessels and/or the sticker-electrodes used to measure your heart rate may cause a skin irritation however, this risk is minimal.

Please contact one of the study investigators if you feel any adverse effects from completing any portion of the study, and/or if you have any questions or concerns. Exercise leaders and study investigators will reinforce proper exercise technique throughout the study. If you experience any adverse effects during any testing procedure, first line response will be provided.

POTENTIAL BENEFITS TO SUBJECTS AND/OR TO SOCIETY

You may or may NOT experience a lower blood pressure at rest or during your activities of daily life after each part of the study. In addition, you may or may not have improvements in the ability of your heart to vary its rhythm, a decrease in the activity of your nervous system, reduced tension in certain blood vessels of your body, an increase in the efficiency of the work of your heart, and/or reduced oxidative stress.

If we prove our theories, isometric handgrip training may be a possible prevention and/or treatment option for older males and females, like you.

PAYMENT FOR PARTICIPATION

You will receive a Human Kinetics T-shirt for your participation, and will be reimbursed for health care provider forms and/or parking costs over the course of the study.

CONFIDENTIALITY

Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission.

To ensure your confidentiality, following your consent, you will be assigned an identification number. Your name will not be mentioned in any publication or presentation, and you will be identified with only your identification number on all collection tools (electronic or otherwise). All paper data and all electronic data will be stored in the locked laboratory (PACR Lab, Room #240, Human Kinetics Building, University of Windsor) of the study investigators. Information stored on computer will be password-accessible only. With respect to final disposal, all paper records (including medical and physical activity readiness questionnaires) will be shredded.

PARTICIPATION AND WITHDRAWAL

You can choose whether to be in this study or not. If you volunteer to be in this study, you may withdraw at any time without consequences of any kind. You may also refuse to answer any questions you do not wish to answer and

still remain in the study. The investigator may withdraw you from this research if circumstances arise which warrant doing so (e.g., medication, nutrition and/or physical activity change).

FEEDBACK OF THE RESULTS OF THIS STUDY TO THE PARTICIPANTS

You will have the option of receiving results from the study either verbally, by mail or email. Results will also be posted on the University of Windsor's Research Ethics Board (REB) website (http://www.uwindsor.ca/reb) at the completion of the study.

SUBSEQUENT USE OF DATA

This data may be used in subsequent studies however your privacy will be upheld with the use of your unique subject identification number under all circumstances.

RIGHTS OF RESEARCH PARTICIPANTS

You may withdraw your consent at any time and discontinue participation without penalty. If you have questions regarding your rights as a research subject, contact: Research Ethics Coordinator, University of Windsor, Windsor, Ontario, N9B 3P4; Telephone: 519-253-3000, ext. 3948; e mail: ethics@uwindsor.ca

SIGNATURE OF RESEARCH PARTICIPANT/LEGAL REPRESENTATIVE

I understand the information provided for the study: Gender differences in the vascular response to acute IHG exercise as described herein. My questions have been answered to my satisfaction, and I agree to participate in this study. I have been given a copy of this form.

_____

Name of Participant

_____ _____

Signature of Participant Date

SIGNATURE OF INVESTIGATOR

These are the terms under which I will conduct research.

_____ _____

Signature of Investigator Date

Version Date: 03/12/13

Appendix B: LETTER OF INFORMATION

LETTER OF INFORMATION

Title of Study: Gender differences in the neurovascular response to acute isometric handgrip exercise and a complimentary ischemic-reperfusion cuff protocol

You are asked to participate in a research study conducted by *Josh Seifarth, BHk*, & *Dr. Cheri McGowan*, PhD, from the Department of Kinesiology at the University of Windsor.

If you have any questions or concerns about the research, please feel to contact Josh Seifarth via telephone (519-253-3000 ext. 4979).

PURPOSE OF THE STUDY

Hypertension increases the risk of cardiovascular disease. Isometric handgrip exercise (constant squeeze) training is a type of exercise training that lowers blood pressure (BP) in people with and without high BP. The goal of this study is to see if men and women respond differently (i.e., blood pressure, heart rate, blood vessels) to a single bout of handgrip exercise. We will also see if inflating a cuff around your lower arm will have the same results as a single bout of handgrip exercise.

Familiarization Session (30 minutes):

In the familiarization session, you will be introduced to all the techniques that will be used in this study. To minimize anything that may influence results, we will ask you to refrain from the consumption of alcohol and participation in vigorous exercise for 24 hours prior to each testing session, and to avoid caffeine consumption for 12 hours prior. Further, we ask that you refrain from eating for 4 hours prior to all testing sessions. All testing will be conducted in a quiet, temperature-controlled room at the same time

of day. You will be asked to void their bladder prior to all testing sessions to minimize the effects of a full bladder on resting BP.

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After 30 minutes of rest, blood vessel function will be measured using a technique called venous strain-gauge plethysmography. In this procedure, your non-dominant arm will be elevated above heart level and a rubber strain gauge (similar to an elastic) will be placed around the largest part of your forearm. A cuff will be placed on your wrist and will be inflated to a pressure above systolic BP (generally close to 200mmHg). The cuff on your upper arm will be inflated for 10 seconds and then released for 5 seconds, and this will continue for 1 minute. Next, the upper arm cuff will be inflated to 200mmHg for 5 minutes and then released. Forearm blood flow will be measured every 10 seconds for 1 minute after the cuff is released. You will then engage in 4 bouts of handgrip exercise in your non-dominant arm comprising 2-minute squeezes with 4-minute breaks between each.

Following the exercise, the blood vessel function technique will be repeated as described above.

Testing Day 2 (approximately 3 hours):

Testing Day 2 will mirror Testing Day 1 with the following exception: testing measures will be performed in the non-exercised limb. You will exercise using the same arm as Testing Day 1 (non-dominant arm), but we will measure all of our variables in your non-exercising arm (dominant arm).

Testing Day 3 (approximately 3 hours):

Testing Day 3 Procedures are identical to Testing Day 1 procedures with the exception of the hand-grip bout. The hand-grip bout will be replaced by a single bout of cuff inflation and deflation. You will have an inflatable cuff positioned on your non-dominant forearm below the elbow. The cuff will be inflated above systolic BP and held at a constant pressure for 2 minutes, after which time the cuff will be released for 4 minutes. This sequence will be repeated 4 times in a row to complete the bout so to mimic what occurs during the hand grip exercise. This protocol should produce minimal discomfort as the duration and pressure is well below what has been found to cause even low discomfort.

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Please contact one of the study investigators if you feel any adverse effects from completing any portion of the study, and/or if you have any questions or concerns. Exercise leaders and study investigators will reinforce proper exercise technique throughout the study. If you experience any adverse effects during any testing procedure, first line response will be provided.

POTENTIAL BENEFITS TO SUBJECTS AND/OR TO SOCIETY

You may or may NOT experience a lower blood pressure at rest or during your activities of daily life after each part of the study. In addition, you may or may not have improvements in the ability of your heart to vary its rhythm, a decrease in the activity of your nervous system, reduced tension in certain blood vessels of your body, an increase in the efficiency of the work of your heart, and/or reduced oxidative stress.

If we prove our theories, isometric handgrip training may be a possible prevention and/or treatment option for older males and females, like you.

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You will receive a Human Kinetics T-shirt for your participation, and will be reimbursed for health care provider forms and/or parking costs over the course of the study.

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Any information that is obtained in connection with this study and that can be identified with you will remain confidential and will be disclosed only with your permission.

To ensure your confidentiality, following your consent, you will be assigned an identification number. Your name will not be mentioned in any publication or presentation, and you will be identified with only your identification number on all collection tools (electronic or otherwise). All paper data and all electronic data will be stored in the locked laboratory (PACR Lab, Room #240, Human Kinetics Building, University of Windsor) of the study investigators. Information stored on computer will be password-accessible only. With respect to final disposal, all paper records (including medical and physical activity readiness questionnaires) will be shredded.

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Version Date: 03/12/13

Appendix C: Medical Questionnaire

Last Name _____ First Name _____

Height:_____ Weight: _____ Date of Birth (Month/Yr) _____

Phone (_____) _____ Postal Code _____

FOR EMERGENCY NOTIFY: Name _____ Relationship _____

Address_____ Phone _____

Family Doctor's Name_____ Date of Last Physical _____

Please Check Yes or No:

	Yes	No
1. Have you ever been hospitalized?	❑	❑
If yes, please specify? _____		
Have you ever had surgery?	❑	❑
If yes, please specify? _____		
2. Are you presently taking any medications or pills (including aspirin and other over-the-counter medication)?	❑	❑
If yes, please specify? _____		
Are you presently taking any vitamins, supplements, and/or herbal supplements?	❑	❑
3. Do you have any allergies (medicine, food, bees or other stinging insects)?	❑	❑
If yes, please specify? _____		
4. Have you ever passed out during or after exercise?	❑	❑
Have you ever been dizzy during or after exercise?	❑	❑
Have you ever had chest pain during or after exercise?	❑	❑
Do you have high blood pressure (hypertension) or low blood pressure (hypotension)?	❑	❑
Have you ever been told that you have a kidney problem?	❑	❑
Have you ever been told that you have joint instability?	❑	❑

Have you ever been told that you have a stomach problem? ..... ❑ ❑

Have you ever been told that you have a heart problem? ..... ❑ ❑

Have you ever been told that you have a heart murmur? ..... ❑ ❑

Do you have a machine that regulated your heart beat? ..... ❑ ❑

Have you ever had racing of your heart or skipped heartbeats? ..... ❑ ❑

Has anyone in your family died of heart problems or a sudden death before age 50? ..... ❑ ❑

5. Do you have any skin problems (itching, rashes, acne)? ..... ❑ ❑

If you get a cut, does it take you a long time to stop bleeding? ..... ❑ ❑

If you experience a blow to a muscle, to you bruise easily? ..... ❑ ❑

6. Do you have Diabetes? ..... ❑ ❑

7. Do you have Asthma or any other breathing problems? ..... ❑ ❑

If yes, please specify?

