Low-Flow Mediated Constriction: Environmental Modifiers And The Role Of Shear Rate In Young Males

By

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The COVID-19 pandemic had a significant impact on my thesis, particularly on the experimental work presented in Chapter 6. This study was originally designed different than that currently presented in the thesis (e.g., more experimental trials), but due to pandemic-related restrictions, the study design had to be altered. Unfortunately, the closure of the University as a safety measure to prevent the spread of the virus disturbed the progress of my experimental work (Chapter 6). The inability to resume my laboratory-based research created significant challenges. Furthermore, the recruitment of participants was additionally challenging, as many potential participants were not willing to attend in-person testing sessions due to concerns about the virus. These unforeseen circumstances demanded a modified experimental format than that originally planned and delayed the completion of my thesis.

ABSTRACT

Low-flow mediated constriction (L-FMC) is a recently developed method of assessing conduit artery vasoconstrictor function. L-FMC is suggested to provide complimentary information to that provided by the more traditional flow mediated dilation (FMD) technique. However, unlike FMD, the mechanisms underlining the L-FMC response and the influence of acute environmental and physiological stimuli is not completely understood. The aim of this thesis was to determine the impact of environmental modifiers and shear rate (SR) manipulation on radial artery L-FMC. Three experimental studies investigated the L-FMC response to heat stress, leg cycling exercise and simulated hyper/hypotension (i.e., lower body pressure).

The first experimental study examined the influence of the acute whole-body passive heat stress (+1°C core temperature), and manipulation of shear rate via partial wrist cuff (WC) inflation, on radial artery function. Heat significantly increased radial artery anterograde and mean SR, blood flow, and diameter ($P < 0.05$). Heat + WC diminished the heat-induced increase in mean and anterograde SR ($P > 0.05$), but markedly increased retrograde SR ($P < 0.05$), while increases in radial artery diameter and blood flow were reduced (heat + WC vs. heat, $P < 0.05$). Heat diminished FMD (8.6 ± 1.2% vs. 2.2 ± 1.4%, $P < 0.05$), however no change in FMD was observed in heat + WC (7.8± 1.2% vs. 10.8± 1.2%, $P > 0.05$). In contrast, L-FMC was not different in either trial ($P > 0.05$).

In study two, the acute impact of leg cycling exercise and SR manipulation on radial artery functional characteristics was determined. Exercise improved radial artery anterograde and mean SR, along with the blood flow, diameter, velocity, and conductance ($P < 0.05$). By design, Exercise + WC reduced the exercise-induced

increase in anterograde and mean SR ($P > 0.05$), whereas it increased retrograde SR ($P < 0.05$). Moreover, increases in radial artery diameter and blood flow were reduced during Exercise + WC (Exercise + WC vs. Exercise, $P < 0.05$). After Exercise, L-FMC was increased (-4.4 ± 1.4 vs. -13.1 ± 1.6%, $P < 0.05$), whereas no change in L-FMC after Exercise + WC (-5.2 ± 2.0 vs. -3.0 ± 1.6%, $P > 0.05$).

The final experimental study determined the impact of acute increases and decreases in sympathetic nerve activity (SNA), utilised by lower body negative pressure (LBNP) and lower body positive pressure (LBPP) respectively, on radial artery function. Results indicated that radial artery characteristics (blood flow, velocity and mean SR) decreased (Time, $P<0.05$) acutely following both trials. However, radial artery L-FMC was not altered following either LBNP or LBPP ($P>0.05$).

Collectively, these findings demonstrate the diverse impact of environmental stimuli (heat, exercise, lower body pressure), and the mediating effects of SR, on conduit artery function.

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M.H.A contribution: performed experiments.

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LIST OF ABBREVIATIONS

ACh - acetylcholine

ANS - autonomic nervous system

BP - Blood pressure

Ca^{2+}- calcium ion

CV - coefficient of variation

CAD - coronary artery disease

cNOS - constitutive NOS

cGMP - cyclic guanosine monophosphate

ECG - Electrocardiograph

EDHF - endothelial-derived hyperpolarizing factor

eNOS - endothelial nitric oxide synthase

ECE - endothelin converting enzyme

IFN-γ - interferon-γ

ET-1 - endothelin-1

FMD - flow-mediated dilation

GC - guanylate cyclase

Heat - whole body heat stress sufficient to raise core temperature by 1 °C

Heat + WC - whole body heat stress sufficient to raise core temperature by 1 °C

with concurrent inflation of a cuff placed around the right wrist to 75 mmHg

HR - Heart rate

ICC - intraclass correlation coefficient

iNOS - inducible NOS

LDL - lipoproteins

L-FMC – Low-flow mediated constriction

L-NMMA - L-NG-monomethyl arginine

LBNP - Lower body negative pressure

LBPP - Lower body positive pressure

MAP - Mean arterial pressure

NO - nitric oxide

PG12 - Prostacyclin

SD - standard deviation

SR - shear rate

SRAUC - Shear rate area under the curve

SNA - sympathetic nerve activity

SNS - sympathetic nervous system

T_{pill} - temperature pill telemetry system

T_{sk} - Mean skin temperature

TNF-α - tumour necrosis factor alpha

TVR - Total vessel reactivity

PGI2 - prostacyclin

PNS - parasympathetic nervous system

VCAM-1 - vascular cell adhesion molecular-1

WC - wrist cuff

CHAPTER 1: GENERAL INTRODUCTION

1.1 Background

Endothelial cells make numerous remarkable contributions to cardiovascular function and health (Watts et al., 2004). In the arteries, the endothelium senses the shear stress generated by blood flow and is responsible for releasing vasoactive agents (Thijssen et al., 2009). These vasoactive agents are released in response to a systemic or local increase or decrease in local blood flow/shear stress and contribute to vascular haemostasis (Sandoo et al., 2010, Green et al., 2017). Endothelial dysfunction and changes in blood flow pattern are valuable biomarkers of atherosclerotic development and other cardiovascular diseases (Hambrecht et al., 2003).

A common and widely used method of assessing endothelial function is flow-mediated dilation (FMD), which is conducted non-invasively via Doppler ultrasonography (Corretti et al., 2002a). It is well recognized that FMD occurs in response to an acute increase in shear stress, is endothelium mediated, and is at least partially nitric oxide-dependent (Joannides et al., 1995, Carter et al., 2013). More recently, low-flow mediated constriction (L-FMC) has gained prominence as a biomarker of vascular function that complements the information provided by FMD.

Notably, many studies have carefully elucidated the influence of modulating factors (e.g., physical activity, environmental stressors) on FMD. This thesis will determine, for the first time, the influence of exercise, heat stress and simulated hyper/hypotension on L-FMC.

CHAPTER 2: LITERATURE REVIEW

2.1 The cardiovascular system

The human cardiovascular system is a closed tubular network through which blood is carried from the heart all over the body, and vice versa. Blood is circulated through two circuits: the pulmonary circuit and the systemic circuit (Figure 2.1). In the pulmonary circuit, deoxygenated blood is pumped from the right ventricle of the heart via pulmonary arteries to the lungs where gas exchange occurs. The oxygenated blood then returns from the lungs to the left atrium of the heart via pulmonary veins. In the systemic circuit, the left ventricle of the heart pumps oxygenated blood to the body's tissues via the aorta and the deoxygenated blood returns to the right atrium of the heart via the vena cavae, thus enabling the circulatory process to be repeated.

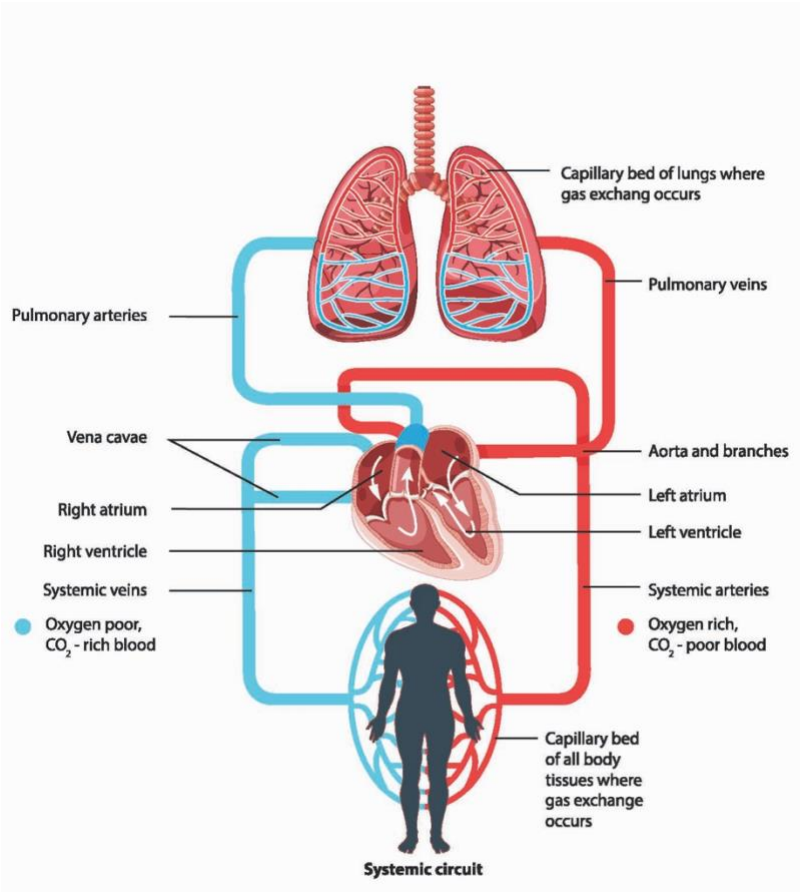


Figure 2.1 Diagram of the pulmonary and systemic circulation. Licensed under Public Domain via GraphicsRF - stock.adobe.com

2.2 Vascular anatomy and physiology

The blood vessels are composed of five general classes: arteries, arterioles, capillaries, venules, and veins. Arteries and veins vary in function, but they share some general features. The vasculature wall for both arteries and veins consists of three layers: (1) the tunica externa (outer layer), which contains elastin, collagen, nerves, and blood vessels; (2) the tunica media (middle layer), which comprises of vascular smooth muscle cells, elastin, and collagen; and (3) the tunica intima (inner layer), which is the thinner layer and is made of endothelial cells (Figure 2.2).

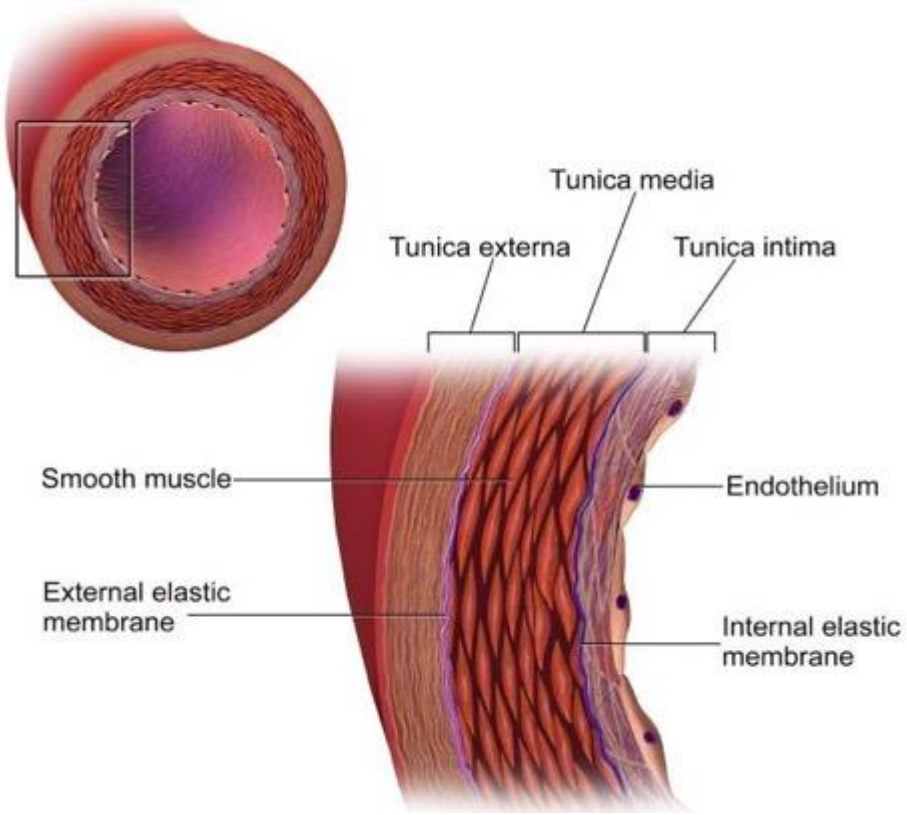


Figure 2.2 Cross-section diagram of an artery and vein anatomy. Licensed under Public Domain via (Staff, 2014)

Arteries (or efferent vessels) are elastic vessels that are able to carry blood away from the heart under high pressure and decrease in diameter as they shift further away from the heart. Moreover, arteries can be divided into three categories based on

their location in the arterial system: conducting arteries, conduit arteries, and resistance arteries. Conducting arteries are the largest arteries in the body and are centrally located (i.e. the aorta, pulmonary artery, and carotid artery), with thick walls and rich in elastic tissue. This allows them to expand and recoil quickly in response to high blood pressure ejected from the heart.

From the conducting arteries, conduit or muscular arteries such as the femoral, brachial and radial arteries branch out. These arteries are classified as medium-sized arteries, and they are responsible for transporting the blood to specific parts of the body. Additionally, conduit arteries contain more smooth muscle in which to generate greater vasodilatory and vasoconstrictive ability for local control of blood flow. Conduit arteries branch out further into resistance arteries (i.e. small arteries and arterioles), which are responsible for delivering blood to organ tissue. Resistance arteries play an important role in regulating the arterial pressure and local blood flow (Pugsley and Tabrizchi, 2000). Arterioles are the smallest artery vessel and deliver blood to the capillaries where gases, nutrients, and waste are exchanged with the target tissue.

Venules and veins are capacitance vessels that are responsible for carrying blood from the capillaries back to the heart in low-pressure flow. The venous system act as a blood reservoir and hence can control the returning blood to the heart. Blood pressure is higher in the arterial system than in veins and they typically have smaller lumen (the hollow passageway through which blood flows) than veins.