_____

8. Do you have any type of cardiovascular disease? ..... ❑ ❑

If yes, please specify? _____

9. Have you had any other medical problems (infectious mononucleosis, etc.)? ..... ❑ ❑

10. Have you had any medical problems since your last physical? ..... ❑ ❑

11. Do you smoke? ..... ❑ ❑

12. Do you aerobically exercise (e.g., walking) for $\geq$ 30 minutes, > 2 times per week? ..... ❑ ❑

Please explain any physical limitations that may prevent you from completing this study:

_____

Appendix D: Resting BP Device

Dinamap Carescape v100, Critikon, Tampa, Florida, USA

Appendix E: Physical Activity Readiness Medical Examination (PAR MED-X)

PARmed-X PHYSICAL ACTIVITY READINESS MEDICAL EXAMINATION

Appendix F: Physical Activity Readiness Questionnaire (PAR-Q)

Appendix G: Healthcare Provider Form

Date: _____.

Dear _____,

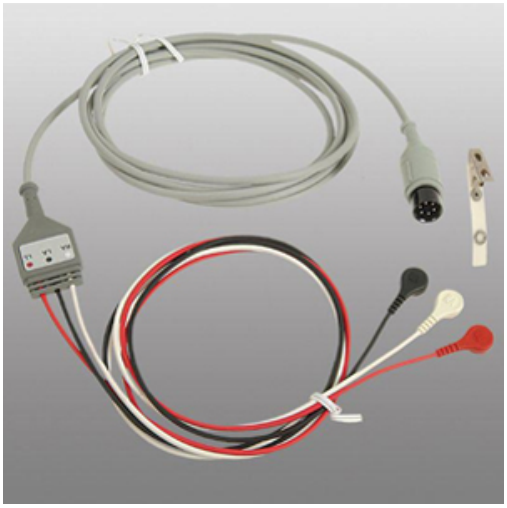
Your patient, _____, has expressed interest in participating in our research study in the Department of Kinesiology at the University of Windsor entitled: Gender differences in the vascular response to acute IHG exercise (see attached Letter of Information for Consent for details). If you approve your patient's participation in our study (via PAR-medX), we kindly request that you or a representative inform us of any changes in medically-endorsed exercise, nutrition and/or medication status, as these changes may influence our findings. We ask that you sign the attached form for return with the PAR Med-X to acknowledge your receipt of this request.

Thank you for your help, and we appreciate your support. Please do not hesitate to contact us if you have any questions or concerns.

Sincerely,

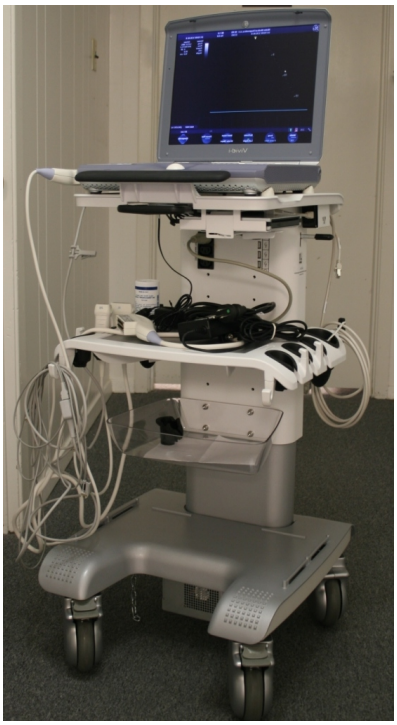
Dr. Cheri McGowan, Ph.D
Assistant Professor
Department of Kinesiology
Faculty of Human Kinetics
University of Windsor

Appendix H: ECG & PowerLab (Data Recording Device)



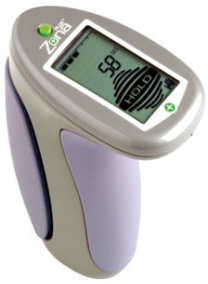
Powerlab ML 870/P, AD Instruments, Colorado Springs, Colorado, USA

Appendix I: Ultrasound Machine



Vivid *i*, GE Healthcare, Pittsburgh, Pennsylvania, USA

Appendix J: Isometric Handgrip



ZonaPLUS, Zona HEALTH, Boise, Idaho, USA

Appendix K: Plethysmography System

Hokanson EC6 Strain Gauge and Photo Plethysmograph, D.E. Hokanson, Inc., Bellevue, WA, USA

Appendix L: Hokanson Inflatable Cuff

Hokanson E20 Cuff Inflator, D.E. Hokanson, Inc., Bellevue, Washington, USA

Appendix M: Recruitment Script & Poster

Local media/newspaper/email recruitment script:

" Attention all men and women over the age of 60 years with normal blood pressure. You may be eligible to participate in a research study at the University of Windsor being conducted by Josh Seifarth, BHK and Dr. Cheri McGowan, PhD. We are investigating the effects of a single bout of isometric handgrip exercise on your blood pressure, heart rate and blood vessels. For more information please contact Josh Seifarth at 519-253-3000 ex. 4979 or seifart@uwindsor.ca."

Josh Seifarth, BHK, and Dr. Cheri McGowan, PhD, at the University of Windsor is currently looking for individuals over the age of 60 years with normal blood pressure to participate in a study examining the effects of isometric handgrip exercise on blood pressure, heart rate and blood vessels.

If you are interested and would like more information please contact

5	5	5	5	5	5	5	5	5	5	5	5	5
1	1	1	1	1	1	1	1	1	1	1	1	1
9	9	9	9	9	9	9	9	9	9	9	9	9
-	-	-	-	-	-	-	-	-	-	-	-	-
2	2	2	2	2	2	2	2	2	2	2	2	2
5	5	5	5	5	5	5	5	5	5	5	5	5
3	3	3	3	3	3	3	3	3	3	3	3	3
-	-	-	-	-	-	-	-	-	-	-	-	-
3	3	3	3	3	3	3	3	3	3	3	3	3
0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0
ext.	ext.	ext.	ext.	ext.	ext.	ext.	ext.	ext.	ext.	ext.	ext.	ext.
4979	4979	4979	4979	4979	4979	4979	4979	4979	4979	4979	4979	4979

Josh Seifarth, BHK: (519)-253-3000 ext. 4979 or seifart@uwindsor.ca

This study has been cleared by the University of Windsor's Research Ethics Board

Appendix N: Emergency Action Plan (EAP)

EMERGENCY ACTION PLAN (EAP)

FOR MEDICAL EMERGENCIES DURING EXERCISE TESTING

STEP 1: **REMAIN CALM.**

CONTROL and **ASSESS** the situation.

Campus Police EXT. 4444 OR **911**
(they will dispatch required authorities)

OUR ADDRESS/DIRECTIONS:

The University of Windsor
Human Kinetics Building
2555 College Ave.
Main Entrance off College Ave.

Room# 240 (uppermost floor)

Go in through the main doors of the Human Kinetics Building. There will be doors on the left hand side. Go Up the stairs, and go to the end of the hallway. Turn left and walk to the end of the short hallway, turn left, room 240 will be the first door on your right hand side.

OUR PHONE#:

STEP 2: **PERFORM** all measures (CPR/First Aid) to ensure safety of subject.

ATTEND to subject until replaced by emergency personnel.

Appendix O: Raw Data for Chapter 2

Participant Characteristics

Participant Characteristics					
Participant	**Age (years)**	**Sex**	**Height (cm)**	**Weight (kg)**	**BMI (kg/m2)**
1	63	M	180.3	73.9	22.7
2	63	F	156.2	72.6	29.8
3	63	F	160.0	67.1	26.2
4	68	F	162.6	65.8	24.9
5	72	F	157.5	53.5	21.6
6	63	F	170.2	65.8	22.7
7	59	F	167.6	73.5	26.2
8	64	F	154.9	79.4	33.1

Resting Systolic Blood Pressure Data (mmHg)					
	Testing Day				
Participant	**IV**	**FAM**	**CUFF**	**E IHG**	**NE IHG**
1	108.0	101.3	122.0	88.3	97.0
2	102.0	97.7	98.3	96.7	95.7
3	102.3	136.0	114.0	96.7	100.3
4	96.3	100.7	84.3	85.0	85.3
5	106.7	94.3	91.3	93.7	90.3
6	99.0	104.3	94.7	98.0	100.3
7	99.3	105.3	100.7	96.7	110.3
8	110.7	101.3	112.3	108.7	105.7

Resting Diastolic Blood Pressure Data (mmHg)					
	Testing Day				
Participant	IV	FAM	CUFF	E IHG	NE IHG
1	64.7	70.7	78.3	60.7	65.7
2	61.7	60.7	57.7	68.0	64.3
3	56.7	65.3	64.3	54.7	59.0
4	54.3	58.3	54.7	53.3	50.0
5	58.7	57.7	58.3	61.3	55.7
6	63.7	65.3	60.0	62.7	64.0
7	64.0	61.0	62.3	61.3	63.0
8	65.3	70.7	62.3	56.3	57.7

Mean Arterial Pressure Data (mmHg)					
	Testing Day				
Participant	IV	FAM	CUFF	E IHG	NE IHG
1	79.1	80.9	92.9	69.9	76.1
2	75.1	73.0	71.2	77.6	74.8
3	71.9	88.9	80.9	68.7	72.8
4	68.3	72.4	64.6	63.9	61.8
5	74.7	69.9	69.3	72.1	67.2
6	75.4	78.3	71.6	74.4	76.1
7	75.8	75.8	75.1	73.1	78.8
8	80.4	80.9	79.0	73.8	73.7