Vascular smooth muscle cells consist of unstriated myocytes with a single nuclei and are located in the tunica media layer, which is the thickest layer in the arterial wall (Figure 2.2) (Pappano and Wier, 2019). Vascular smooth muscle contraction and relaxation is regulated by multiple factors, e.g. electrical, chemical, and mechanical stimuli (Mohrman and Heller, 2018). The contractile characteristics of

arterial smooth muscle is formed by the sliding of actin and myosin filaments, which is regulated primarily by the calcium ion (Ca^{2+}) concentration in the myocyte that is released and stored in the sarcoplasmic reticulum. Myocytes are connected by electrical conductive gap junctions that allow ionic current transmission between them. Another gap junction present in the inner wall of the tunica media is the myoendothelial gap junction, which connects the middle layer with the tunica intima (inner layer). The tunica intima is the innermost layer of the blood vessel wall mainly composed of a monolayer of endothelial cells (endothelium) that rest on thin connective tissue called intimal connective tissue. The endothelium is in contact with the blood to facilitate the transfer of nutrients, chemical substances, and exchange of gases between blood and tissue, and to maintain vascular homeostasis (Sandoo et al., 2010).

2.3 Blood flow regulation

Blood pressure, viscosity and vascular diameter are essential components in regulating the blood flow that passes through the peripheral arteries (Pugsley and Tabrizchi, 2000). The alteration of the vascular diameter by either constriction or relaxation of vascular smooth muscle plays an important role in the healthy circulation of nutrients and waste removal to and from the tissue bed (Sandoo et al., 2010). Artery diameter changes in response to neural, paracrine and endothelial factors (Pugsley and Tabrizchi, 2000), are described in more detail below.

2.3.1 Sympathetic vascular control

The neurogenic control of vascular tone refers to the regulation of the blood vessel diameter by the autonomic nervous system (ANS). The ANS performs a variety

of functions through numerous cells and is composed of two main components: (1) the sympathetic nervous system (SNS) and (2) the parasympathetic nervous system (PNS). The SNS consists of preganglionic neurons that are located in the lateral gray column of the spinal cord between T1 to L2 (Levick, 2010). These neurons synapse with ganglia and use acetylcholine (ACh) to activate nicotinic receptors on postganglionic neurons. The postganglionic neurons then release norepinephrine to activate adrenergic receptors at the target site (Pappano and Wier, 2019). Of note, though norepinephrine is the most common neurotransmitter between the postganglionic neuron and the effector cell, a few sympathetic postganglionic endings release acetylcholine (e.g., sympathetic pathways regulating sweating). The PNS is composed of two parts: the cranial portion and the pelvic splanchnic nerves. The cranial portion includes cranial nerves III, VII, IX, and X, while the pelvic splanchnic nerves exit from S2 to S4. Like the SNS, preganglionic fibers in the PNS synapse on postganglionic fiber, which then innervate target sites (Levick, 2010). The present thesis will focus on the role of SNS in regulating blood flow.

The SNS plays an important role in regulating cardiovascular homeostatic function, helping to maintain blood pressure and heart rate. The SNS also plays an important role in the body's response to stress (i.e., fight-or-flight response). Most arterioles are innervated by sympathetic postganglionic neurons. Therefore, when the SNS is activated, it causes vasoconstriction in blood vessels by releasing norepinephrine, which activates alpha-adrenergic receptors on the smooth muscle cells in the target vessels (Davis and Hill, 1999). This vasoconstriction increases blood pressure and redirects blood flow to the areas of the body that need it most during times of stress or physical activity (Hansen, 2002). The SNS can also produce vasodilation. At rest there is some degree of sympathetic tonic discharge (in addition

to the vessels' intrinsic tone). Dilation can occur by decreasing sympathetic activity rate to below resting levels. Moreover, the sympathetic nerve activity (SNA) also regulates body temperature by causing vasoconstriction or vasodilation in the blood vessels, skin and other tissues. This helps to maintain the body's internal environment by responding to changes in the external environment and preparing the body for appropriate action (Crandall and Wilson, 2015).

Overall, the SNS innervates the vasculature primarily responsible for controlling vasoconstriction to regulate blood pressure and local blood flow. SNA is crucial in mediating vasomotor control of vasculature in response to changes in blood pressure, baroreceptor perturbation and in maintaining vascular tone (Davis and Hill, 1999, Hansen, 2002). Whereas, the parasympathetic nervous system (PNS) is associated with reductions in heart rate, digestion and taking control during the resting state (sleep).

2.3.2 Endothelium vascular control

The endothelium makes numerous remarkable contributions to vascular function and the regulation of vasomotor tone. Vascular tone is regulated by endothelial secretion of several vasodilators (e.g. nitric oxide (NO) and prostacyclin (PGI2)) bradykinin, and endothelial-derived hyperpolarizing factor (EDHF) and vasoconstrictors (e.g. endothelin-1 and angiotensin II) (Pappano and Wier, 2019).

2.3.2.1 Vasodilator factors

NO is a primary mediator of vasodilation (Furchgott and Zawadzki, 1980, Feelisch et al., 1994, Zanzinger et al., 1994). NO was first discovered in 1980 in an *in*

vitro study (Furchgott and Zawadzki, 1980). During this study authors observed that ACh to the arterial vasculature with the presence of endothelium caused smooth muscle relaxation, with the substance responsible initially termed endothelial-derived releasing factor (EDRF) (Furchgott and Zawadzki, 1980). Later, EDRF and NO were shown to be identical (Feelisch et al., 1994, Zanzinger et al., 1994). NO synthesis in the endothelium involves nitrogen oxidation processed from L-arginine to generate L-citrulline and NO, which is catalyzed by endothelial nitric oxide synthase (eNOS) (Palmer et al., 1988, Forstermann and Munzel, 2006). In detail, there are two types of NOS production in the endothelium: 1) inducible NOS (iNOS) and 2) constitutive NOS (cNOS) (Forstermann and Munzel, 2006). NO is constantly being produced by cNOS under ordinary conditions. However, production of NO production via iNOS is commonly observed during inflammation (Forstermann and Munzel, 2006). Once NO is generated, it diffuses into the smooth muscle layer and activates guanylate cyclase (GC), which causes an increase in the production of cyclic guanosine monophosphate (cGMP) (Forstermann and Munzel, 2006). Subsequently, cGMP allows calcium ion entrapment, resulting in the relaxation of smooth muscle cells and artery diameter increase (dilation) (Pugsley and Tabrizchi, 2000).

Prostacyclin (PG12) is another vasodilator substance known to be released to maintain vascular haemostasis (Moncada, 1988). PG12 is also related to inflammation and can be activated through arachidonic acid metabolism (Moncada, 1988, Deanfield et al., 2007). Bradykinin is a vasodilator associated with the release of NO, prostacyclin and the so-called endothelial-derived hyperpolarizing factor (EDHF) (Mombouli et al., 1992, O'Kane et al., 1994, Hornig et al., 1997). EDHF is expressed as a non-characterized endothelial factor that is linked to intracellular Ca^{2+} concentration and membrane hyperpolarisation in endothelial and vascular smooth

muscle cells. Mechanistically, hyperpolarisation of vascular smooth muscle membrane leads to a reduction in Ca^{2+}, which consequently results in smooth muscle relaxation via the release of EDHF (Hasunuma et al., 1991, Suzuki et al., 1992). NO and prostacyclin are also known as antithrombotic and platelet aggregation inhibitor factors, which prevent or limit vascular cell adhesion molecular-1 (VCAM-1) and blood clotting on the endothelium (Grover-Paez and Zavalza-Gomez, 2009).

2.3.2.2 Vasoconstrictor factors

The vascular endothelium factors responsible for causing contraction in smooth muscles are thromboxane, angiotensin II and endothelin-1 (ET-1) (Masaki et al., 1990). The most dominant factor in mediating vascular vasoconstriction is ET-1 (Tabuchi et al., 1989, Masaki et al., 1990). In detail, ET-1 is a 21-amino acid peptide formed by the endothelin converting enzyme (ECE) (Masaki et al., 1990). ET-1 acts on adjacent smooth muscle membrane receptors in which to cause an elevation in Ca^{2+} release through the phospholipase C-inositol triphosphate pathway, which results in smooth muscle contraction (Masaki et al., 1990, Pugsley and Tabrizchi, 2000). The contribution of ET-1 production is essential in maintaining basal vascular tone (Pugsley and Tabrizchi, 2000). However, a high level of ET-1 production can also lead to oxidative stress, which can lead to endothelial dysfunction and inward structural remodelling of the vasculature (Dong et al., 2005).

Angiotensin II is another vasoconstriction factor that produces a cascade effect through the G protein signalling pathway on smooth muscle (Mohrman and Heller, 2018). Angiotensin II promotes enhancements in Ca^{2+} production to induce vasoconstriction (Mehta and Griendling, 2007). Furthermore, thromboxane behaves similarly to prostacyclin in that it is formed by arachidonic acid. Nevertheless,

thromboxane induces contractile influence on smooth muscle of the vasculature (Shirahase et al., 1987).

2.3.2.3 Mechanical factor

While the blood passes through the vasculature, it creates a frictional force that acts tangential to the vasculature wall and particularly the endothelium surface, known as shear stress. The endothelial cells can sense shear stress mechanically through specialized mechanosensors such as ion channels, integrins and glycocalyx, which activates PI3 kinase and produces Akt-mediated phosphorylation of eNOS, and subsequently enhances eNOS activation and lead to the production of NO (Malek et al., 1999, Topper and Gimbrone Jr, 1999). Exposure of endothelial cells to repeated shear stress stimulus alters endothelial cell phenotype, eNOS protein expression and NO bioavailability (Laughlin et al., 2008). More importantly, the pattern of blood flow and/or shear rate (SR) through an artery is known as an important mechanical factor and can induce structural (e.g., arterial remodelling) and functional vasculature adaptations (e.g., alter NO release) (Woodman et al., 2005, Thijssen et al., 2009, Tinken et al., 2010). The recent development of technologies such as Doppler ultrasound provided a better understanding and accurate readings of arteries and specifically blood flow and/or SR. The normal blood flow/SR pattern of most peripheral arteries (e.g. brachial and radial) in humans at rest is described as a pulsatile flow, which is characterized by dominant anterograde (forward) flow with a small amount of retrograde (backward) flow (Halliwill and Minson, 2010). However, the irregularity or disturbed blood flow/SR pattern including oscillatory flow characterized by low anterograde and/or high dominant retrograde blood flow/SR pattern, is found by

various reports to contribute to impaired NO production and endothelial dysfunction (Malek et al., 1999, Thijssen et al., 2009, Johnson et al., 2012).

2.4 Endothelial dysfunction and cardiovascular risk

A disturbance in the balance between endothelial-derived vasodilators and vasoconstrictors is defined as endothelial dysfunction (Rajendran et al., 2013). Endothelial dysfunction leads to leukocyte adhesion, thrombosis, vascular inflammation, platelet activation, pro-oxidation, vasoconstriction and atherosclerosis (Haller et al., 2002, Watts et al., 2004). Several cardiovascular risk factors and diseases are associated with endothelial dysfunction (Furumoto et al., 2002). Accordingly, the association between endothelial function, inflammation and atherosclerosis will be reviewed.

2.4.1 Inflammation

Endothelial cells also play a role in immunity by defending against pathogens by changing size to form larger gaps that allow immunoglobulins to flood the inflamed tissue. However, endothelial dysfunction is associated with increases in inflammatory responses (Pappano and Wier, 2019). Moreover, cardiovascular disease risks are associated with the appearance of inflammatory responses (Furumoto et al., 2002, Arnold and Koenig, 2019). At a cellular level, the exposure of cytokines, which are known as a pro-inflammatory substance to the endothelial, has been observed to impact negatively endothelial dependant dilation and increase adhesion molecules on endothelial cells (Bhagat and Vallance, 1997). The presence of these cytokines was reported in multiple conditions such as smoking, diabetes and obesity and can cause

atherosclerosis (Wassmann et al., 2004, Wannamethee et al., 2005, Barbieri et al., 2011).

2.4.2 Atherosclerosis

The existence of Inflammation and vascular endothelial dysfunction is recognised as a significant precursor to atherosclerosis (Arnold and Koenig, 2019). Although endothelial-dependent vasoactive agents represent vascular function normality, shear stress is also vital for normal endothelial activation and adaptation (Malek et al., 1999, Topper and Gimbrone Jr, 1999, Tinken et al., 2010). Earlier studies observed that low net shear stress and/or oscillatory shear stress downgrade endothelial cells function and reactivity (Malek et al., 1999). Specifically, low shear stress and oscillatory shear stress have been shown to increase ET-1 and reduce eNOS expression, both of which are associated with plaque formation, plaque vulnerability and atherosclerotic lesions (Topper et al., 1996, Greyling et al., 2015). Excessive ET-1 circulation along with low shear stress may also lead to oxidative stress, which stimulates molecule adhesion onto the endothelial cells (Dong et al., 2005). Moreover, increases in ET-1 are correlated with the presence of pro-inflammatory cytokines such as tumour necrosis factor α (TNF-α) and interferon-γ (IFN-γ) (Klemm et al., 1995, Saleh et al., 1997, Lozano et al., 2010). Conclusively, disturbed vascular shear stimulus is associated with the presence of endothelial dysfunction and is an essential factor in the development of atherosclerosis.

The arterial endothelium senses the shear stress generated by blood flow and is responsible for releasing the above-described vasoactive agents (Malek et al., 1999). However, during blood flow/shear stress disturbance, leukocyte adhesion

molecules increase in production (Topper and Gimbrone Jr, 1999). Leukocyte molecules were found to attach to VCAM-1 during atherosclerotic cascade (e.g. plaque of fatty material and atheroma) (Deanfield et al., 2007, Grover-Paez and Zavalza-Gomez, 2009, Arnold and Koenig, 2019). Once leukocytes are attached to VCAM-1 and enter the intima, they bind to low-density lipoproteins (LDL) and form oxidized LDL, which leads to foam cell formation (Grover-Paez and Zavalza-Gomez, 2009). Additionally, elevation in LDL disturbs endothelial eNOS production and subsequently reduces NO bioavailability. Accumulation of foam cell formation in the endothelium along with the presence of pro-inflammatory cytokines can lead to more dense extracellular formation by the release of peptide growth factors, which over time may form a plaque. This observation is categorised as an advanced level of atherosclerosis (Grover-Paez and Zavalza-Gomez, 2009). Over time the plaque can develop into a thrombus and subsequently cause embolism. During this atherosclerotic phase and based on the embolism location, stroke, myocardial infarction and ischemia or cardiac arrest may occur (Verstraete, 1990, Qiao and Fishbein, 1991).

NO plays a crucial role in vascular wall regulation via the endothelium. In addition to its role in vascular tone regulation, substantial evidence indicates that NO also has vital antiatherogenic effects, including (1) Antiplatelet effects. Specifically, NO has an inhibitory effect on platelet adhesion, aggregation and thrombin-induced expression of platelet-activating factor (Arnal et al., 1999). (2) Antiproliferative effects. In detail, NO can also inhibit vascular smooth muscle cell proliferation, migration and extracellular matrix synthesis (Sandoo et al., 2010). (3) Anti-inflammatory effects. Furthermore, NO block cytokine-stimulated endothelial adhesion molecule expression, inhibit the activation and nuclear translocation of factor-κB and reduce

adhesion and activation of monocytes and neutrophils (Arnold and Koenig, 2019). (4) Antioxidant effects. In detail, low-level of NO bioavailability can directly inhibit lipid oxidation through hunting of free radicals (Forstermann and Munzel, 2006). Collectively, the reduction of NO bioavailability and/or bioactivity affects vascular tone regulation, which subsequently leads to vascular dysfunction, and this is found to be the earliest feature of the development of the atherosclerosis cascade (Grover-Paez and Zavalza-Gomez, 2009).