Resting Heart Data (bpm)					
	Testing Day				
Participant	**IV**	**FAM**	**CUFF**	**E IHG**	**NE IHG**
1	56.0	69.7	60.0	59.0	57.3
2	77.0	74.7	77.3	75.0	74.0
3	65.3	60.3	64.3	70.0	71.3
4	74.0	76.0	66.7	68.3	69.7
5	74.3	67.3	73.3	70.3	73.3
6	68.7	69.0	74.0	75.0	66.0
7	70.7	62.0	63.0	63.3	68.0
8	62.0	69.7	66.3	58.3	57.3

Resting and Peak Blood Flow Data

Pre CUFF Testing Flows Female		
Participant	Resting Flow (cm/s)	Peak Flow (cm/s)
2	22.22519733	404.5078673
3	20.35982427	382.7482208
4	23.99261081	220.9790071
5	47.81266657	570.7514923
6	73.14511132	366.1467813
7	18.4721024	279.9516878
8	21.63822891	709.7212716

Pre EIHG Testing Flows Female		
Participant	Resting Flow (cm/s)	Peak Flow (cm/s)
2	14.60205232	205.5673214
3	19.59163852	434.3219139
4	28.77236416	581.7644794
5	48.13869258	847.6186324
6	31.1149305	413.5066312
7	28.10489987	304.8371524
8	68.35749489	460.7339396

Pre NEIHG Testing Flows Female		
Participant	Resting Flow (cm/s)	Peak Flow (cm/s)
2	22.29328193	425.2542218
3	21.63324871	376.0325289
4	25.33594238	403.88686
5	45.90914855	561.0371047
6	53.16600149	265.6374032
7	18.11859245	391.7234726
8	49.63268045	535.50808

Post CUFF Testing Flows Female		
Participant	Resting Flow (cm/s)	Peak Flow (cm/s)
2	17.73813899	270.1877329
3	21.42480324	356.5324867
4	20.83494608	327.5638129
5	23.27570226	435.5061611
6	91.06042693	528.5279628
7	19.57201412	320.3457525
8	25.25685597	424.5895606

Post EIHG Testing Flows Female		
Participant	Resting Flow (cm/s)	Peak Flow (cm/s)
2	56.16773976	246.2693679
3	33.79627911	377.0957874
4	22.34190045	605.5760602
5	27.60999991	624.277575
6	41.10740014	416.1127884
7	27.95703032	368.7801058
8	46.62203405	487.0128601

Post NEIHG Testing Flows Female		
Participant	Resting Flow (cm/s)	Peak Flow (cm/s)
2	17.11948178	257.0308573
3	26.2993078	482.7056463
4	18.87184917	313.8152303
5	30.25820127	728.2940992
6	25.25452248	477.5617903
7	21.06789363	375.2653923
8	37.44781531	661.9019017

Pre CUFF Testing Flows Male		
Participant	Resting Flow (cm/s)	Peak Flow (cm/s)
1	188.0804487	1054.058321

Pre EIHG Testing Flows Male		
Participant	Resting Flow (cm/s)	Peak Flow (cm/s)
1	217.2368179	1072.10085

Pre NEIHG Testing Flows Male		
Participant	Resting Flow (cm/s)	Peak Flow (cm/s)
1	68.0977749	967.7796548

Post CUFF Testing Flows Male		
Participant	Resting Flow (cm/s)	Peak Flow (cm/s)
1	123.6441267	1114.223419

Post EIHG Testing Flows Male		
Participant	Resting Flow (cm/s)	Peak Flow (cm/s)
1	168.586634	964.9555118

Post NEIHG Testing Flows Male		
Participant	Resting Flow (cm/s)	Peak Flow (cm/s)
1	102.3508548	733.0389512

Resting Diameter, Peak Diameter, and Flow-Mediated Dilation Data

Pre CUFF Testing FMD Female				
Participant	Resting Diameter (mm)	Peak Diameter (mm)	FMD Absolute (mm)	Non-Normalized FMD Relative (%)
2	2.3242	2.858	0.5338	22.96704242
3	2.6812	2.8473	0.1661	6.194987319
4	2.8376	2.926	0.0884	3.115308712
5	3.4228	3.8877	0.4649	13.58244712
6	2.9678	3.2867	0.3189	10.74533324
7	2.9076	3.0443	0.1367	4.701472004
8	3.1154	3.5743	0.4589	14.73005072

Pre EIHG Testing FMD Female				
Participant	Resting Diameter (mm)	Peak Diameter (mm)	FMD Absolute (mm)	Non-Normalized FMD Relative (%)
2	2.1224	2.5393	0.4169	19.64285714
3	2.6128	2.9745	0.3617	13.84338641
4	2.8872	3.9437	1.0565	36.59254641
5	3.4878	4.8547	1.3669	39.19089397
6	3.1057	3.2863	0.1806	5.815114145
7	2.9293	3.21	0.2807	9.582494111
8	3.4487	3.4605	0.0118	0.342157915

Pre NEIHG Testing FMD Female				
Participant	Resting Diameter (mm)	Peak Diameter (mm)	FMD Absolute (mm)	Non-Normalized FMD Relative (%)
2	2.9457	3.3747	0.429	14.56360118
3	2.7104	3.5517	0.8413	17.20410272
4	2.9095	3.3167	0.4072	13.99553188
5	3.9078	3.988	0.0802	2.052305645
6	3.03675	3.095	0.05825	0.019181691
7	2.8101	3.0547	0.2446	8.704316572
8	3.362	3.4393	0.0773	2.299226651

Post CUFF Testing FMD Female				
Participant	Resting Diameter (mm)	Peak Diameter (mm)	FMD Absolute (mm)	Non-Normalized FMD Relative (%)
2	2.3633	2.9017	0.5384	22.78170355
3	2.7064	2.8693	0.1629	6.019065918
4	2.7724	3	0.2276	8.20949358
5	3.4544	3.701	0.2466	7.13872163
6	2.8989	3.3313	0.4324	14.91600262
7	2.7911	2.8907	0.0996	3.568485543
8	3.4373	3.1407	-0.2966	-8.628865679

Post EIHG Testing FMD Female				
Participant	Resting Diameter (mm)	Peak Diameter (mm)	FMD Absolute (mm)	Non-Normalized FMD Relative (%)
2	2.5347	2.4783	-0.0564	-2.225115398
3	2.6277	2.8103	0.1826	6.949042889
4	2.7241	3.7595	1.0354	38.00888367
5	3.3155	4.0787	0.7632	23.01915247
6	3.0084	3.4393	0.4309	14.32322829
7	2.9966	3.1883	0.1917	6.397250217
8	3.2932	3.515	0.2218	6.735090489

Post NEIHG Testing FMD Female				
Participant	Resting Diameter (mm)	Peak Diameter (mm)	FMD Absolute (mm)	Non-Normalized FMD Relative (%)
2	2.817	2.9943	0.1773	6.293929712
3	2.7487	3.0203	0.2716	9.881034671
4	2.9071	3.1957	0.2886	9.927419077
5	3.7906	3.8293	0.0387	1.020946552
6	3.1089	3.2127	0.1038	3.338801505
7	2.7402	3.199	0.4588	16.74330341
8	3.2487	3.476	0.2273	6.996644812

Pre CUFF Testing FMD Male				
Participant	Resting Diameter (mm)	Peak Diameter (mm)	FMD Absolute (mm)	Non-Normalized FMD Relative (%)
1	4.9424	4.942	-0.0004	-0.008093234

Pre EIHG Testing FMD Male				
Participant	Resting Diameter (mm)	Peak Diameter (mm)	FMD Absolute (mm)	Non-Normalized FMD Relative (%)
1	5.0536	4.8643	-0.1893	-3.745844546

Pre NEIHG Testing FMD Male				
Participant	Resting Diameter (mm)	Peak Diameter (mm)	FMD Absolute (mm)	Non-Normalized FMD Relative (%)
1	5.0748	5.143	0.0682	1.343895326

Post CUFF Testing FMD Male				
Participant	Resting Diameter (mm)	Peak Diameter (mm)	FMD Absolute (mm)	Non-Normalized FMD Relative (%)
1	4.7075	5.1787	0.4712	10.00955921

Post EIHG Testing FMD Male				
Participant	Resting Diameter (mm)	Peak Diameter (mm)	FMD Absolute (mm)	Non-Normalized FMD Relative (%)
1	4.7855	5.5133	0.7278	15.20844217

Post NEIHG Testing FMD Male				
Participant	Resting Diameter (mm)	Peak Diameter (mm)	FMD Absolute (mm)	Non-Normalized FMD Relative (%)
1	5.3078	4.951	-0.3568	-6.722182448

Resting and Peak Shear Data

Pre CUFF Shear Flows Female		
Participant	Resting Shear (s^{-1})	Peak Shear (s^{-1})
2	30.05204222	361.7244643
3	17.93226913	298.9278434
4	17.82677726	154.4187576
5	20.24171244	187.2961708
6	47.5040097	193.887279
7	12.75737149	176.3690558
8	12.14868075	302.719822

Pre EIHG Testing Shear Female		
Participant	Resting Shear (s^{-1})	Peak Shear (s^{-1})
2	25.92871801	255.0043975
3	18.64666258	318.9528475
4	20.29522154	219.9436594
5	19.26142554	175.05438
6	17.63352688	209.2943083
7	18.98188522	171.4521103
8	28.29233181	189.3938779

Pre NEIHG Testing Shear Female		
Participant	Resting Shear (s^{-1})	Peak Shear (s^{-1})
2	14.80666735	215.1981985
3	18.44465059	233.3933491
4	17.4634817	214.2287907
5	13.0602385	153.2490574
6	32.22966073	155.0271398
7	13.86142842	253.6118525
8	22.172949	228.6004362

Post CUFF Shear Flows Female		
Participant	Resting Shear (s^{-1})	Peak Shear (s^{-1})
2	22.81394257	230.5104444
3	18.34804273	271.6464676
4	16.5986805	222.8673111
5	9.585919407	156.2544388
6	63.45689744	278.9048721
7	15.28122553	233.1773614
8	10.55785829	212.5924417

Post EIHG Shear Flows Female		
Participant	Resting Shear (s^{-1})	Peak Shear (s^{-1})
2	58.55393801	268.5498613
3	31.62207761	308.4745848
4	18.76289417	267.0141821
5	12.86080531	192.1471875
6	25.63079787	198.5108363
7	17.63817175	205.527153
8	22.16081623	203.1985101

Post NEIHG Shear Flows Female		
Participant	Resting Shear (s^{-1})	Peak Shear (s^{-1})
2	13.00106496	172.7653532
3	21.49864785	326.8163132
4	13.04017437	179.4448075
5	9.431224608	222.4379606
6	14.268153	252.6569955
7	17.38293821	227.1834173
8	18.54157047	286.2683535

Normalized Flow-Mediated Dilation to Area-Under-Curve Data

Normalized FMD Pre CUFF Female			
Participant	AUC (s^-1)	FMD (% Change)	Normalized FMD (FMD:AUC)
2	5812.3165	22.96704242	3.951443873
3	5031.7688	6.194987319	1.231174874
4	430.9138	3.115308712	7.229540367
5	3938.7774	13.58244712	3.448391656
6	2956.0875	10.74533324	3.634984838
7	2859.1754	4.701472004	1.644345431
8	9186.8551	14.73005072	1.603383373

Normalized FMD Pre EIHG Female			
Participant	AUC (s^-1)	FMD (% Change)	Normalized FMD (FMD:AUC)
2	1892.3211	19.64285714	10.38029811
3	7845.7111	13.84338641	1.764452735
4	4164.2788	36.59254641	8.787247005
5	979.6796	10.5195252	10.73771997
6	5102.4433	5.815114145	1.139672467
7	3102.5985	9.582494111	3.08853824
8	6153.2461	0.342157915	0.055606083

Normalized FMD Pre NEIHG Female			
Participant	AUC (s^-1)	FMD (% Change)	Normalized FMD (FMD:AUC)
2	679.2549	14.56360118	21.44055373
3	1597.6749	17.20410272	10.76821243
4	3096.7299	13.99553188	4.519455145
5	2118.9745	2.052305645	0.968537208
6	225.3807	0.019181691	0.085107957
7	754.1978	8.704316572	11.54115879
8	8992.5004	2.299226651	0.255682685

Normalized FMD Post CUFF Female			
Participant	AUC (s^-1)	FMD (% Change)	Normalized FMD (FMD:AUC)
2	2835.0948	22.78170355	8.035605564
3	1759.0219	6.019065918	3.421825458
4	2842.9362	8.20949358	2.887681257
5	1076.4615	7.13872163	6.631655317
6	8317.5512	14.91600262	1.793316598
7	2480.3568	8.54860091	3.446520642
8	4438.903	-5.419951706	-1.221011522

Normalized FMD Post EIHG Female			
Participant	AUC (s^-1)	FMD (% Change)	Normalized FMD (FMD:AUC)
2	5623.3676	-2.225115398	-0.395690902
3	9017.9755	6.949042889	0.770576821
4	3862.8437	38.00888367	9.839612115
5	3641.2896	23.01915247	6.321703296
6	1929.4074	14.32322829	7.423641214
7	2495.8424	6.397250217	2.563162729
8	4346.8623	6.735090489	1.549414273

Normalized FMD Post NEIHG Female			
Participant	AUC (s^-1)	FMD (% Change)	Normalized FMD (FMD:AUC)
2	1987.3845	6.293929712	3.166941129
3	6375.3274	9.881034671	1.549886626
4	3230.7513	9.927419077	3.072789626
5	1927.8325	1.020946552	0.529582602
6	2350.3771	3.338801505	1.420538647
7	2370.1924	16.74330341	7.064111508
8	4763.426	6.996644812	1.468826179

Normalized FMD Pre CUFF Male			
Participant	AUC (s^-1)	FMD (% Change)	Normalized FMD (FMD:AUC)
1	4360.5706	-0.008093234	-0.001856003

Normalized FMD Pre EIHG Male			
Participant	AUC (s^-1)	FMD (% Change)	Normalized FMD (FMD:AUC)
1	1814.5938	-3.745844546	-2.064288188

Normalized FMD Pre NEIHG Male			
Participant	AUC (s^-1)	FMD (% Change)	Normalized FMD (FMD:AUC)
1	3498.6218	1.343895326	0.384121349

Normalized FMD Post CUFF Male			
Participant	AUC (s^-1)	FMD (% Change)	Normalized FMD (FMD:AUC)
1	2319.6843	10.00955921	4.31505236

Normalized FMD Post EIHG Male			
Participant	AUC (s^-1)	FMD (% Change)	Normalized FMD (FMD:AUC)
1	2691.0906	15.20844217	5.651404739

Normalized FMD Post NEIHG Male			
Participant	AUC (s^-1)	FMD (% Change)	Normalized FMD (FMD:AUC)
1	2133.3905	-6.722182448	-3.150938587

Resistance Vessel Function Plethysmography Data

	Pre NEIHG (ml/min/100mL)				
Participant	Pre1	Pre2	Pre3	PreAVG	Post
1	NA	NA	NA	NA	NA
2	NA	NA	NA	NA	NA
3	11.80039	18.5501	12.04631	14.13226667	47.09532
4	0.84006	1.93445	1.63782	1.470776667	5.82215
5	3.31918	3.90108	3.45281	3.55769	43.17275
6	8.29485	6.02313	3.05427	5.79075	39.6503
7	4.48572	8.80818	7.40931	6.90107	28.40552
8	8.6471	7.29131	5.1879	7.042103333	41.76925

	Pre EIHG (ml/min/100mL)				
Participant	Pre1	Pre2	Pre3	PreAVG	Post
1	NA	NA	NA	NA	NA
2	NA	NA	NA	NA	NA
3	20.2617	42.9035	32.82851	31.99790333	66.83889
4	6.01138	6.4931	5.82604	6.110173333	26.12393
5	14.72074	10.00891	10.02425	11.58463333	77.38969
6	13.01243	9.7227	5.40492	9.380016667	45.4065
7	7.54566	5.73339	4.1882	5.822416667	26.28721
8	5.2134	6.32424	2.85904	4.798893333	50.08815

	Pre CUFF (ml/min/100mL)				
Participant	Pre1	Pre2	Pre3	PreAVG	Post
1	NA	NA	NA	NA	NA
2	3.32829	2.64789	1.74952	2.575233333	38.10101
3	2.24736	2.10095	1.65911	2.002473333	46.1206
4	7.45178	10.1916	9.57748	9.07362	23.05956
5	7.68731	4.95402	6.7541	6.465143333	46.33809
6	5.06635	3.86301	5.13548	4.68828	45.69986
7	2.44427	1.94474	2.53613	2.30838	41.53496
8	2.89552	2.57575	3.27492	2.915396667	34.83446

	Post NEIHG (ml/min/100mL)				
Participant	Pre1	Pre2	Pre3	PreAVG	Post
1	NA	NA	NA	NA	NA
2	NA	NA	NA	NA	NA
3	4.70431	6.96192	7.93385	6.53336	58.8782
4	4.321385	4.082782	1.640562	3.348243	35.80695
5	10.72153	10.64342	13.5788	11.64792	106.1506
6	2.7511	3.57957	2.89176	3.074143333	43.05418
7	1.29277	4.07373	4.66594	3.344146667	32.47578
8	7.52166	6.67188	6.1386	6.77738	33.91461

	Post EIHG (ml/min/100mL)				
Participant	Pre1	Pre2	Pre3	PreAVG	Post
1	NA	NA	NA	NA	NA
2	NA	NA	NA	NA	NA
3	17.55736	12.71173	8.84376	13.03761667	53.45543
4	10.10476	12.93273	12.70704	11.91484333	31.34942
5	7.60458	8.81746	9.41001	8.610683333	70.57919
6	12.73225	22.50849	9.72716	14.9893	23.19122
7	7.5286	8.23375	8.23236	7.998236667	40.37915
8	10.70901	11.0428	12.142	11.29793667	50.67992

	Post CUFF (ml/min/100mL)				
Participant	Pre1	Pre2	Pre3	PreAVG	Post
1	NA	NA	NA	NA	NA
2	2.74114	2.61477	1.8213	2.392403333	32.99766
3	3.2693	3.9803	5.45247	4.234023333	39.13814
4	19.0933	12.4226	14.5353	15.3504	28.83334
5	15.25128	14.48485	12.56437	14.10016667	76.01387
6	5.1017	6.77356	4.39923	5.42483	52.4203
7	1.77377	1.88094	2.0721	1.908936667	27.34554
8	0.94592	8.32322	9.2521	6.173746667	66.09124

*All missing data for participant 1 and 2 was due to equipment malfunction at the time of testing.