2.5 Assessment of endothelial function

Endothelial dysfunction is an early manifestation of atherosclerosis (Grover-Paez and Zavalza-Gomez, 2009). Epidemiological studies reported that more than 25 million individuals in Europe and North America are diagnosed with coronary artery disease (CAD) peripheral artery disease (Belch et al., 2003). Accordingly, assessing vascular function can provide significant clinical data and may help to detect early and/or predict future diseases. Several techniques have been developed to assess vascular function and dysfunction. The most common method used to evaluate vascular function is flow mediated dilation (FMD) assessment via Doppler ultrasound (Corretti et al., 2002a, Harris et al., 2010, Thijssen et al., 2011). Lately, low-flow mediated constriction (L-FMC) has been introduced, which has been suggested to provide complimentary information to that in traditional vascular function assessment (e.g., FMD) (Gori et al., 2008).

2.5.1 Flow mediated dilation (FMD)

FMD is a non-invasive approach developed to assess the vasodilation response of peripheral conduit arteries in response to reactive hyperaemia that occurs following a period of distal ischemia application (Celermajer et al., 1992). The FMD assessment is investigated in peripheral conduit arteries in the upper (e.g., brachial and radial artery) or lower (e.g., femoral artery) limb via Doppler ultrasound (Thijssen et al., 2008). The application of an FMD protocol includes baseline measurement of the conduit artery (e.g., 1-2 minutes) followed by an occlusion period to block downstream blood flow via inflation of supra-systolic cuff pressure placed distal to the measurement site (e.g., over either the arm or leg). Occlusion is maintained for a period of time (e.g. 5 minutes), and then the cuff is released, which initiates a reactive increase in downstream blood flow (reactive hyperaemia shear stimulus), resulting in an elevation in unidirectional shear stress (anterograde) on the vasculature wall and inducing vasodilation. The change in diameter from the pre-occlusion period is recorded and a change is calculated to indicate the vascular function (Corretti et al., 2002a).

The mechanism underlying the FMD response is widely accepted across the literature to be largely mediated by NO release (Joannides et al., 1995, Mullen et al., 2001, Green et al., 2014). In detail, infusion of L-NG-monomethyl arginine citrate (L-NMMA; NOS blocker) was found to diminish or severely attenuate FMD response in several conduit arteries such as femoral, brachial and radial arteries (Doshi et al., 2001, Mullen et al., 2001, Kooijman et al., 2008). Therefore, FMD can be recognized as a vital marker of the conduit artery endothelium-dependent NO function (Yeboah et al., 2008).

The FMD assessment is widely used as a reliable method to evaluate the conduit artery endothelial function and the brachial artery is the most frequent site for FMD assessment across the literature (Corretti et al., 2002a, Harris et al., 2010, Thijssen et al., 2011). However, FMD assessment is also found reliable in different conduit arteries such as femoral and radial artery (Mullen et al., 2001, Thijssen et al., 2008). In clinical examination trials, a positive correlation was observed between FMD and coronary ACh responses. In detail, FMD from the brachial artery and intracoronary ACh responses were investigated in 50 patients with coronary angiography appointment (Anderson et al., 1995). Participants who were characterized with low FMD response (less than 3%), were observed also with coronary endothelial dysfunction, and the conclusion was that FMD values were significantly associated with the presence of coronary endothelial dysfunction. Moreover, participants with cardiovascular risk manifest lower FMD response compared to the control participants (Hambrecht et al., 2003, Watts et al., 2004). Conclusive evidence indicates that FMD is a reliable method to evaluate vascular function and is an independent predictor of future cardiovascular events (Hashimoto et al., 2000, Furumoto et al., 2002).

The impact of various interventions on the FMD response has been widely explored. Furthermore, It is widely recognised that chronic physical activity, exercise and heating are interventions that are associated with improvement in endothelial function, which is primarily mediated by the exposure to increases in blood flow and/or shear stress (Watts et al., 2004, Laughlin et al., 2008, Tinken et al., 2009, Birk et al., 2012, Fletcher et al., 2013, Brunt et al., 2016). According to several studies, it has become appreciated that exercise training and heating therapy can enhance conduit artery endothelium-dependent function, as assessed using FMD (Padilla et al., 2011, Birk et al., 2012, Birk et al., 2013, Brunt et al., 2016). For example, chronic heat

application (4 – 5 times per week) for 8 weeks via hot water immersion in a 40.5 °C bath for 60 minutes per session, significantly improved FMD in sedentary individuals (Brunt et al., 2016). This study revealed that passive heat therapy improves endothelial function in sedentary individuals, suggesting its potential as a nonpharmacological intervention for promoting cardiovascular health. Like heat therapy, exercise training programs (including cycling and running on a treadmill for 15 minutes three times per week for 8 weeks at 80% heart rate reserve) significantly enhanced brachial artery FMD in the 2^{nd}, 4^{th} and 6^{th} week of the program (Tinken et al., 2008). Moreover, increases in brachial artery FMD was also noted after an exercise protocol involving 30 minutes of lower limb cycling exercise per week at 80% of maximal heart rate for 8 weeks (Birk et al., 2012). The underlying mechanisms for these improvements were attributed to enhanced endothelial function, increased NO availability, and improved vascular function. However, such increases in the brachial artery characteristics and FMD were not observed when exercise-induced increased in shear stress were disrupted using a partial pneumatic cuff inflation (60 mmHg), confirming that enhancement of FMD during exercise is at least partly mediated by shear stimulus (Birk et al., 2012). The application of pneumatic cuff inflation placed distal to the measurement area is a popular non-invasive technique to manipulate shear stress to prevent the increase in anterograde shear stress and elevate retrograde shear stress (Thijssen et al., 2009). Several studies reported that acute and chronic shear stress manipulation characterized by retrograde shear stress elevation via partial cuff inflation at rest and during various interventions (e.g. cycling, handgrip exercise and forearm heating) prevent endothelial function improvement (FMD) (Tinken et al., 2009, Thijssen et al., 2009, Birk et al., 2012). Indeed, acute SNA induced by lower body negative pressure (LBNP) along with partial cuff inflation (75 mmHg) increased

retrograde shear stress and attenuated brachial artery FMD responses (Thijssen et al., 2014). However, local forearm heat application prevented the increase in retrograde shear stress during LBNP and FMD was preserved. These results support the notion that changes in SR play a key role in mediating FMD responses (Thijssen et al., 2014).

2.5.2 Low-flow mediated constriction (L-FMC)

While FMD assesses the ability of the conduit artery to dilate in response to hyperaemia, low-flow mediated constriction (L-FMC) assesses the ability of a conduit artery to constrict during a low blood flow state. It was noted first by Levenson et al. (1987) that an acute reduction of blood flow applied distally to the brachial artery, by an inflatable cuff, caused brachial artery vasoconstriction. Later, the relationship between a low blood flow state and vasoconstriction was revisited and confirmed that when a supra-systolic forearm cuff was applied distally to different conduit arteries such as brachial and radial artery for period of time (5-10 minutes), a significant vasoconstriction response in the brachial artery was noted in several studies (Gori et al., 2008, Humphreys et al., 2014). Gori et al. (2008) and colleagues coined the term L-FMC and suggested that it provides complimentary vascular function information when assessed along with FMD in healthy and clinical populations, despite the lack of correlation between L-FMC and FMD responses (Gori et al., 2008, Gori et al., 2010), as described in detail in section 2.5.2.3.

Like FMD, L-FMC is monitored and assessed via Doppler ultrasound. A 5 minute period of distal blood flow occlusion via supra-systolic cuff is recommended for assessing L-FMC, during which images of diameter and blood velocity of the conduit

artery are recorded continuously (Spiro et al., 2011, Elliott et al., 2018). Specifically, during the last 30 seconds of blood flow occlusion, diameter reactivity is considered as the period of L-FMC response and calculated as the change in diameter during the L-FMC period in relation to baseline diameter (pre occlusion), with L-FMC response data presented in absolute (mm) and (%) relative terms (Gori et al., 2008). Furthermore, several reports proposed a link between the reduction in conduit artery diameter during a low blood flow state and basal tone (Gori et al., 2011, Gori et al., 2012). Thus, L-FMC may provide a description of an integrated score of basal and stimulated vasoconstriction function.

2.5.2.1 L-FMC underlying mechanisms

The mechanisms mediating the L-FMC response are proposed to generally arise from the endothelium, whereby the low-flow elicits either increased vasoconstrictor and/or reduced vasodilator factors. In support of this, a mechanistic *in vivo* study reported an attenuation in L-FMC following inhibition of vasodilators agents (e.g. EDHF and prostaglandin). This suggests that L-FMC is at least partly mediated by the endothelium (Gori et al., 2008, Dawson et al., 2012). However, inhibiting NO activity by infusing NO-blocker (NG-monomethyl-L-arginine) has been found not to alter L-FMC response, indicating that L-FMC and FMD are not similarly mediated (Gori et al., 2008). Alternatively, blockade of vasoconstrictor agent ET-1 was found to diminish L-FMC response of radial artery, proposing that L-FMC is mediated by endothelium-derived vasoconstrictor pathways (Spieker et al., 2003). Nevertheless, alterations in plasma ET-1 levels were found to cause no influence on the brachial artery L-FMC response (Spiro et al., 2011). A role for sympathetic vasoconstrictor tone

has also been suggested, but not examined (Gori et al., 2008, Elliott et al., 2018). Hence, the mechanisms underlying L-FMC remain incompletely understood.

2.5.2.2 Site specificity of L-FMC

L-FMC can be measured simultaneously with FMD, and as mentioned above the brachial artery is the most common and reliable measurement site for FMD assessment across the literature. However, heterogeneity in L-FMC response was noted when assessed at the brachial artery (Weissgerber et al., 2010, Spiro et al., 2011). For example, a reduction in the L-FMC response was observed in healthy participants when measured at the brachial artery (Spiro et al., 2011). Whereas Thijssen et al. (2008), reported an increase in brachial artery diameter during forearm occlusion (e.g., L-FMC period) in healthy children and adults. A comparable study of L-FMC response in two different arteries (brachial and radial artery) was conducted in pregnant and non-pregnant females, which confirmed the presence of a L-FMC response in the radial artery but not in the brachial artery in the same participants (Weissgerber et al., 2010). Moreover, L-FMC was significantly reduced when measured at the radial artery in participants with cardiovascular diseases compared to healthy volunteers, suggesting that L-FMC at the radial artery can possibly provide additional clinical insight into the traditional FMD measurement (Gori et al., 2010). These contradictory findings and limited evidence needs additional research investigation to quantify the heterogeneity between different arteries so an appropriate interpretation of L-FMC can be carried out.

2.5.2.3 L-FMC and cardiovascular disease

The L-FMC response has been investigated in both healthy (Gori et al., 2008, Weissgerber et al., 2010, Harrison et al., 2011, Rakobowchuk et al., 2012) and clinical (Gori et al., 2008, Gori et al., 2010, Spiro et al., 2011) populations to quantify its association with cardiovascular health (Gori et al., 2008, Gori et al., 2010, Dawson et al., 2012, Rakobowchuk et al., 2012, Spiro et al., 2011). Indeed, several reports confirmed the reduction in L-FMC response of radial artery in patients with cardiovascular diseases as well as in participants with cardiovascular risk factors (Gori et al., 2008, Gori et al., 2010, Harrison et al., 2011). Although L-FMC and FMD are suggested to be mediated by different mechanisms, the reduction in L-FMC in clinical populations is also observed to be accompanied by a reduction in FMD response (Gori et al., 2008, Gori et al., 2010, Harrison et al., 2011). Furthermore, patients with different severities of coronary artery disease (CAD; 1, 2 and 3 vessels) showed a dose-dependent attenuation in both radial artery L-FMC and FMD responses (%) based on the severity of CAD (the more severe the CAD the lower the L-FMC and the FMD % responses) (Gori et al., 2012). These findings support the theory that L-FMC provides complementary information to FMD and can be used as a marker of vascular dysfunction.

2.5.2.4 Impact of interventions on L-FMC

Non-pharmacological interventions such as exercise training and heat therapy are well-known to enhance cardiovascular health and more specifically endothelial function (Watts et al., 2004, Ohori et al., 2012, Fletcher et al., 2013, Brunt et al., 2016). Parallel to this, similar improvements in L-FMC response were also noted following exercise training when the influence of two 6-week exercise training programs

conducted at different exercise intensities (moderate and high intensity) investigated the L-FMC response of the brachial artery in healthy adults (Dawson et al., 2012, Rakobowchuk et al., 2012). L-FMC of the brachial artery was enhanced following the exercise in both protocols to a similar magnitude, which supports the notion that L-FMC measurement can be used as a determinant of vascular function. The similarity of the magnitude of improvement in L-FMC between the exercise groups was suggested to not be attributable to metabolic stress as this was different between the groups, but potentially shear stress, which was similar in both training groups (Rakobowchuk et al., 2012).

Dawson et al. (2012) examined the impact of 6-week handgrip exercise training on radial artery L-FMC following radial artery catheterization in patients scheduled for coronary angiography or angioplasty appointment. It was observed that L-FMC was diminished post-catheterization in the catheterized arm, but not altered in the non-catheterized arm, suggesting that L-FMC response is endothelium-dependent. Additionally, the vascular function of the radial artery and particularly L-FMC recovered faster in a group undergoing handgrip exercise training, compared to a non-exercise control group. Among the various explanations of the mechanism responsible for preserving L-FMC response post-catheterization in the exercise group, repeated increases in blood flow and shear stress were suggested to be the prime mediators (Dawson et al., 2012).

Although longitudinal exercise interventions were observed to be beneficial in improving L-FMC response, there is a lack of understanding of the acute impact on L-FMC response across the literature. However, the effect of acute dynamic cycling exercise for 30 minutes on L-FMC in healthy young adults has been investigated (Elliott et al., 2018). In this study, L-FMC was measured before and after an acute bout

of exercise and radial artery characteristics were monitored throughout the trial. Although radial artery characteristics such as blood flow, mean SR and diameter were augmented during exercise, the L-FMC response of the radial artery was significantly increased versus the pre-exercise and control group. The underlying mechanisms were not explored in this study, however, an increase in retrograde shear during exercise was noted. Retrograde shear is well known to attenuate vascular function and alter endothelial cells bioreactivity in various studies (Ziegler et al., 1998, Padilla et al., 2010). Nevertheless, L-FMC was significantly augmented following an acute bout of dynamic exercise (Elliott et al., 2018) suggesting that L-FMC response may be mediated by factors that are independent of SR and/ or the endothelium, such as SNA. Exercise is a well-known intervention for increasing SNA (Fisher et al., 2015). Currently the role of SR on L-FMC response is not completely understood and whether the L-FMC response is associated with and/or mediated by SNA needs further investigation.