Appendix P: Statistics for Chapter 2

Resting SBP

One Way Repeated Measures Analysis of Variance Monday, October 21, 2013
Data source: Data 1 in Resting SBP.JNB
Normality Test (Shapiro-Wilk) Passed (P = 0.100)
Equal Variance Test: Passed (P = 0.466)

Treatment Name	N	Missing	Mean	Std Dev	SEM
Rest IV	7	0	102.333	4.907	1.854
Rest Fam	7	0	105.667	13.894	5.251
Rest CUFF	7	0	99.381	10.783	4.076
Rest EIHG	7	0	96.476	6.960	2.631
Rest NEIHG	7	0	98.286	8.620	3.258

Source of Variation	DF	SS	MS	F	P
Between Subjects	6	1410.394	235.066		
Between Treatments	4	366.635	91.659	1.658	0.192
Residual	24	1326.432	55.268		
Total	34	3103.460			

Power of performed test with alpha = 0.050: 0.181

Resting DBP

One Way Repeated Measures Analysis of Variance Monday, October 21, 2013
Data source: Data 1 in Resting DBP.JNB
Normality Test (Shapiro-Wilk) Passed (P = 0.388)
Equal Variance Test: Passed (P = 0.252)

Treatment Name	N	Missing	Mean	Std Dev	SEM
Rest IV	7	0	60.619	4.143	1.566
Rest Fam	7	0	62.714	4.633	1.751
Rest CUFF	7	0	59.952	3.325	1.257
Rest EIHG	7	0	59.667	5.168	1.953
REST NEIHG	7	0	59.095	5.216	1.972

Source of Variation	DF	SS	MS	F	P
Between Subjects	6	317.263	52.877		
Between Treatments	4	54.908	13.727	1.083	0.387
Residual	24	304.292	12.679		
Total	34	676.463			

Power of performed test with alpha = 0.050: 0.063

Resting MAP

One Way Repeated Measures Analysis of Variance Monday, October 21, 2013
Data source: Data 1 in Resting MAP.JNB
Normality Test (Shapiro-Wilk) Passed (P = 0.742)
Equal Variance Test: Passed (P = 0.688)

Treatment Name	N	Missing	Mean	Std Dev	SEM
Rest IV	7	0	74.524	3.722	1.407
Rest Fam	7	0	77.032	6.417	2.425
Rest CUFF	7	0	73.095	5.666	2.142
Rest EIHG	7	0	71.937	4.437	1.677
REST NEIHG	7	0	72.159	5.789	2.188

Source of Variation	DF	SS	MS	F	P
Between Subjects	6	478.018	79.670		
Between Treatments	4	123.328	30.832	2.033	0.122
Residual	24	363.971	15.165		
Total	34	965.317			

Power of performed test with alpha = 0.050: 0.270

Resting HR

One Way Repeated Measures Analysis of Variance Monday, October 21, 2013
Data source: Data 1 in Resting HR.JNB
Normality Test (Shapiro-Wilk) Passed (P = 0.278)
Equal Variance Test: Passed (P = 0.669)

Treatment Name	N	Missing	Mean	Std Dev	SEM
Rest IV	7	0	70.286	5.342	2.019
Rest Fam	7	0	68.429	5.862	2.216
Rest CUFF	7	0	69.286	5.522	2.087
Rest EIHG	7	0	68.619	6.057	2.289
REST NEIHG	7	0	68.524	5.689	2.150

Source of Variation	DF	SS	MS	F	P
Between Subjects	6	584.083	97.347		
Between Treatments	4	17.003	4.251	0.261	0.900
Residual	24	390.552	16.273		
Total	34	991.638			

Power of performed test with alpha = 0.050: 0.050

Resting BA Diameter

Two Way Repeated Measures ANOVA (Two Factor Repetition) Monday, October 21, 2013
Data source: Data 1 in Resting Diameters.JNB
Balanced Design
Dependent Variable: Resting Diameter
Normality Test (Shapiro-Wilk) Passed (P = 0.932)
Equal Variance Test: Passed (P = 0.994)

Source of Variation	DF	SS	MS	F	P
Participant	6	4.749	0.792		
Day	2	0.228	0.114	2.671	0.110
Day x Participant	12	0.511	0.0426		
Pre-Post	1	0.00146	0.00146	0.213	0.661
Pre-Post x Participant	6	0.0412	0.00686		
Day x Pre-Post	2	0.00853	0.00426	0.295	0.749
Residual	12	0.173	0.0144		
Total	41	5.712	0.139		

Power of performed test with alpha = 0.0500: for Day : 0.276
Power of performed test with alpha = 0.0500: for Pre-Post : 0.0500
Power of performed test with alpha = 0.0500: for Day x Pre-Post : 0.0500

Least square means for Day :

Group	Mean
CUFF	2.906
EIHG	2.935
NEIHG	3.075

Std Err of LS Mean = 0.0551

Least square means for Pre-Post :

Group	Mean
Pre	2.978
Post	2.966

Std Err of LS Mean = 0.0181

Least square means for Day x Pre-Post :

Group	Mean
CUFF x Pre	2.894
CUFF x Post	2.918
EIHG x Pre	2.942
EIHG x Post	2.929
NEIHG x Pre	3.097
NEIHG x Post	3.052

Std Err of LS Mean = 0.0454

Resting Mean Blood Flow

Two Way Repeated Measures ANOVA (Two Factor Repetition) Monday, October 21, 2013
Data source: Data 1 in Resting Flows.JNB
Balanced Design
Dependent Variable: Resting Flow
Normality Test (Shapiro-Wilk) Passed (P = 0.968)
Equal Variance Test: Passed (P = 0.998)

Source of Variation	DF	SS	MS	F	P
Participant	6	4926.916	821.153		
Day	2	241.455	120.728	0.357	0.707
Day x Participant	12	4053.200	337.767		
Pre-Post	1	62.738	62.738	0.382	0.559
Pre-Post x Participant	6	986.085	164.348		
Day x Pre-Post	2	218.025	109.013	0.980	0.404
Residual	12	1335.461	111.288		
Total	41	11823.881	288.387		

Power of performed test with alpha = 0.0500: for Day : 0.0500
Power of performed test with alpha = 0.0500: for Pre-Post : 0.0500
Power of performed test with alpha = 0.0500: for Day x Pre-Post : 0.0500

Least square means for Day :

Group	Mean
CUFF	31.915
EIHG	35.306
NEIHG	29.458

Std Err of LS Mean = 4.912

Least square means for Pre-Post :

Group	Mean
Pre	33.448
Post	31.004

Std Err of LS Mean = 2.798

Least square means for Day x Pre-Post :

Group	Mean
CUFF x Pre	32.521
CUFF x Post	31.309
EIHG x Pre	34.097
EIHG x Post	36.515
NEIHG x Pre	33.727
NEIHG x Post	25.188

Std Err of LS Mean = 3.987

Peak Mean Blood Flow

Two Way Repeated Measures ANOVA (Two Factor Repetition) Friday, October 25, 2013
Data source: Data 1 in Peak Flows.JNB
Balanced Design
Dependent Variable: Peak Flow
Normality Test (Shapiro-Wilk) Passed (P = 0.529)
Equal Variance Test: Passed (P = 0.810)

Source of Variation	DF	SS	MS	F	P
Participant	6	473076.739	78846.123		
Day	2	24942.395	12471.198	0.788	0.477
Day x Participant	12	190008.550	15834.046		
Pre-Post	1	78.128	78.128	0.0103	0.923
Pre-Post x Participant	6	45699.238	7616.540		
Day x Pre-Post	2	14409.624	7204.812	0.738	0.499
Residual	12	117198.136	9766.511		
Total	41	865412.811	21107.630		

Power of performed test with alpha = 0.0500: for Day : 0.0500
Power of performed test with alpha = 0.0500: for Pre-Post : 0.0500
Power of performed test with alpha = 0.0500: for Day x Pre-Post : 0.0500

Least square means for Day :

Group	Mean
CUFF	399.861
EIHG	455.248
NEIHG	446.832

Std Err of LS Mean = 33.630

Least square means for Pre-Post :

Group	Mean
Pre	435.345
Post	432.617

Std Err of LS Mean = 19.044

Least square means for Day x Pre-Post :

Group	Mean
CUFF x Pre	419.258
CUFF x Post	380.465
EIHG x Pre	464.050
EIHG x Post	446.446
NEIHG x Pre	422.726
NEIHG x Post	470.939

Std Err of LS Mean = 37.353

Resting Shear Rate

Two Way Repeated Measures ANOVA (Two Factor Repetition) Friday, November 01, 2013
Data source: Data 1 in Notebook1
Balanced Design
Dependent Variable: Resting Shear
Normality Test (Shapiro-Wilk) Passed (P = 0.468)
Equal Variance Test: Passed (P = 1.000)

Source of Variation	DF	SS	MS	F	P
Participant	6	1728.506	288.084		
Day	2	372.167	186.083	1.012	0.392
Day x Participant	12	2205.789	183.816		
Pre-Post	1	3.146	3.146	0.0768	0.791
Pre-Post x Participant	6	245.671	40.945		
Day x Pre-Post	2	145.465	72.733	1.233	0.326
Residual	12	707.707	58.976		
Total	41	5408.451	131.913		

Power of performed test with alpha = 0.0500: for Day : 0.0508
Power of performed test with alpha = 0.0500: for Pre-Post : 0.0500
Power of performed test with alpha = 0.0500: for Day x Pre-Post : 0.0772

Least square means for Day :

Group	Mean
CUFF	22.508
EIHG	24.019
NEIHG	17.086

Std Err of LS Mean = 3.623

Least square means for Pre-Post :

Group	Mean
Pre	20.931
Post	21.478

Std Err of LS Mean = 1.396

Least square means for Day x Pre-Post :

Group	Mean
CUFF x Pre	22.638
CUFF x Post	22.378
EIHG x Pre	21.291
EIHG x Post	26.747
NEIHG x Pre	18.863
NEIHG x Post	15.309

Std Err of LS Mean = 2.903

Peak Shear Rate

Two Way Repeated Measures ANOVA (Two Factor Repetition) Friday, November 01, 2013
Data source: Data 1 in Notebook1
Balanced Design
Dependent Variable: Peak Shear
Normality Test (Shapiro-Wilk) Failed (P < 0.050)
Equal Variance Test: Passed (P = 0.854)

Source of Variation	DF	SS	MS	F	P
Participant	6	46928.172	7821.362		
Day	2	935.455	467.728	0.232	0.796
Day x Participant	12	24187.570	2015.631		
Time	1	1478.599	1478.599	0.839	0.395
Time x Participant	6	10578.091	1763.015		
Day x Time	2	2921.992	1460.996	0.753	0.492
Residual	12	23290.520	1940.877		
Total	41	110320.399	2690.741		

Power of performed test with alpha = 0.0500: for Day : 0.0500
Power of performed test with alpha = 0.0500: for Time : 0.0500
Power of performed test with alpha = 0.0500: for Day x Time : 0.0500