2.6 Summary

This review puts a spotlight on the impact of non-pharmacological therapeutic interventions and acute stimuli on shear stress and mediating FMD and L-FMC responses. Further research is needed to fully understand the physiological mechanisms underpinning SR modification and vascular function. Such research will inform basic physiological understanding as well as cardiovascular function and health.

The current thesis aims to target this scarcity of research within the field of vascular physiology. By utilising the long-established technique of FMD, alongside the

novel technique of L-FMC, this thesis will determine the acute impact of different environmental modifiers (heat stress, exercise and lower body pressure) and SR modification on vascular function, specifically L-FMC.

2.7 Thesis Aims

In light of the background provided, the specific study aims addressed in the thesis are:

Experimental Chapter 4:

To investigate the acute effect of whole-body passive heat stress on radial artery blood flow characteristics, FMD and L-FMC. Also, to determine whether the influence of whole-body passive heat stress on FMD and L-FMC is mediated by a change in local SR.

a. Hypothesis 1: whole-body passive heat stress augments anterograde SR and subsequently increases FMD and L-FMC via endothelium-mediated mechanisms.
b. Hypothesis 2: increases in FMD and L-FMC would be prevented if increases in anterograde SR were minimized, and retrograde SR augmented (i.e., with a wrist cuff), during whole-body passive heat stress.

Experimental Chapter 5:

To determine the impact of acute exercise and SR modification on radial artery L-FMC in young healthy.

a. Hypothesis 1: wrist cuff inflation to reduce mean SR and augment retrograde SR during leg cycling exercise will attenuate radial artery L-FMC.

Experimental Chapter 6:

To examine the effect of acute SNA (sympathoexcitatory and sympathoinhibitory manoeuvres) on radial artery characteristics and L-FMC response.

a. Hypothesis 1: Lower body negative pressure (LBNP) would increase radial artery retrograde SR and L-FMC response and attenuates FMD.
b. Hypothesis 2: Lower body positive pressure (LBPP) would decrease retrograde SR and attenuate L-FMC response, and augments FMD.

CHAPTER 3: GENERAL METHODS

The measurements and procedures detailed in this chapter were conducted across all research chapters in the present thesis. Accordingly, information regarding participants characteristics, as well as key techniques and measures applied to evaluate this thesis's hypothesises are described in this chapter. Techniques and measures that were only used in one research chapter are not detailed here but instead, are described in the associated chapter.

3.1 Ethical Approval and consent

All procedures were approved by the University of Birmingham, Science Technology Engineering and Mathematics Ethical Review (approval number ERN_18-0523) and conformed to the *Declaration of Helsinki* ethical standards, with the exception of the registration of research in a clinical trials database. Prior to participants' enrolment in a study, an information sheet was provided containing details of the experimental procedures and participants were given the opportunity to ask questions. Additional to this, all study participants gave written informed consent prior to enrollment.

3.2 Inclusion criteria

Participants characteristics for each research chapter are outlined in Table 3.1. All included participants were healthy young men; none reported having been diagnosed with pulmonary, metabolic and cardiovascular disease or cardiovascular risk such as hypertension. Participants who were on any type of medication and/or tobacco smokers were excluded. It is well accepted that there are variances in the cardiovascular system between males and females, and recent research has been directed towards identifying and comprehending these biological distinctions. Differences in the baseline diameter of boys and girls as young as 6 years old has previously been demonstrated, and disparities in FMD between the sexes are noticeable at around age 17 (Hopkins et al., 2015). One notable variation between adult males and females is that unlike males, females experience fluctuations in ovarian sex hormones, primarily oestrogen and progesterone, throughout their menstrual cycle (Abraham et al., 1972). The menstrual cycle consists of three phases: the follicular phase, the periovulatory phase and the luteal phase (Draper et al., 2018). Oestrogen and progesterone levels are low during the early follicular phase (menses) (Draper et al., 2018). During the late follicular phase, oestrogen levels increase and reach their peak just before ovulation, while in the luteal phase, oestrogen levels decline, and progesterone levels rise (Abraham et al., 1972, Draper et al., 2018). Oestrogen plays a crucial role in influencing endothelial function through two primary pathways known as the genomic and non-genomic pathways (Chakrabarti et al., 2014). Notably, oestrogen receptors, predominantly oestrogen receptor alpha (ERα) and oestrogen receptor beta (ERβ), are expressed in endothelial cells (Chakrabarti et al., 2014). In the genomic pathway, oestrogen enters the endothelial cell plasma

membrane and binds to ERα or ERβ, causing the receptors to form homo- or heterodimers. These dimers then enter the nucleus and trigger gene transcription, which increases eNOS expression (Chakrabarti et al., 2014). Thus, in females the genomic effect of their elevated oestrogen levels is an increased gene expression of eNos and prostacyclin synthase, which elevates NO bioavailability (Arnal et al., 1999). In the non-genomic pathway, oestrogen binds to cell surface ERα, leading to eNOS phosphorylation, which enhances the production of NO (Chakrabarti et al., 2014). Expression of ERα changes across the menstrual cycle, with Erα low when oestrogens are low (early follicular) and vice versa (Gavin et al., 2009). This determines a rapid change in the peripheral vascular responsiveness across the menstrual cycle due to the influence of oestrogens on ERα expression and NO production (Gavin et al., 2009). Accordingly, females were excluded in the current thesis to avoid the confounding effect of the menstrual cycle on endothelial function (Hashimoto et al., 1995). All participants who engaged in regular exercise were instructed to stop vigorous exercise for at least 48 hours prior to each experimental test. Dietary instructions identified to impact vasculature responses were given for all participants to follow (Harris et al., 2010). Specifically, these instructions included: no vitamin supplementation for at least 72 hours prior to an experimental session, no food and beverages with polyphenol enrichment for at least 18 hours and no alcohol or caffeine for at least 12 hours prior to an experimental session.

Table 3.1 Participant characteristics for each research chapter.

	Chapter 4	**Chapter 5**	**Chapter 6**
n	11	10	10
Age (years)	25 ± 5	23 ± 2	23 ± 6
Weight (kg)	73 ± 6	75 ± 6	71 ± 9
Height (cm)	176 ± 3	177 ± 6	175 ± 8
BMI (kg/m^2)	23 ± 2	23 ± 5	22 ± 2

Values are means ± SD.

3.3 Measured Variables

3.3.1 Haemodynamics

Heart rate (HR) was measured using the R wave peaks from a standard lead II electrocardiogram (ECG) (Tango+, SunTech Medical Instruments, Raleigh, NC, USA). The use of the lead II ECG model was selected because it provides a superior-inferior plane reference of the heart via the placement of the electrodes on the base of the right upper limb and left lower limb, which has been identified to show a reliable and accurate visualisation of the upward P waves and the amplitude of R wave peak (Meek and Morris, 2002). Blood pressure (BP) was assessed from the left arm by placing the cuff on the brachial artery and using an automated auscultatory R-wave gating algorithm method (Tango+, SunTech Medical Instruments, Raleigh, NC, USA). Systolic and diastolic BP were recorded, and mean arterial pressure (MAP) was calculated according to the formula: MAP (mmHg)=Diastolic BP(mmHg)+[0.33+(HR x 0.0012)] x [Systolic BP(mmHg)-Diastolic BP (mmHg)] (Razminia et al., 2004).

3.3.2 Radial artery characteristics

High-resolution duplex Doppler ultrasound (Terason uSmart 3300, Teratech Corporation, Burlington, MA, USA) was used in all studies (Chapters 4, 5 and 6) to obtain radial artery diameter and blood flow velocity at rest, during and following all interventions (time points described within each experimental chapter). From these measures, functional changes in the radial artery were monitored and recorded throughout all experimental trials and vascular function (L-FMC and FMD) was assessed before and after all interventions. A 4-15 MHz multi-frequency linear-array probe (Terason uSmart 15L4) was plugged into the Doppler ultrasound machine to

transmit the images of the radial artery. The measurement of the radial artery diameter and blood flow velocity was obtained in all studies from the right forearm, specifically 10-15 cm distal to the medial epicondyle. Following obtaining an optimal image of the radial artery, the probe was held stable via an adjustable holder and the insonation angle of the ultrasound was maintained close to 60 degrees throughout the entire experiment. The mode used on Doppler ultrasound to obtain radial artery diameter image was B-mode, whereas pulse-wave mode was used for radial artery flow velocity, with both modes running simultaneously during the entire experiment. Doppler ultrasound images were recorded in FMD studio software (Cardiovascular Suite Version 3.4.1, FMD Studio, Pisa, Italy) and saved as video files. The radial artery blood flow (ml/min) was calculated from diameter and blood velocity variables as mean blood velocity (cm/s) x π x r (cm) ^{2} x 60, where r is the radius of the radial artery lumen. Vascular conductance was calculated as the following equation:

$$\text{Radial artery vascular conductance} = \frac{\text{Radial artery blood flow}}{\text{MAP}}$$

Radial artery SR (s^{-1}) was calculated with respect to the blood viscosity derived from Poiseuillies law accordingly:

$$\text{Radial artery SR} = 4 \text{ x } \frac{\text{Radial artery blood velocity}}{\text{Radial artery diameter}}$$

Note: Anterograde and retrograde SR were calculated using positive and negative blood velocities, respectively.

3.4 General Experimental Procedures

All visits took place in a temperature-controlled laboratory (23 °C) at the University of Birmingham. Prior to any experimental trial, participants were invited for a familiarisation session where the experimental procedures along with the experimental measures were explained and applied. In all experimental studies (Chapter 4, 5 and 6), trials within each study were performed at the same time of the day for each participant to minimize the potential confounding influences of diurnal variations in the measured variables (Facer-Childs et al., 2019) and trials were separated by at least 24 hours and completed within 14 days. The order of the experimental trials was randomised by a simple random allocation technique (coin toss). Measured variables mentioned above were obtained at the baseline following 20 minutes of resting and continuously every 5 minutes throughout the trials (Chapter 4, 5 and 6).

3.5 Interventions

3.5.1 Assessment of radial artery function (FMD and L-FMC)

Assessment of radial artery function (FMD and L-FMC) was measured in accordance with recent guidelines and technical recommendations (Gori et al., 2008, Gori et al., 2010, Thijssen et al., 2011). FMD and L-FMC assessments were applied twice per experimental trial, first after 20 minutes of rest following instrumentation and then again at a specific time point durin g/following the intervention (details of second measurement/assessment time point explained within individual experimental chapters). The vascular function assessment (FMD and L-FMC) procedure was

initiated with a 60-second recording of the radial artery to obtain resting artery diameter and blood flow velocity (baseline period; Figure 3.1). A pressure cuff wrapped distally to the radial artery measurement area around the wrist was then inflated to a supra systolic pressure (>220 mmHg) to induce an ischemic stimulus (occlusion period; Figure 3.1). After five minutes of occlusion, the cuff was then deflated, and recording of the radial artery was continued for an additional three minutes following cuff deflation (recovery period; Figure 3.1). The change in radial artery diameter and velocity from the baseline period to the last 30 seconds of the cuff occlusion period (5 min) was determined as the L-FMC response (Gori et al., 2008). The FMD response was defined as the change from resting radial artery diameter to the peak diameter in the post occlusion period (3 min, recovery period) (Thijssen et al., 2011). Both L-FMC and FMD responses are presented relative (%) and absolute (mm) change (Thijssen et al., 2011). The time-to-peak was calculated as the total time (seconds) taken to reach peak diameter from cuff deflation. The SR area under the curve (SR_{AUC}) was defined as an individual area (FMD, mm), this area calculated from cuff deflation to the peak diameter (FMD, mm) (Padilla et al., 2008). The sum-up of FMD (%) and L-FMC (%) response was identified as the composite end or total vessel reactivity (TVR, %) and calculated accordingly (Rakobowchuk et al., 2012):

$$\frac{\text{FMD diameter (mm)} - \text{LFMC diameter(mm)}}{\text{Baseline average diameter (mm)}} \text{ x } 100$$

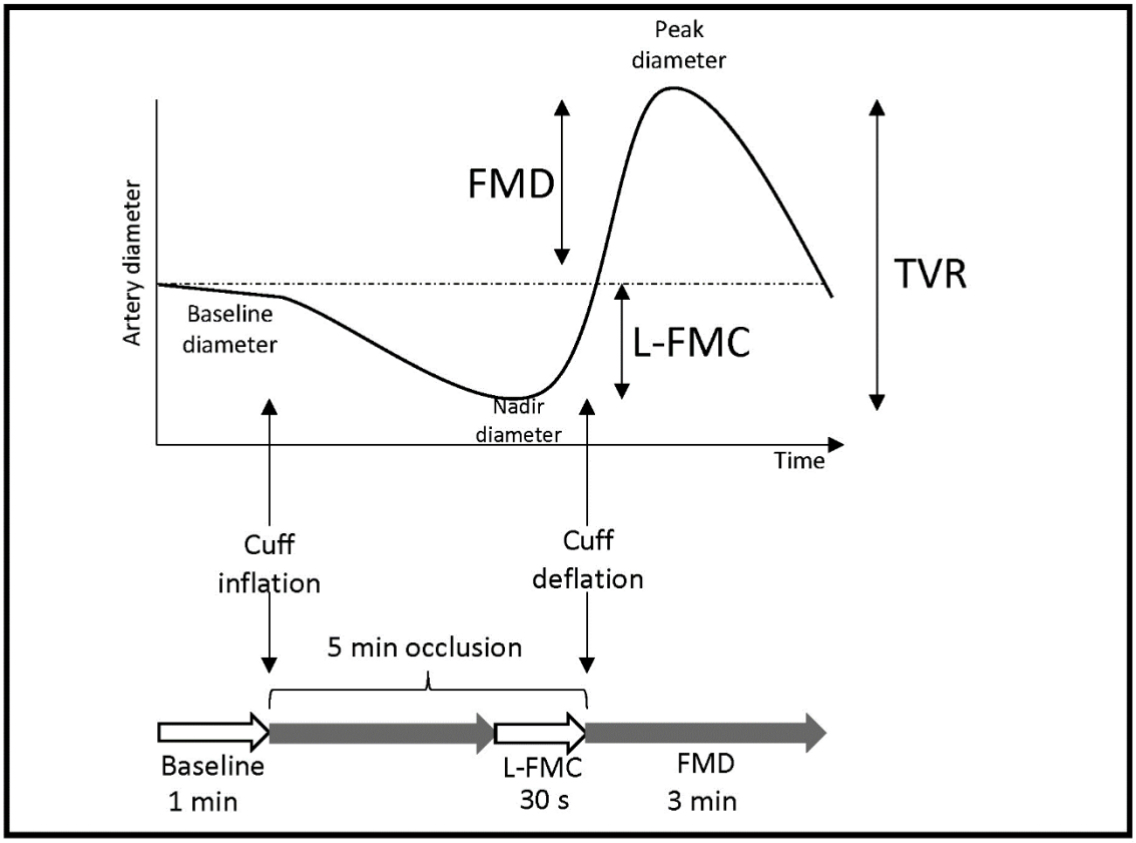


Figure 3.1 Schematic representation of different parameters of vascular function assessment. Adapted from (Gori et al., 2008).

3.5.2 Shear rate manipulation

SR manipulation of the right radial artery was done non-invasively to modify the SR pattern of blood flow through the right radial artery measurement area. Changes in SR on the endothelium surface is an important stimulus for vascular function adaptation (described in Chapter 2). The SR pattern is modified by increasing the retrograde SR, which was achieved through patrial inflation of the wrist cuff (75 mmHg) in Chapters 4 and 5 (Thijssen et al., 2009) and by using the lower body negative pressure model (-20 mmHg) in Chapter 6 (Thijssen et al., 2014).

3.6 Data acquisition and analysis

In all studies, haemodynamic data were sampled and recorded manually according to standard operating procedures described in detail within experimental Chapters 3, 4 and 5. Radial artery diameter and velocity were analysed offline via automated edge detection and wall tracking algorithms software (Cardiovascular Suite Version 3.4.1, FMD Studio, Pisa, Italy) by the primary researcher of the current thesis. During this analysis, the researcher was blinded to participants' identities as well as the time point of the measurement/assessment. The acquired data were then transported to a digital spreadsheet (Excel, Microsoft, WA, USA), where averages of variables' at each time point for each participant, mean, standard deviation and standard error were determined. Statistical analysis was performed using scientific data analysis and graphing software (SPSS 21, IBM, NY, USA; Sigmaplot 13, Systat software, London, UK – Chapter 4 and GraphPad Prism 8, GraphPad Software, San Diego, CA – Chapters 3 and 5.

3.7 Reliability of L-FMC and FMD measures

This section aims to evaluate the between-day repeatability of the radial artery variables measurement obtained by Doppler ultrasound (mentioned above) in the studies described in Chapters 4, 5 and 6. The repeatability of these data were assessed to determine how reproducible and reliable baseline measures of L-FMC and FMD were when collected by the same operative (M Alali).

Measurements were conducted on 10 healthy young individuals (aged 18 to 33 years) at the same time of the day on two separate visits, separated by at least 24 hours but completed within 7 days. Participants were instructed to adhere to the following instructions prior to the test: no alcohol or caffeine beverages and no vigorous exercise for 12 hours. Participants then came to a temperature-controlled quiet laboratory (23 °C) on the study day and rested in a supine position throughout the session. Measurement of radial artery measures obtained by Doppler ultrasound is described above, with radial artery baseline diameter, L-FMC (mm) and FMD (mm) analysed offline using FMD studio software (described above). Radial artery blood flow was calculated as mentioned above.

The relative reliability of inter-day (two measurements; on separated days) radial artery baseline diameter, nadir diameter, peak diameter, L-FMC (mm), FMD (mm) and baseline blood flow measures were analysed using intraclass correlation coefficient (ICC) (Shrout and Fleiss, 1979). ICC less than 0.50, between 0.50 and 0.75, between 0.75 and 0.90 and greater than 0.90 were taken to be indicative of poor, moderate, good and excellent reliability, respectively (Koo and Li, 2016). The error of measurement of inter-day radial artery baseline diameter, nadir diameter, peak diameter, L-FMC (mm), FMD (mm) and baseline blood flow measures was assessed

using the coefficient of variation (CV) and calculated in excel as the following equation: CV = (SD/x)*100, where SD is the standard deviation of the sample and x represents the sample mean. Inter-day CV of <10% was considered to indicate that the data (baseline diameter, L-FMC and FMD) was reproducible (Gori et al., 2008). Agreement between inter-day baseline blood flow to evaluate bias between the mean differences and to estimate an agreement interval was also assessed using the Bland-Altman plot (Bland and Altman, 1986). Statistical analysis was performed in GraphPad Prism 8 (GraphPad Software, San Diego, CA).

3.7.1 Results

Doppler ultrasound measurement of 10 radial arteries was conducted in two separate occasions as described above and stored for offline analysis. Radial artery diameter was measured and averaged at the baseline, the last 30 seconds of cuff occlusion (nadir diameter) and 3 minutes post occlusion (peak diameter). Radial artery blood flow of each participant was calculated as described previously. The overall correlation of radial artery diameter between days was good, with an ICC of 0.80 (95% CI 0.33 to 0.95), 0.88 (95% CI 0.58 to 0.95) and 0.83 (95% CI 0.41 to 0.91) for baseline diameter (mm), nadir diameter (mm) and peak diameter (mm), respectively (Table 3.2). The correlation of radial artery L-FMC (mm) and FMD (mm) between days was also good, with an ICC of 0.76 (95% CI 0.25 to 0.94) and 0.64 (95% CI 0.20 to 0.90), respectively (Table 3.2). Radial artery baseline blood flow correlation between days was good, with an ICC of 0.81 (95% CI 0.37 to 0.95). The CV between days was acceptable for all radial artery main measures, with a CV of 3.2% for baseline diameter, 3.7% for nadir diameter, 3.4% for peak diameter, 6.4% for L-FMC (mm),

12.1% for FMD (mm) and 23.4% for blood flow (Table 3.2). The Bland-Altman plot for radial artery diameter and blood flow shows no systemic bias and good agreement, with a mean difference of 0.04 mm and limits of agreement ranging -0.06 to 0.14 mm at baseline diameter (Figure 3.2A), a mean difference of 0.05 mm and limits of agreement ranging -0.33 to 0.44 mm at peak diameter (Figure 3.2B), a mean difference of 0.03 mm and limits of agreement ranging -0.27 to 0.34 mm at nadir diameter (Figure 3.2C), a mean difference of 0.03 mm and limits of agreement ranging -0.13 to 0.19 at FMD (Figure 3.2D), a mean difference of 0.02 mm and limits of agreement ranging -0.29 to 0.33 mm at L-FMC (Figure 3.2E) and a mean difference of -0.10 mm and limits of agreement ranging -31.57 to 31.37 at blood flow (Figure 3.2F).

Table 3.1 Inter-day reproducibility of radial artery variables.

	Visit 1	**Visit 2**	**CV(%)**	**ICC**	**(Gori et al., 2008) ICC**
Baseline Diameter (mm)	2.61 ± 0.18	2.62 ± 0.22	3.2	0.80	0.85
Baseline blood flow (ml min$^{-1)}$	46.35 ± 16.97	46.45 ± 26.35	23.4	0.81	-
Nadir Diameter (mm)	2.49 ± 0.23	2.46 ± 0.22	3.7	0.88	-
L-FMC (mm)	0.11 ± 0.11	0.15 ± 0.09	6.4	0.76	0.80
Peak Diameter (mm)	2.81 ± 0.24	2.75 ± 0.23	3.4	0.83	-
FMD (mm)	0.19 ± 0.10	0.13 ± 0.13	12.1	0.64	0.68

Values are means ± SD. L-FMC, low-flow mediated constriction; FMD, flow mediated dilatation; CV, coefficient of variation; ICC, Intraclass correlation coefficient.

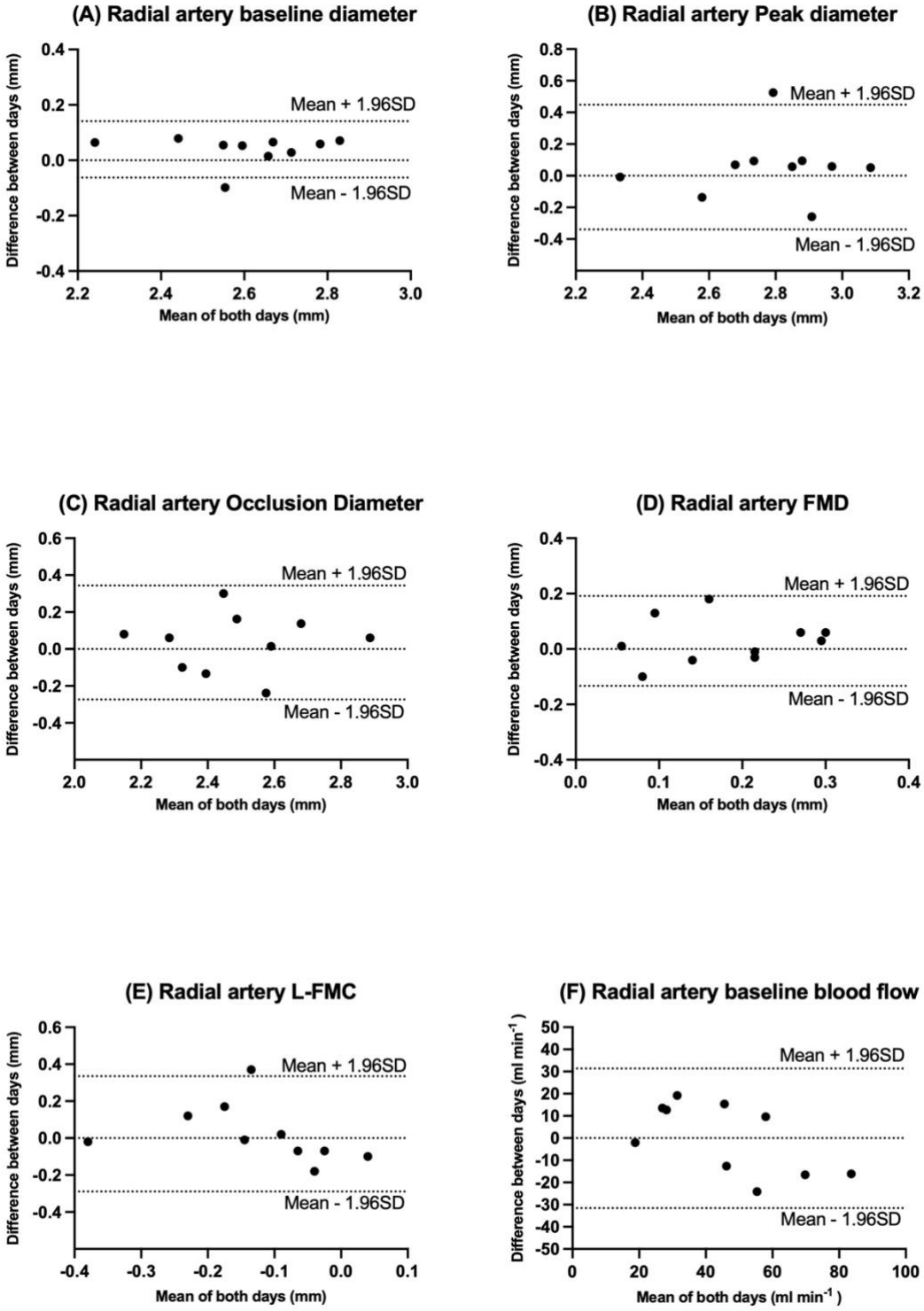


Figure 3.2 Bland-Altman plots of the between days reproducibility of radial artery baseline diameter (A), peak diameter (B), occlusion diameter (C), FMD (D), L-FMC (E) and baseline blood flow (F) measurements. Dashed lines indicate the mean of differences and limits of agreement.

3.7.2 Conclusions

In the present thesis, the assessment of radial artery baseline, nadir and peak diameter and function (L-FMC and FMD (mm)) via Doppler ultrasound was shown to be reliable with an acceptable agreement between days. If the mean difference is zero or close to zero, it indicates good agreement between the visit 1 and visit 2 measurements. A large mean difference may suggest a systemic bias between the sets of measurements (Bland and Altman, 1986). In addition, variability of the parameters in the radial artery were within expected limits (Gori et al., 2008).

CHAPTER 4: IMPACT OF WHOLE-BODY PASSIVE HEAT STRESS AND ARTERIAL SHEAR RATE MODIFICATION ON RADIAL ARTERY FUNCTION IN YOUNG MEN

This chapter was published in the Journal of Applied Physiology (Alali et al., 2020) and has undergone minor modifications to adapt to the thesis.

Alali, M. H., Vianna, L. C., Lucas, R. A., Junejo, R. T. & Fisher, J. P. 2020. Impact of whole body passive heat stress and arterial shear rate modification on radial artery function in young men. *Journal of Applied Physiology,* 129(6): 1373-1382. doi:10.1152/japplphysiol.00296.2020

M.H.A contributions: M.H.A conceived and designed research; M.H.A performed experiments; M.H.A analysed data, interpreted results of experiments, prepared figures, drafted manuscript, edited, and revised manuscript.

4.1 Abstract

We sought to determine how whole-body heating acutely influences radial artery function, characterized using flow mediated dilation (FMD) and low-flow mediated constriction (L-FMC), and the mechanistic role of shear rate modification on radial artery functional characteristics during heating. Eleven young healthy men underwent whole-body heating (water-perfused suit) sufficient to raise core temperature +1°C. Trials were repeated with (Heat+WC) and without (Heat) the application of a wrist cuff located distal to the radial artery examined, known to prevent increases in mean and anterograde shear rate but increase retrograde shear. Radial artery characteristics were assessed throughout each trial, with FMD and L-FMC assessed prior to and upon reaching the target core temperature. Heat markedly increased radial artery mean and anterograde shear rate, along with radial artery diameter and blood flow ($P<0.05$). Heat+WC abolished the heat-induced increase mean and anterograde shear rate ($P<0.05$), but markedly increased retrograde shear ($P<0.05$). Concomitantly, increases in radial artery diameter and blood flow were decreased (Heat+WC vs Heat, $P<0.05$). Heat attenuated FMD (8.6±1.2 vs. 2.2±1.4%, $P<0.05$), whereas no change in FMD was observed in Heat+WC (7.8±1.2 vs. 10.8±1.2%, $P>0.05$). In contrast, L-FMC was not different in either trial ($P>0.05$). In summary, acute whole-body heating markedly elevates radial artery shear rate, diameter and blood flow, and diminishes FMD. However, marked radial artery vasodilation and diminished FMD are absent when these shear rate changes are prevented. Shear rate modifications underpin the radial artery response to acute whole-body heat-stress, but further endothelial-dependent vasodilation (e.g., FMD) is attenuated likely as the vasodilatory range limit is approached.