Least square means for Day :

Group	Mean
CUFF	234.378
EIHG	227.323
NEIHG	222.920

Std Err of LS Mean = 11.999

Least square means for Time :

Group	Mean
Pre	222.274
Post	234.140

Std Err of LS Mean = 9.163

Least square means for Day x Time :

Group	Mean
CUFF x Pre	239.335
CUFF x Post	229.422
EIHG x Pre	219.871
EIHG x Post	234.775
NEIHG x Pre	207.616
NEIHG x Post	238.225

Std Err of LS Mean = 16.651

Non-Normalized FMD (% Change)

Two Way Repeated Measures ANOVA (Two Factor Repetition) Friday, October 25, 2013
Data source: Data 1 in Non-Normalized FMD.JNB
Balanced Design
Dependent Variable: FMD %Change
Normality Test (Shapiro-Wilk) Passed (P = 1.000)
Equal Variance Test: Passed (P = 0.569)

Source of Variation	DF	SS	MS	F	P
Subject	6	721.807	120.301		
Day	2	217.163	108.582	0.809	0.468
Day x Subject	12	1610.924	134.244		
PrePost	1	11.119	11.119	0.266	0.624
PrePost x Subject	6	250.814	41.802		
Day x PrePost	2	4.805	2.402	0.0567	0.945
Residual	12	508.505	42.375		
Total	41	3325.136	81.101		

Power of performed test with alpha = 0.0500: for Day : 0.0500
Power of performed test with alpha = 0.0500: for PrePost : 0.0500
Power of performed test with alpha = 0.0500: for Day x PrePost : 0.0500

Least square means for Day :

Group	Mean
CUFF	9.874
EIHG	13.539
NEIHG	8.074

Std Err of LS Mean = 3.097

Least square means for PrePost :

Group	Mean
Pre	11.010
Post	9.981

Std Err of LS Mean = 1.411

Least square means for Day x PrePost :

Group	Mean
CUFF x Pre	10.862
CUFF x Post	8.885
EIHG x Pre	13.763
EIHG x Post	13.315
NEIHG x Pre	8.405
NEIHG x Post	7.743

Std Err of LS Mean = 2.460

Normalized FMD (FMD:AUC)

Two Way Repeated Measures ANOVA (Two Factor Repetition) Friday, October 25, 2013
Data source: Data 1 in Normalized FMD.JNB
Balanced Design
Dependent Variable: FMDnorm
Normality Test (Shapiro-Wilk) Passed (P = 0.998)
Equal Variance Test: Passed (P = 0.447)

Source of Variation	DF	SS	MS	F	P
Participant	6	199.489	33.248		
Time	1	32.480	32.480	2.082	0.199
Time x Participant	6	93.600	15.600		
Day	2	16.296	8.148	0.410	0.673
Day x Participant	12	238.740	19.895		
Time x Day	2	42.323	21.162	1.441	0.275
Residual	12	176.238	14.686		
Total	41	799.166	19.492		

Power of performed test with alpha = 0.0500: for Time : 0.137
Power of performed test with alpha = 0.0500: for Day : 0.0500
Power of performed test with alpha = 0.0500: for Time x Day : 0.103

Least square means for Time :

Group	Mean
Pre	5.156
Post	3.397

Std Err of LS Mean = 0.862

Least square means for Day :

Group	Mean
CUFF	3.410
EIHG	4.573
NEIHG	4.847

Std Err of LS Mean = 1.192

Least square means for Time x Day :

Group	Mean
Pre x CUFF	3.249
Pre x EIHG	5.136
Pre x NEIHG	7.083
Post x CUFF	3.571
Post x EIHG	4.010
Post x NEIHG	2.610

Std Err of LS Mean = 1.448

RVF (% Change)

Two Way Repeated Measures ANOVA (Two Factor Repetition) Friday, October 25, 2013
Data source: Data 1 in Plethysmography.JNB
General Linear Model
Dependent Variable: % Change
The following subject was deleted from calculations because of missing data: 2
Normality Test (Shapiro-Wilk) Passed (P = 0.997)
Equal Variance Test: Passed (P = 0.997)

Source of Variation	DF	SS	MS	F	P
Participant	5	886899.241	177379.848	0.741	0.624
Day	2	1752817.802	876408.901	2.876	0.103
Day x Participant	10	3046818.347	304681.835		
Pre-Post	1	12967.762	12967.762	0.266	0.628
Pre-Post x Participant	5	243483.085	48696.617		
Day x Pre-Post	2	776468.553	388234.277	3.407	0.074
Residual	10	1139471.415	113947.141		
Total	35	7858926.206	224540.749		

Power of performed test with alpha = 0.0500: for Day : 0.294
Power of performed test with alpha = 0.0500: for Pre-Post : 0.0500
Power of performed test with alpha = 0.0500: for Day x Pre-Post : 0.368

Expected Mean Squares:
Approximate DF Residual for Day = 10.000
Approximate DF Residual for Pre-Post = 5.000
Approximate DF Residual for Participant = 5.185

Expected MS(Day) = var(res) + 2.000 var(Day x Participant) + var(Day)
Expected MS(Pre-Post) = var(res) + 3.000 var(Pre-Post x Participant) + var(Pre-Post)
Expected MS(Participant) = var(res) + 2.000 var(Day x Participant) +3.000 var(Pre-Post x Participant) +6.000 var(Participant)
Expected MS(Day x Participant) = var(res) + 2.000 var(Day x Participant)
Expected MS(Day x Pre-Post) = var(res) + var(Day x Pre-Post)
Expected MS(Pre-Post x Participant) = var(res) + 3.000 var(Pre-Post x Participant)
Expected MS(Residual) = var(res)

Least square means for Day :

Group	Mean	SEM
NEIHG	682.173	159.343
EIHG	390.393	159.343
CUFF	930.301	159.343

Least square means for Pre-Post :

Group	Mean	SEM
Pre	686.602	52.013
Post	648.643	52.013

Least square means for Day x Pre-Post :

Group	Mean	SEM
NEIHG x Pre	505.345	137.809
NEIHG x Post	859.001	137.809
EIHG x Pre	447.296	137.809
EIHG x Post	333.490	137.809
CUFF x Pre	1107.164	137.809
CUFF x Post	753.438	137.809

Correlations between FMD and AUC

Pre CUFF

Pearson Product Moment Correlation Tuesday, November 05, 2013
Data source: Data 1 in Pre Cuff Correlation.JNB
Cell Contents:
Correlation Coefficient
P Value
Number of Samples

	FMD
Shear	0.625
	0.133
	7

Post CUFF

Pearson Product Moment Correlation Tuesday, November 05, 2013
Data source: Data 1 in Notebook1

Cell Contents:
Correlation Coefficient
P Value
Number of Samples

	FMD
Shear	0.132
	0.779
	7

Pre EIHG

Pearson Product Moment Correlation Tuesday, November 05, 2013
Data source: Data 1 in Notebook1
Cell Contents:
Correlation Coefficient
P Value
Number of Samples

	FMD
Shear	-0.191
	0.681
	7

Post EIHG

Pearson Product Moment Correlation Tuesday, November 05, 2013
Data source: Data 1 in Notebook1
Cell Contents:
Correlation Coefficient
P Value
Number of Samples

	FMD
Shear	-0.306
	0.505
	7

Pre NEIHG

Pearson Product Moment Correlation Tuesday, November 05, 2013
Data source: Data 1 in Notebook1
Cell Contents:
Correlation Coefficient
P Value
Number of Samples

	FMD
SHEAR	-0.271
	0.557
	7

Post NEIHG

Pearson Product Moment Correlation Tuesday, November 05, 2013
Data source: Data 1 in Notebook1
Cell Contents:
Correlation Coefficient
P Value
Number of Samples

	FMD
SHEAR	0.237
	0.608
	7

Correlations of FMD between EIHG and CUFF Testing Days

Pearson Product Moment Correlation Wednesday, November 20, 2013
Data source: Data 1 in Non-Normalized FMD.JNB

Cell Contents:
Correlation Coefficient
P Value
Number of Samples

	EIHG Pre
CUFF Pre	-0.287
	0.533
	7

Pearson Product Moment Correlation Wednesday, November 20, 2013

Cell Contents:
Correlation Coefficient
P Value
Number of Samples

	EIHG Pre
CUFF Pre	-0.346
	0.502
	6

Appendix Q: Reproducibility

Inter-Observer Reproducibility

ID	Testing Day	Investigator 1	Investigator 2	X	SD	Cv
2	Pre CUFF	2.32	2.21	2.27	0.08	3.53
3	Pre CUFF	2.68	2.73	2.71	0.04	1.36
4	Pre CUFF	2.84	2.73	2.78	0.08	2.73
5	Pre CUFF	3.42	3.32	3.37	0.07	2.20
6	Pre CUFF	2.97	3.09	3.03	0.09	2.94
7	Pre CUFF	2.91	2.93	2.92	0.02	0.60
8	Pre CUFF	3.12	3.11	3.11	0.01	0.22
2	Post CUFF	2.36	2.20	2.28	0.11	4.97
3	Post CUFF	2.71	2.76	2.73	0.04	1.30
4	Post CUFF	2.77	2.77	2.77	0.00	0.10
5	Post CUFF	3.45	3.47	3.46	0.01	0.37
6	Post CUFF	2.90	2.87	2.88	0.02	0.81
7	Post CUFF	2.79	2.65	2.72	0.10	3.55
8	Post CUFF	3.44	3.27	3.35	0.12	3.53
			CC	**0.97**	**Cv**	**2.01**

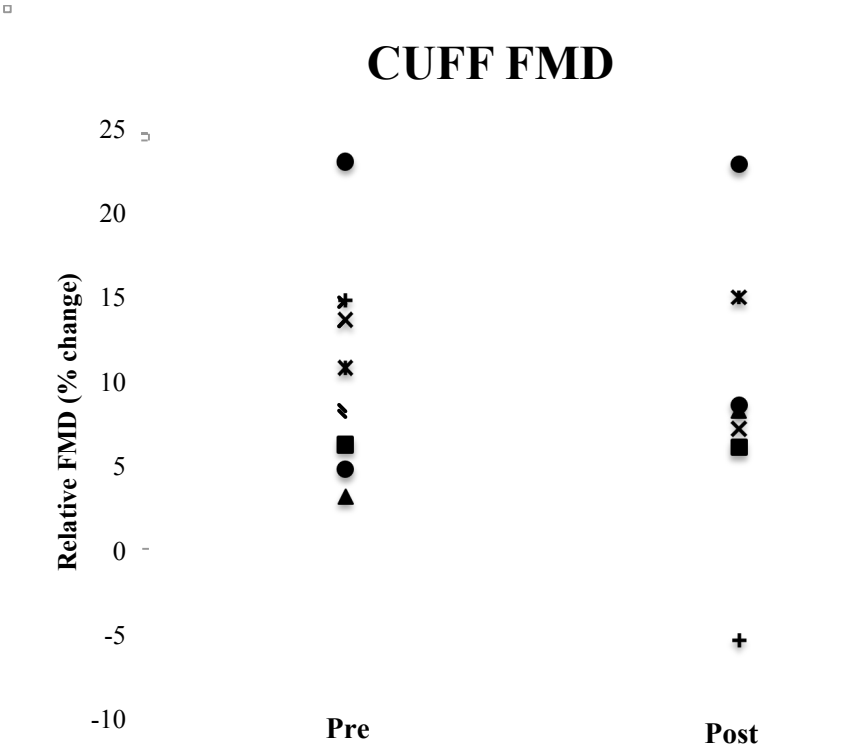
ID	Testing Day	Investigator 1	Investigator 2	Difference
2	Pre CUFF	2.32	2.21	0.11
3	Pre CUFF	2.68	2.73	-0.05
4	Pre CUFF	2.84	2.73	0.11
5	Pre CUFF	3.42	3.32	0.10
6	Pre CUFF	2.97	3.09	-0.13
7	Pre CUFF	2.91	2.93	-0.02
8	Pre CUFF	3.12	3.11	0.01
2	Post CUFF	2.36	2.20	0.16
3	Post CUFF	2.71	2.76	-0.05
4	Post CUFF	2.77	2.77	0.00
5	Post CUFF	3.45	3.47	-0.02
6	Post CUFF	2.90	2.87	0.03
7	Post CUFF	2.79	2.65	0.14
8	Post CUFF	3.44	3.27	0.17
2		2.21		
3		2.73		
4		2.73		
5		3.32		
6		3.09		
7		2.93		
8		3.11		
2		2.20		
3		2.76		
4		2.77		
5		3.47		
6		2.87		
7		2.65		
8		3.27		
	Grand Mean	**2.89**	**Mean Difference**	**0.04**
			SD Difference	**0.09**
			Method Error	**3.16**

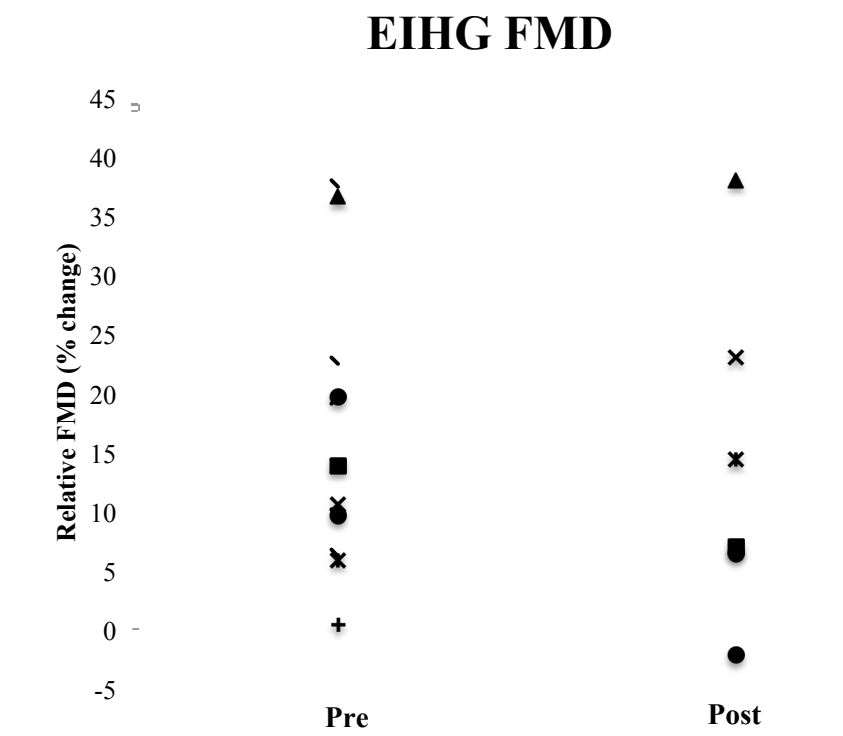
Intra-Observer Reproducibility

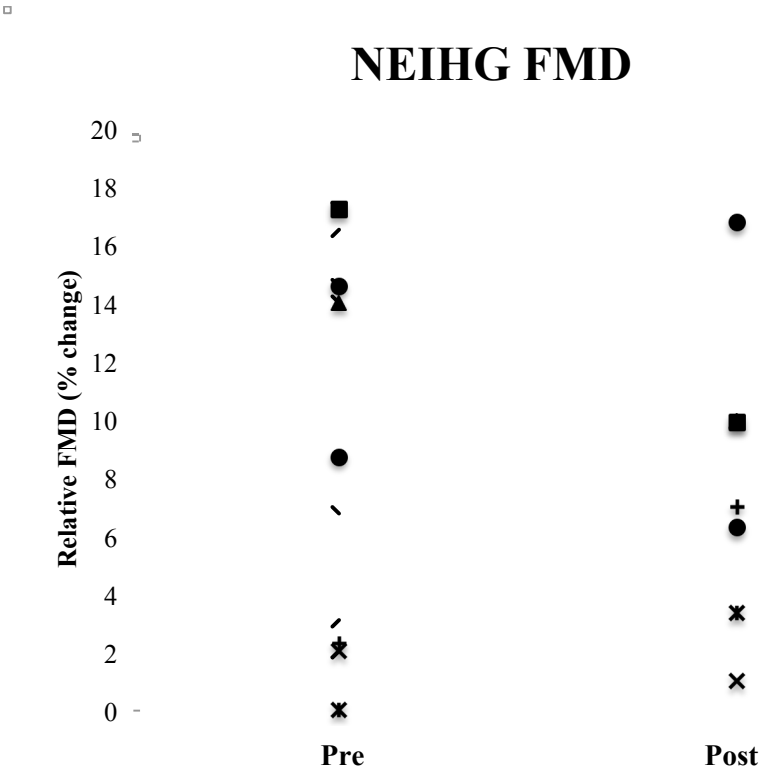
ID	CUFF	EIHG	X	SD	Cv
2	2.3242	2.1224	2.22	0.14	6.41812389
3	2.6812	2.6128	2.65	0.05	1.827204527
4	2.8376	2.8872	2.86	0.04	1.225282852
5	3.4228	3.4878	3.46	0.05	1.330186692
6	2.9678	3.1057	3.04	0.10	3.210999428
7	2.9076	2.9293	2.92	0.02	0.525765977
8	3.1154	3.4487	3.28	0.24	7.180837896
		CC	**0.97**	**Cv**	**3.102628752**

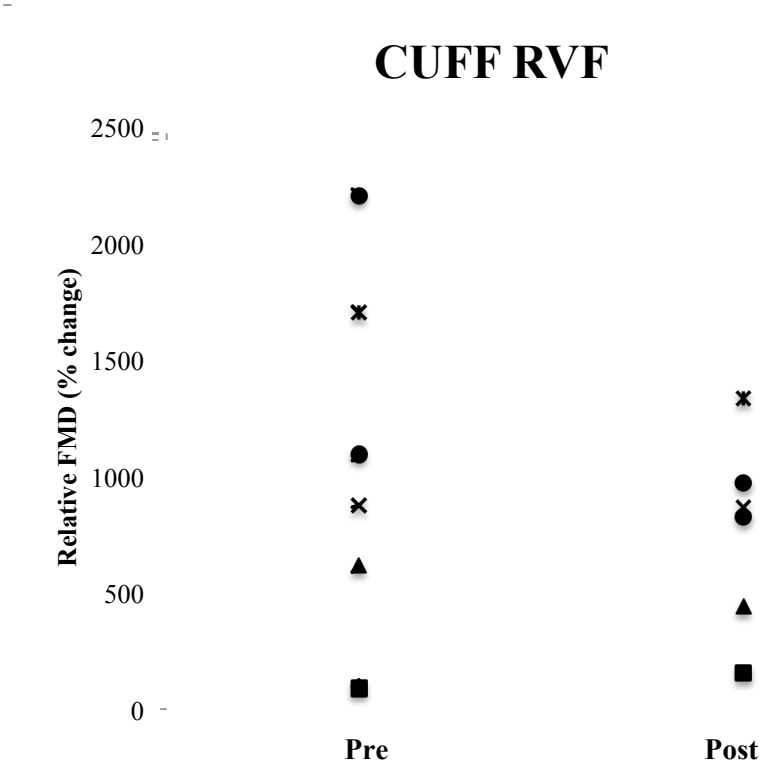
ID	CUFF	EIHG	Difference
2	2.3242	2.1224	0.2018
3	2.6812	2.6128	0.0684
4	2.8376	2.8872	-0.0496
5	3.4228	3.4878	-0.065
6	2.9678	3.1057	-0.1379
7	2.9076	2.9293	-0.0217
8	3.1154	3.4487	-0.3333
2	2.1224		
3	2.6128		
4	2.8872		
5	3.4878		
6	3.1057		
7	2.9293		
8	3.4487		
	Grand Mean	**2.92**	**Mean Difference**	**-0.05**
			SD Difference	**0.17**
			Method Error	**5.71**