4.2 Introduction

Endothelial-dependent processes provide an important mechanism whereby arterial diameter adapts in response to localized changes in blood flow (Birk et al., 2012, Green et al., 2017). Conversely, endothelial dysfunction disrupts vascular homeostasis and is integral to the pathophysiology of many cardiovascular diseases (Hambrecht et al., 2003, Watts et al., 2004). The flow mediated dilatation (FMD) technique provides a widely-used, non-invasive method of assessing endothelial function in response to an acute, marked increase in blood flow shear stress (Corretti et al., 2002a). However, it is less widely recognized that the acute reductions in arterial blood flow shear stress can evoke a low-flow mediated constriction (L-FMC) (Levenson et al., 1987, Gori et al., 2008). L-FMC has promising clinical utility and compliments the information provided by FMD (Gori et al., 2010, Gori et al., 2012). However, in contrast to FMD, limited work has explored the mechanisms underlying L-FMC or considered how it is affected by environmental factors, such as temperature.

The consequences of climate change, particularly increasing global temperatures and heat waves, are recognised to directly and indirectly increases cardiovascular morbidity and mortality (Aitken et al., 2022). Exposure to a hot environment results in pronounced cardiovascular autonomic adjustments that includes an increase in sympathetic nervous system activity, heart rate, and cardiac output, along with elevations in conduit artery and skin blood flow (Crandall and Wilson, 2015). Notably, local forearm heating increases brachial artery diameter, anterograde shear rate and FMD (Thijssen et al., 2014). While studies in animals and *in-vitro* studies of human endothelial cell cultures have shown an increased anterograde shear rate upregulates the release of endothelial nitric oxide synthase

(eNOS) and cytochrome-related endothelium-derived hyperpolarizing factors (EDHF) (Kinlay et al., 2001, Bellien et al., 2010, Green et al., 2010, Casey et al., 2017), this fails to occur with increases in retrograde shear rate, and instead there is an augmented release of endothelial derived vasoconstrictor molecules, such as ET-1 (Topper et al., 1996, Ziegler et al., 1998, Topper and Gimbrone Jr, 1999). Experimental induction of an increase in retrograde arterial shear rate in the human brachial artery can be achieved by inflation of pneumatic cuff (30-75 mmHg) placed distal to the site of investigation (Thijssen et al., 2009, Carter et al., 2013, Thijssen et al., 2014), and this maneuver prevents the brachial artery vasodilation during local heating (Pyke et al., 2008). Acute increases in sympathetic vasoconstrictor activity can also increase retrograde shear rate and attenuate FMD (Hijmering et al., 2002, Padilla et al., 2010, Thijssen et al., 2014). Unlike local forearm heating, acute whole-body passive heat stress evokes major systemic cardiovascular effects along with sympatho-excitation, both of which have the potential to modify artery blood flow pattern and functional characteristics. However, the influence of whole-body passive heat stress on radial arterial shear rate and function is incompletely understood.

In contrast to FMD, the influence of heat stress on L-FMC has not been considered, and whether L-FMC is modulated by the manipulation of local shear rate either independently or with concomitant heat stress is unknown. The L-FMC response to heating cannot be assumed to track that of FMD. While FMD and L-FMC responses complement one another in healthy and clinical populations , they are not significantly correlated (Gori et al., 2008, Gori et al., 2010). Like FMD, L-FMC is at least partly endothelium mediated (Dawson et al., 2012), but unlike FMD, L-FMC is not altered by pharmacological antagonism of nitric oxide synthase (Gori et al., 2008). Therefore, non-endothelial factors, such as an increase in SNA, cannot be discounted as

contributing to L-FMC (Elliott et al., 2018). Thus, during whole-body passive heat stress, both increases in SNA and anterograde shear rate could potentially modify L-FMC.

The objectives of this investigation were twofold. First, to characterize the effect of whole-body passive heat stress on radial artery blood flow pattern, FMD and L-FMC. Secondly, to determine whether the influence of whole-body passive heat stress on FMD and L-FMC is mediated by a change in local shear rate. To achieve this, the influence of whole-body passive heat stress (sufficient to raise core temperature +1 °C) on radial artery blood flow pattern, FMD and L-FMC was investigated. Heating trials were conducted both with and without the addition of a cuff, inflated to 75 mmHg, placed around the wrist that was distal to the radial artery being examined. We hypothesized that; 1) whole-body passive heat stress would augment anterograde shear rate and subsequently increase FMD and L-FMC via endothelium mediated mechanisms, and 2) such increases in FMD and L-FMC would be prevented if increases in anterograde shear rate were prevented, and retrograde shear rate augmented, during whole-body passive heat stress (i.e., with a wrist cuff).

4.3 Methods

4.3.1 Ethical approval

Ethical Approval for this study was received from the University of Birmingham, Science Technology Engineering and Mathematics Ethical Review (approval number ERN_18-0523). All study procedures were undertaken in accordance with the ethical standards outlined in the *Declaration of Helsinki*, except for registration in a database. Written informed consent was obtained from all study participants following a verbal and written explanation of the study objectives and procedures.

4.3.2 Participant characteristics

Thirteen healthy men were recruited. All participants were normotensive, normothermic (36.2 – 37.6 °C), non-smokers and medication free. Prior to experimental trials participants were requested to adhere to the following instructions: no food or beverages ≥6 hours, no alcohol or caffeine for ≥12 hours, no polyphenol rich food/beverages for ≥18 hours, no vigorous exercise for ≥48 hours and no vitamin supplements for ≥72 hours. Eleven participants completed the experiment, with two participants withdrawing from the study after first trial due to personal reasons.

4.3.3 Experimental measures

Heart rate (HR) was measured using a standard lead II surface electrocardiogram, and systolic and diastolic blood pressure (BP) obtained non-invasively from left brachial artery by automated sphygmomanometer (Tango+, SunTech Medical Instruments, Raleigh, NC, USA). Core (intestinal) temperature was measured using an ingestible temperature pill telemetry system (T_{pill}; Jonah™ Core

Body Temperature, Respironics, Bend, OR, USA). Data were transmitted wirelessly to monitoring device (EQ02+ LifeMonitor, Equivital, Hidalgo, Cambridge, U.K) and then gathered with embedded application software (eqView mobile, Equivital, Hidalgo, Cambridge, U.K). Skin temperature was measured by using thermistors located at four sites ($chest_{sk}$, $biceps_{sk}$, $thigh_{sk}$ and $calf_{sk}$) (Squirrel SQ2010 Data Logger; Grant, Cambridge, UK).

Right radial artery diameter and blood flow velocity were obtained using duplex Doppler ultrasound (Terason uSmart 3300, Teratech Corporation, Burlington, MA, USA) with the arm supported at heart level. The radial artery was insonated 10 – 15 cm distal to the medial epicondyle using a multi-frequency linear-array probe (Terason uSmart 15L4) operating at 4-15 MHz and fixed on an adjustable holder throughout the experiment. B-mode imaging was used to measure radial artery diameter and pulse-wave mode to obtain radial artery peak blood velocity. Measurements were made in accordance with recent technical recommendations (Padilla et al., 2008, Thijssen et al., 2011). FMD studio software was used to record Doppler images as video files and offline analysis conducted using automated edge detection and wall tracking algorithms (Cardiovascular Suite Version 3.4.1, FMD Studio, Pisa, Italy).

4.3.4 Experimental protocol

Prior to experimental trials, participants attended a familiarization session during which study procedures were explained and methods demonstrated. Participants then returned for three separate experimental trials to investigate the impact of whole-body passive heat stress on radial artery endothelial function and blood flow pattern. Trials were conducted on three days separated by at least 24 hours

and completed within 14 days. The three experimental trials were; 1) whole-body passive heat stress sufficient to raise core temperature by 1 °C (Heat), 2) whole-body passive heat stress sufficient to raise core temperature by 1 °C with concurrent inflation of a cuff placed around the right wrist to 75 mmHg in order to modify the blood flow pattern of the right radial artery (Heat + WC), and 3) a Time Control trial with neither whole body heat stress nor wrist cuff inflation. The order of the Heat and Heat + WC trials was randomized by a coin toss. By necessity the Time Control trial was always performed last; its duration determined by the average of the Heat and Heat + WC trials.

All experimental sessions and data collection were conducted at the same time of day for a given individual. For the Heat and Heat + WC trials, participants came to the laboratory and swallowed the T_{pill} with water two hours prior to testing. The T_{pill} was not provided for Time Control trial. Experimental sessions commenced with securing skin temperature thermistors to the participants and then putting on a tube-lined water-perfused suit covering the entire body surface with the exception of the head and right forearm. Participants then rested in a supine position on a medical examination table and were instrumented for collection of the experimental measures outlined above. An inflatable cuff was placed around the right wrist to modify the blood flow pattern as described above (Heat + WC) and was also used for the assessment of L-FMC and FMD (described below). The suit was perfused with water at a thermo-neutral temperature (34°C) for 15 min and temperature and hemodynamics recorded. An assessment of radial artery function (L-FMC and FMD) was then made, consisting of a 1 min baseline, followed by 5 min wrist cuff inflation to ≥220 mmHg, and a 3 min post-cuff inflation recovery period (Gori et al., 2008). In the Heat trial, the temperature

of the water perfusing the suit was then adjusted to 48 °C and applied until core temperature increased by 1°C. In the Heat + WC trial, the wrist cuff was inflated to 75 mmHg to modify radial artery flow pattern (Thijssen et al., 2009), and the whole body heat stress protocol was replicated as in the Heat trial. Once core temperature was elevated by 1°C (the desired amount) in the Heat and Heat + WC trials, radial artery function testing (L-FMC and FMD) was repeated. During the Time Control trial, the temperature of water perfusing the suit was maintained at a thermo-neutral temperature (34°C) and pre and post intervention recordings of L-FMC and FMD were made as in other two trials (Heat and Heat + WC).

4.3.5 Data analysis

Mean skin temperature (T_{sk}) was calculated as (Ramanathan, 1964):

$$T_{sk}(C°) = 0.3\ x\ (\text{Biceps}_{sk}\,C° + \text{Chest}_{sk}C°) + 0.2\ x\ (\text{Thigh}_{sk}C° + \text{Calf}_{sk}C°)$$

Mean arterial pressure (MAP) was calculated as (Razminia et al., 2004):

$$\text{MAP (mmHg)} = \text{Diastolic BP(mmHg)} + [0.33 + (\text{HR}\ x\ 0.0012)]\ x\ [\text{Systolic BP(mmHg)} - \text{Diastolic BP (mmHg)}]$$

Radial artery blood flow was calculated as:

Blood Flow (ml/min) = Mean Blood Velocity (cm/s) x π x radius (cm) ^{2} x 60 (s/min)

Radial artery vascular conductance was determined by dividing arterial blood flow (ml/min) by mean arterial pressure (mmHg).

Radial artery wall shear rate was defined as:

$$\text{Arterial Wall Shear Rate (SR, s}^{-1}) = \frac{4\ x\ \text{Mean Blood Velocity (cm/s)}}{\text{Diameter (cm)}}$$

Anterograde and retrograde shear rate were calculated using anterograde and retrograde blood velocities, respectively.

Core temperature (Heat and Heat + WC only), skin temperature, HR, BP and radial artery characteristics were obtained prior to the start of intervention, and then every 5 min during the intervention (Heat, Heat + WC, Time Control trials). In order to make between trial comparisons of the temporal response pattern for temperature and cardiovascular variables, values were selected that corresponded to 25%, 50%, 75% and 100% of total trial duration. A 20 s average was used to provide radial artery measure for a given participant at each time point.

For radial artery function testing, L-FMC was defined as the change from average baseline diameter to the average diameter of the last 30 s of wrist cuff occlusion, while FMD was taken as the change from the average baseline diameter to the maximal post cuff occlusion diameter (Gori et al., 2008). L-FMC and FMD responses are presented as relative (%) and absolute (mm) change (Thijssen et al., 2011). Total vessel reactivity (TVR) was calculated as the change from the average diameter of the last 30 s of wrist cuff occlusion to the maximal diameter post cuff deflation divided by the average baseline diameter (Rakobowchuk et al., 2012) and is presented as a relative (%) change. TVR was used to assess the vascular reactivity range (Black et al., 2003). The time-to-peak diameter and shear rate area under the curve (SR_{AUC}), calculated as an integral, were determined from cuff deflation until maximum artery dilation. A ratio of L-FMC against change in mean shear rate (difference between baseline shear rate and shear rate during last 30 s of cuff occlusion; L-FMC-to-mean SR ratio, au) and FMD against SR_{AUC} (FMD-to-SR_{AUC} ratio,

au) were calculated and the values multiplied by 1000 (Padilla et al., 2008, Junejo et al., 2020). Recent guidelines suggest considering whether allometric scaling is necessary when evaluating FMD (Atkinson et al., 2013). Accordingly, baseline and nadir / peak diameters were natural log-transformed for slope and upper bound 95% confidence intervals (CI). Further allometric scaling for baseline diameters was not performed as the slope of the relationship between log(peak diameters) and log(baseline diameters) did not deviate significantly from 1 (i.e., all slopes > 0.86 and all upper bound 95% CI < 1.42).

4.3.6 Statistical analysis

All statistical analyses were conducted using Statistical Package for Social Sciences (SPSS, version 21.0). One-way repeated measures analysis of variance (ANOVA) was used to test for differences in total trial durations (Heat, Heat +WC, Time Control). Two-way repeated measures ANOVA was used to test for differences in core and skin temperature, cardiovascular responses, radial artery characteristics and blood flow pattern with respect to time (baseline, 25%, 50%, 75% and 100% of intervention duration), trial (Heat, Heat + WC, Time Control), and their interaction. Two-way repeated measures ANOVA was also used to investigate main effects of time (Pre vs. Post intervention), trial (Heat, Heat + WC, Time Control) and their interaction, for radial artery function (e.g., L-FMC and FMD). An analysis of covariance (ANCOVA), with SR_{AUC} as covariate, was used to statistically assess the FMD response for the shear rate stimulus (SR_{AUC}-corrected-FMD%) (Thijssen et al., 2019). Significant main effects and interactions were investigated *post hoc* using Students t-

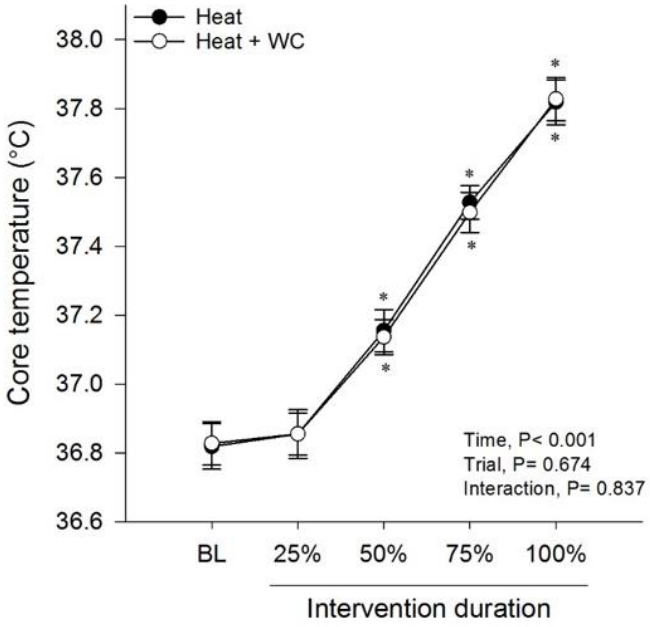
tests with Bonferroni adjustment. P <0.05 was recognized as being statistically significant. Data are presented as mean (SD) unless stated otherwise.

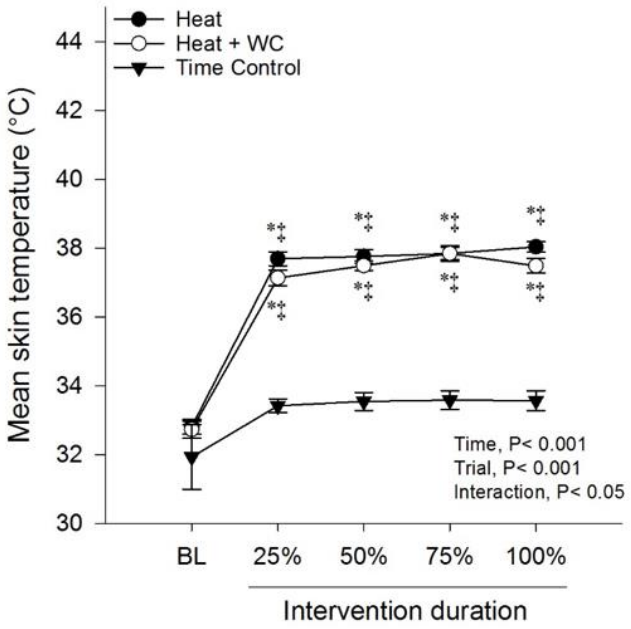
The minimum sample size was determined retrospectively on the basis of a priori power analysis, considering radial artery baseline diameter (within-participant and within-day repeat measure) as the primary efficacy variable. The power analysis was conducted using the G* Power version 3.1 (Buchner, Erdfelder, Faul and Lang, 2014) with an alpha level of 0.05, power = 0.80, and an estimated medium effect size of d= 0.67. Data for calculating the effect size were obtained from a similar previous study and used the mean and standard deviation differences for the control and intervention conditions as well as the between condition correlation of 0.94 (Elliott et al., 2018). These calculations suggested the need for a minimum sample size of 20 participants.

4.4 RESULTS

4.4.1 Core and mean skin temperature

Total duration was not different between the Heat (78.6 ± 9.5 min) and Heat + WC (76.3 ± 12.2 min) and Time Control (77.5 ± 9.1) trials ($P = 0.554$). Core temperature increased progressively from baseline during Heat and Heat + WC trials, with both being different from baseline after 50%, 75% and 100% of intervention duration, respectively (all $P < 0.05$) and this increase in core temperature was similar between heat trials ($P = 0.922$; Figure 4.1). Mean skin temperature also increased from baseline during the Heat (32.8 ± 0.68 °C) and Heat + WC (32.7 ± 0.83 °C) trials ($P < 0.05$ vs. baseline and Time Control trial after 25% of intervention duration and beyond), but was not different between the Heat and Heat + WC trials (Heat vs. Heat + WC: baseline; after 25%, 50%, 75% and 100% of intervention duration, $P = 1.00$; $P = 0.089$; $P = 0.620$; $P = 1.00$; $P = 0.166$, respectively). Mean skin temperature during Time Control trial remained between 32 and 34°C throughout the trial (Time Control after 25%, 50%, 75% and 100% of intervention duration vs. baseline: $P = 1.00$; $P = 0.994$; $P = 0.955$; $P = 0.994$, respectively). Mean skin temperature during FMD and L-FMC assessment was clamped and remained stable throughout the intervention period within each trial ($P > 0.05$; Figure 4.1).


Heat
Heat + WC
Core temperature (°C)
38.0
37.8
37.6
37.4
37.2
37.0
36.8
36.6
Time, P< 0.001
Trial, P= 0.674
Interaction, P= 0.837
BL
25%
50%
75%
100%
Intervention duration


Heat
Heat + WC
Time Control
Mean skin temperature (°C)
44
42
40
38
36
34
32
30
Time, P< 0.001
Trial, P< 0.001
Interaction, P< 0.05
BL
25%
50%
75%
100%
Intervention duration

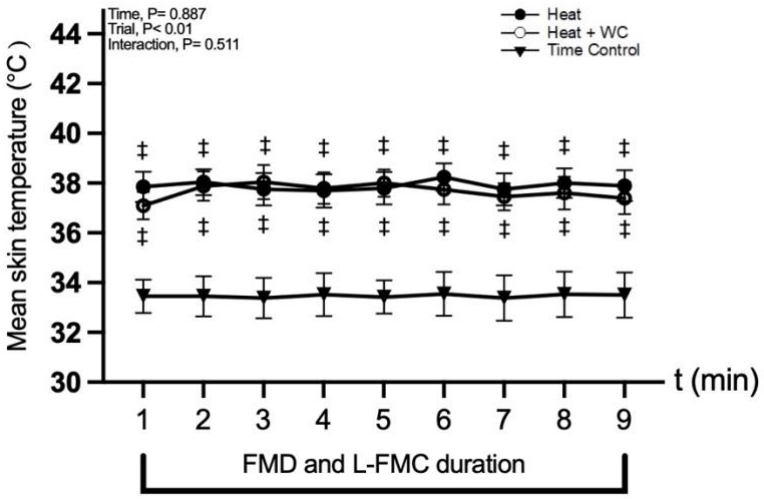


Figure 4.1 Core and skin temperatures during interventions and vascular assessment.

Whole-body passive heat stress (Heat) and whole-body heat stress with wrist cuff (Heat + WC) evoked similar increases in core and skin temperature. Skin temperature was not changed from baseline in the Time Control trial. Values are mean ± SE. * $P < 0.05$ vs. baseline (BL); ‡ $P < 0.05$ vs. Time Control.

4.4.2 HR and BP

HR progressively increased from baseline during both whole-body passive heat stress trials (Heat and Heat + WC, $P < 0.05$ vs. baseline and Time Control at 25% intervention duration and beyond; (Figure 4.2). Systolic BP also increased during the Heat and Heat + WC trials ($P < 0.05$), and diastolic BP fell slightly during the heating trials.

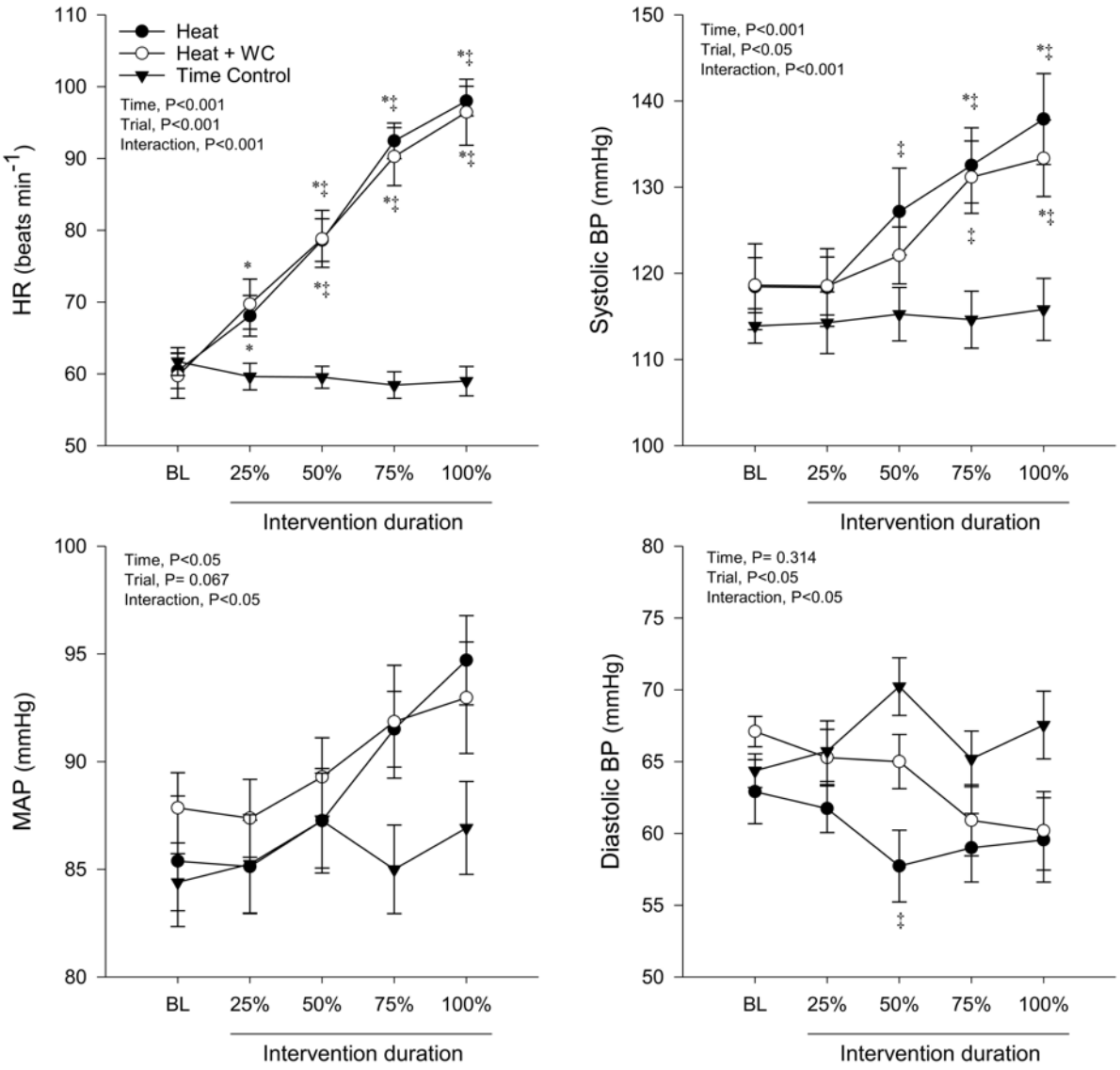


Figure 4.2 Cardiovascular responses.

Heart rate (HR), systolic blood pressure (systolic BP), diastolic blood pressure (Diastolic BP), mean arterial pressure (MAP) responses were similar in the whole-body passive heat stress (Heat) and whole-body heat stress with wrist cuff (Heat + WC) trials. Values are mean ± SE. * P < 0.05 vs. baseline (BL); † P < 0.05 vs. Heat + WC; ‡ P < 0.05 vs. Time Control.

4.4.3 Radial artery characteristics

Mean and anterograde shear rate increased progressively and robustly throughout the Heat trial ($P < 0.05$ vs. baseline and Time Control at 25% intervention duration and beyond; (Figure 4.3), while retrograde shear rate decreased slightly from baseline values and was significantly different to Time Control at 75% and 100% intervention duration ($P < 0.05$ vs. Time Control). In the Heat + WC trial, increases in mean and anterograde shear rate were abolished ($P < 0.05$ vs. Heat), while increases in retrograde shear rate were pronounced ($P < 0.05$ vs. baseline and Heat). In the Time Control trial, radial artery mean, anterograde and retrograde shear rates remained unchanged from baseline ($P > 0.05$).

During the Heat trial, radial artery diameter, velocity, blood flow and vascular conductance all increased progressively and markedly ($P < 0.05$ vs. baseline and Heat + WC at all intervention durations; (Table 4.1 and Figure 4.4). In contrast, these radial artery characteristics remained close to baseline values throughout the Heat + WC and Time Control trials; the exceptions being Heat + WC radial artery diameter which increased slightly at 50% and 75% of intervention duration, and Time Control vascular conductance which fell slightly at 100% of intervention duration (both $P < 0.05$ vs. Baseline). At baseline, radial artery blood flow, velocity and vascular conductance were slightly but significantly elevated in the Time Control trial compared to the Heat trial ($P < 0.05$).

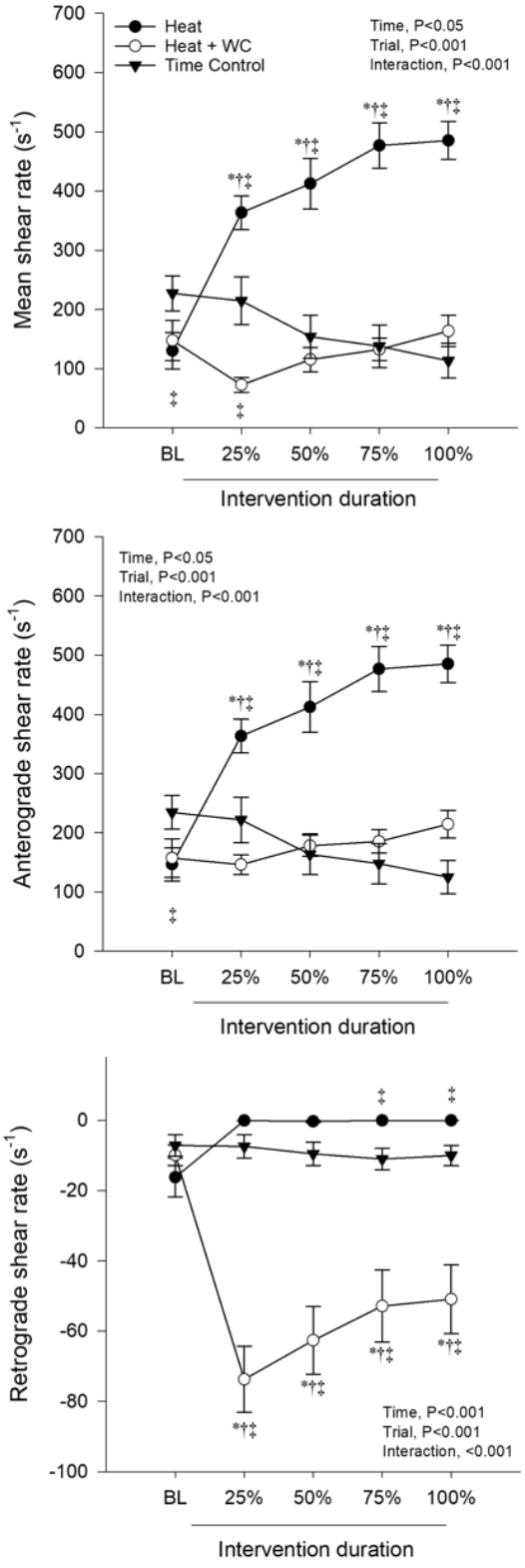


Figure 4.3 Radial artery blood flow pattern.