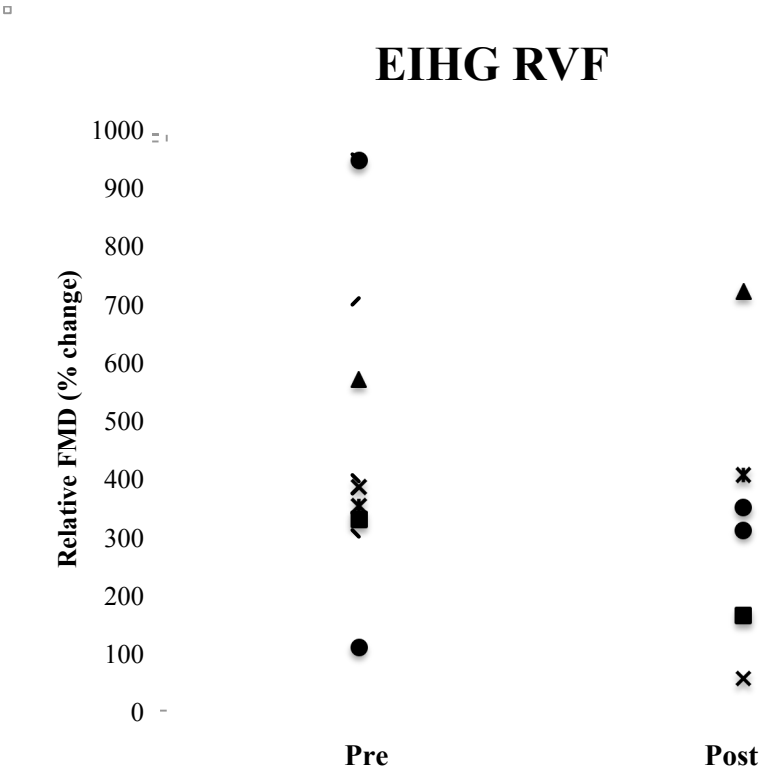
Appendix R: Vascular Function Scatter Plots

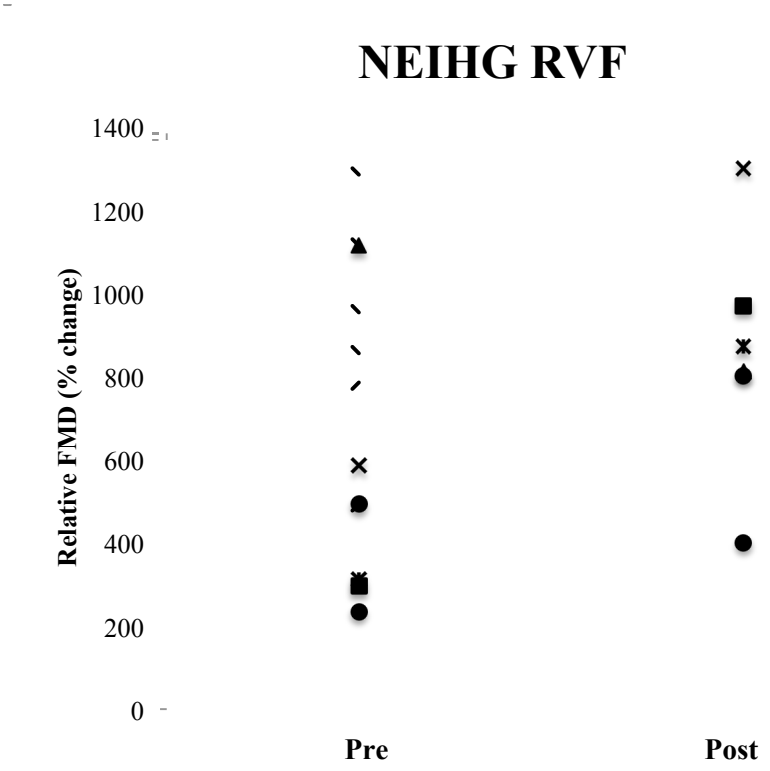





NEIHG FMD
Relative FMD (% change)
20
18
16
14
12
10
8
6
4
2
0
Pre
Post


CUFF RVF
Relative FMD (% change)
2500
2000
1500
1000
500
0
Pre
Post


EIHG RVF
Relative FMD (% change)
1000
900
800
700
600
500
400
300
200
100
0
Pre
Post


NEIHG RVF
Relative FMD (% change)
1400
1200
1000
800
600
400
200
0
Pre
Post

Vita Auctoris

Joshua Eugene Seifarth

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Telephone: 519-253-3000 ex. 4979 (PACR Laboratory)

PERSONAL INFORMATION

Born: Windsor, Ontario, Canada Citizenship: Canadian

DEGREES and RESEARCH TRANING

Master of Human Kinetics - Applied Human Performance (Cardiovascular Physiology)
University of Windsor, Windsor, Ontario, Canada, 2011 - Present
Thesis: Sex differences in the neurovascular response to acute isometric handgrip exercise and a complementary ischemic-reperfusion cuff protocol
Supervisor: Dr. Cheri McGowan

Bachelor of Human Kinetics - Honours Movement Science
University of Windsor, Windsor, Ontario, 2011

OTHER RESEARCH EXPERIENCE

Laboratory Volunteer
Physical Activity and Cardiovascular Research Lab
University of Windsor, Windsor, Ontario, Canada, January 2010 – August 2010

Independent Undergraduate Research Study
Physical Activity and Cardiovascular Research Lab
University of Windsor, Windsor, Ontario, Canada, September 2010 – December 2010

Research Assistant
Physical Activity and Cardiovascular Research Lab
University of Windsor, Windsor, Ontario, Canada, January 2011 – August 2011

SCHOLARSHIPS & AWARDS

James Molnar Graduate Scholarship
University of Windsor, April 2013

RESEARCH SKILLS

Measures of cardiovascular function: Arterial blood pressure measurement using brachial artery oscillometry, measurement of heart rate using electrocardiography

Doppler ultrasound applied for the purpose of assessing endothelium-dependent vasodilation, reactive hyperemia, and resting brachial artery diameters

Venous occlusion plethysmography for the purpose of assessing resistance vessel function

Applanation Tonometry (Millar)

RESEARCH INTERESTS

Cardiovascular and metabolic adjustments to acute and chronic exercise in healthy and diseased populations

Physiological mechanisms underlying the treatment and prevention of disease using aerobic and anaerobic exercise training

Monitoring and modulating exercise prescription in response to changes in physiological and psychological markers of fitness in elite endurance athletes

EXERCISE REHABILITATION EXPERIENCE

C.O.P.D. Rehabilitation Program
University of Windsor, Windsor, Ontario, Canada, February 2012 – April 2012

Windsor-Essex Community Cardiac Rehabilitation Centre
University of Windsor, Windsor, Ontario, Canada, January – April 2010

PUBLICATIONS

Seifarth, J., Milne, K., & McGowan, C. (2012). Sex and life expectancy. *Gender Medicine*. 9(6): 390-401.

PRESENTATIONS

Seifarth, J., Milne, K., & McGowan, C. Changes in Exercise Stress Response and Recovery Rate Using Active Recovery on Days Between Trials: A Proposal (Poster Presentation). Research Day, Department of Kinesiology, University of Windsor, Windsor, Ontario, Canada, 2012.

Hanik, S., Gregory, M., **Seifarth, J.**, Clarke, D., MacDonald, M., McCartney, N., Millar, P., Zinszer, K., Milne, K., & McGowan, C. Investigating the Effects of Isometric Handgrip Training on Ambulatory Blood Pressure and Muscle Sympathetic Nerve Activity in Post Menopausal Women (Poster Presentation). Research Day, Department of Kinesiology, University of Windsor, Windsor, Ontario, Canada, 2012.

Gregory, M., **Seifarth, J.**, Clarke, D., MacDonald, M., McCartney, N., Millar, P., Zinser, K., Milne, K., & McGowan, C. The effects of isometric hand-grip training on ambulatory blood pressure and neurovascular function in post-menopausal women: A thesis proposal (Poster Presentation). Research Day, Department of Kinesiology, University of Windsor, Windsor, Ontario, Canada, 2011.

DiBartolomeo, M., Badrov, M., **Seifarth, J.**, Stiller-Moldovan, C., Ackersviller, J., Clarke, D., & McGowan, C. The effects of acute isometric hand-grip exercise on endothelial-dependent vasodilation and resistance vessel function in normotensive individuals (Poster Presentation). Research Day, Department of Kinesiology, University of Windsor, Windsor, Ontario, Canada, 2011.

TEACHING EXPERIENCE

Graduate Assistant, Introduction to Exercise Physiology, Department of Kinesiology, University of Windsor, Windsor, Ontario, Canada, 2012

Graduate Assistant, Introduction to Exercise Physiology, Department of Kinesiology, University of Windsor, Windsor, Ontario, Canada, 2013

Graduate Assistant, Exercise in Extreme Environments, Department of Kinesiology, University of Windsor, Windsor, Ontario, Canada, 2013

STUDENT ADVISORY EXPERIENCE AS A GRADUATE STUDENT

Sarah Hanik, BSc (Behaviour, Cognition, and Neuroscience), University of Windsor, Windsor, Ontario, Canada, 2011-present

Yasina Somani, BSc (Biology), University of Windsor, Windsor, Ontario, Canada, 2012-present

Shannon Thompson, BSc (Biology), University of Windsor, Windsor, Ontario, Canada, 2011-2013

Kristi Martin, BHK (Movement Science), University of Windsor, Windsor, Ontario, Canada, 2011-2013

Shane Freeman, BHK Candidate (Movement Science), University of Windsor, Windsor, Ontario, Canada, 2011-present