Mean, anterograde and retrograde shear rate during the whole-body passive heat stress (Heat), whole-body heat stress with wrist cuff (Heat + WC) and Time Control trials. Values are the mean ± SE. * $P < 0.05$ vs. baseline (BL); † $P < 0.05$ vs. Heat + WC; ‡ $P < 0.05$ vs. Time Control.

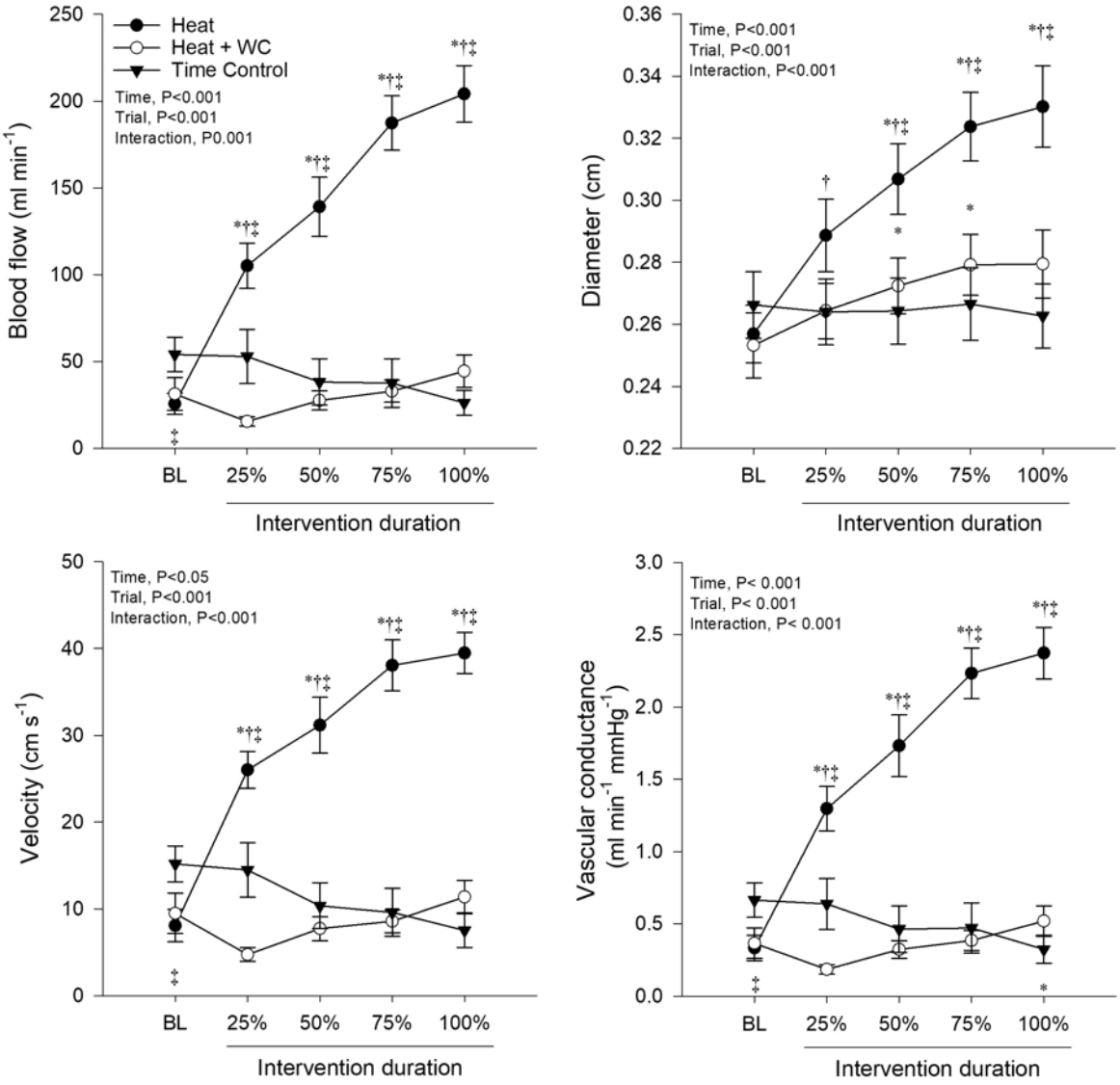


Figure 4.4 Radial artery characteristics

Radial artery blood flow, diameter, velocity and vascular conductance during whole-body passive heat stress (Heat), whole-body heat stress with wrist cuff (Heat + WC) and Time Control trials. Values are the mean ± SE. * P < 0.05 vs. baseline (BL); † P < 0.05 vs. Heat + WC; ‡ P < 0.05 vs. Time Control.

4.4.4 Radial artery function responses

Table 4.1 provides radial artery characteristics before and after intervention in the Time Control, Heat and Heat + WC trials. At baseline, FMD, L-FMC and TVR % were not different between the Heat, Heat + WC and Time Control trials (FMD % between trials at the baseline, all P=1.00; L-FMC % between trials at the baseline, all P=1.00; TVR % at the baseline, Heat vs. Heat + WC P=0.511, Heat vs. Time Control P=1.00, Heat + WC vs. Time Control P=0.764, Figure 4.5). Following whole body heating (Post) in the Heat trial, FMD % was significantly decreased ($P < 0.05$ vs. Pre and Heat + WC, Figure 4.5), while FMD % was unchanged in either the Heat + WC or the Time Control trials (Heat + WC vs. Pre, $P = 0.176$; Time Control vs. Pre, $P = 0.464$). No between trial differences in L-FMC % were observed either at baseline or following intervention in the Heat, Heat + WC and Time Control trials ($P > 0.05$, Figure 4.5). Following intervention in the Heat + WC trial, TVR % was increased (Pre vs Post, $P < 0.05$ vs. baseline and Time Control). Following the Time Control trial, TVR % was significantly decreased (Pre vs. Post, $P < 0.05$).

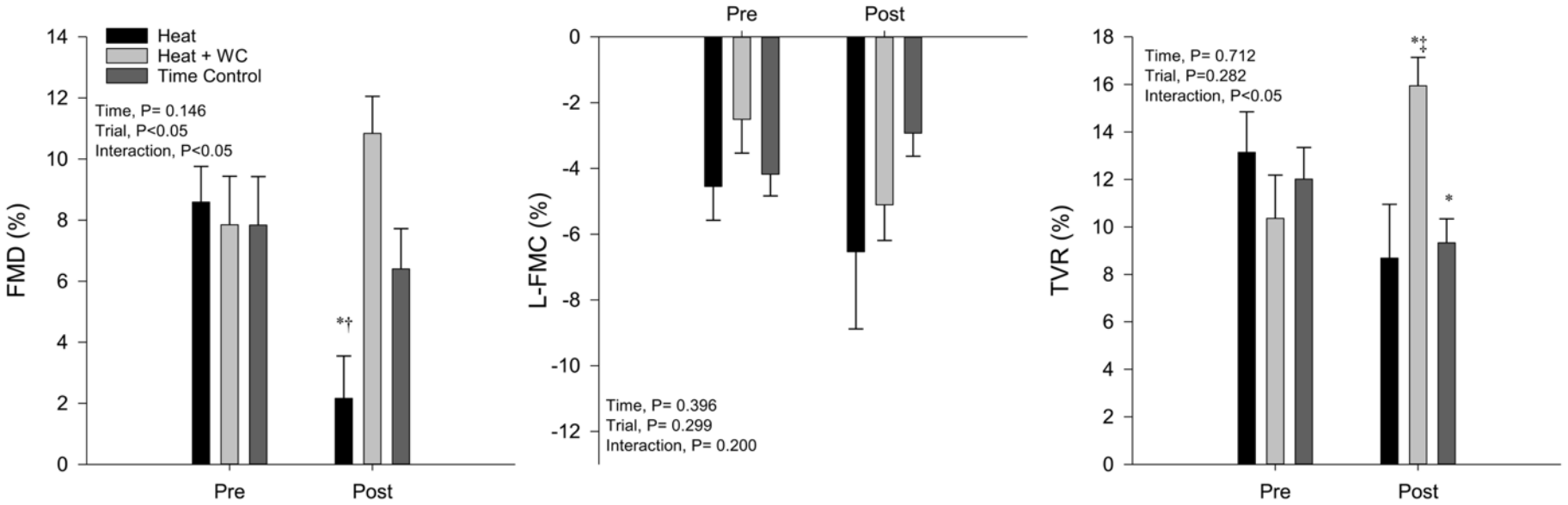


Figure 4.5 Radial artery function

Radial artery flow mediated dilatation (FMD), low-flow mediated constriction (L-FMC), and total vascular range (TVR) during the whole-body passive heat stress (Heat), whole-body heat stress with wrist cuff (Heat + WC) and Time Control trials. Values are the mean ± SE. * P < 0.05 vs. baseline (BL); † P < 0.05 vs. Heat + WC; ‡ P < 0.05 vs. Time Control.

Table 4.1 Radial artery characteristics before [Pre] and after [Post] the Time Control, Heat and eat + WC trials.

	Time Control		Heat		Heat + WC		P values		
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Trial**	**Time**	**Interaction**
Baseline									
Diameter (mm)	2.66 (0.35)	2.63 (0.34)	2.57 (0.31)	3.30 (0.43)*†‡	2.53 (0.35)	2.79 (0.36)*	**<0.001**	**<0.001**	**<0.001**
Velocity (cm/s)	15.17 (6.9)	7.53 (6.6)*	8.07 (6.2)‡	39.48 (7.8)*†‡	9.48 (7.72)	11.59 (6.5)	**<0.001**	**<0.05**	**<0.001**
Blood flow (ml/min)	54.04 (33.0)	26.18 (23.7)*	25.6 (20.3)‡	204.17 (54.2)*†‡	31.32 (31.2)	45.60 (32.7)	**<0.001**	**<0.001**	**<0.001**
Mean shear rate (s^{-1})	227.2 (98.0)	113.3 (96.9)*	130.3 (102.1)‡	485.3 (105.2)*†‡	147.5 (112.4)	165.2 (88.4)	**<0.001**	**0.022**	**<0.001**
L-FMC									
Nadir Diameter (mm)	2.55 (0.34)	2.55 (0.34)	2.45 (0.27)	3.08 (0.41)*†‡	2.47 (0.36)	2.65 (0.31)	**<0.001**	**<0.001**	**<0.001**
Δ Diameter (mm)	-0.11 (0.06)	-0.08 (0.06)	-0.12 (0.09)	-0.22 (0.27)	-0.06 (0.08)	-0.15 (0.12)	**0.174**	**0.195**	**0.162**
Mean shear rate (s^{-1})	22.8 (6.2)	26.2(7.5)	21.4 (11.0)	74.0 (30.6)*†‡	23.5 (8.2)	109.2 (59.9)*‡	**<0.001**	**<0.001**	**0.001**
Δ Mean shear rate (s^{-1})	204.4 (103.0)	87.1 (90.4)*	108.9 (104.5)‡	411.2 (93.6)*†‡	123.9 (111.1)	56.0 (57.2)	**<0.001**	**0.229**	**<0.001**
L-FMC-to- Δ mean SR ratio (au)	-0.029(0.038)	-0.064(0.082)	-0.110 (0.234)	-0.020 (0.025)	-0.004 (0.092)	-0.209 (0.348)	**0.548**	**0.231**	**0.075**
FMD									
Peak Diameter (mm)	2.88 (0.41)	2.79 (0.40)	2.79 (.35)	3.37 (0.46)*†‡	2.73 (0.40)‡	3.10 (0.40)*‡	**<0.001**	**<0.001**	**<0.001**
Δ Diameter (mm)	0.21 (0.14)	0.17 (0.12)	0.22 (0.11)	0.07 (0.15)*†	0.20 (0.14)	0.30 (0.11)	**0.038**	**0.334**	**<0.05**
Time to peak diameter (s)	80.72(32.68)	77.18 (46.95)	90.36 (41.62)	97.72 (152.58)	76.54 (42.49)	118.6 (39.66)	**0.326**	**0.097**	**0.265**
SR_{AUC} ($x10^3$ s^{-1})	19.33 (6.7)	16.58 (5.7)	16.4 (6.56)	7.50 (7.0)†‡	17.8 (8.5)	29.57 (11.6)*‡	**<0.001**	**0.507**	**<0.05**
FMD-to-SR_{AUC} ratio (au)	0.40 (0.26)	0.62 (0.97)	0.63 (0.46)	0.66 (1.24)	0.46 (0.27)	0.42 (0.19)	**0.641**	**0.689**	**0.819**
SR_{AUC}-corrected-FMD (%)	7.696 (4.60)	6.526 (4.60)	8.73 (4.60)	3.156 (5.25)	7.856 (4.60)	9.712 (5.42)	**0.224**	**0.154**	**0.066**

Values are means (SD). L-FMC, low-flow mediated constriction; FMD, flow mediated dilatation; SR_{AUC}, shear rate area under curve. P values represent 2-way repeated ANOVA results (Trial; Time Control, Heat and Heat + WC: Time; Pre and Post: Interaction, Trial x Time). P value for SR_{AUC}-corrected-FMD (%) represent ANCOVA results. * P < 0.05 vs. Pre; † P < 0.05 vs. Heat + WC; ‡ P < 0.05 vs. Time Control.

4.5 DISCUSSION

The objectives of this investigation were to characterize the effect of whole-body passive heat stress on radial artery blood flow pattern and functional characteristics (i.e., FMD and L-FMC), and to determine whether the influence of whole-body passive heat stress on FMD and L-FMC is mediated by a change in local shear rate (as induced via inflation of pneumatic cuff (75 mmHg) placed distal to the site of investigation). We observed that whole-body heating (i.e., Heat trial), sufficient to raise core temperature by +1 °C, markedly and progressively increased radial artery mean and anterograde shear rate, along with radial artery diameter, velocity and blood flow. Contrary to our hypothesis, whole-body passive heat stress attenuated FMD, whereas L-FMC was unchanged. As expected, the addition of a cuff, inflated to 75 mmHg around the wrist distal to the radial artery being examined (i.e., Heat + WC trial), abolished the heat-induced increase mean and anterograde shear rate, but markedly increased retrograde shear. Associated with this, no changes in either radial artery blood velocity, diameter, blood flow or vascular conductance were observed. Moreover, neither FMD nor L-FMC were different following Heat + WC. Collectively, these findings suggest that whole-body passive heat stress (+1 °C core temperature) acutely elevates radial artery mean and anterograde shear rate, leading to radial artery vasodilatation and diminished FMD, but unchanged L-FMC. However, when whole-body heating induced increases in radial artery mean and anterograde shear rate are prevented, and instead retrograde shear is increased, both radial artery vasodilation and the diminished FMD are prevented.

In healthy adults brachial artery FMD has been shown to be enhanced following whole-body passive heat therapy (4 - 5 times, 60 minutes session per week for 8 weeks) (Brunt et al., 2016). Moreover, regular whole-body heating for 3-4 weeks improves endothelial function, maximal O_2 uptake (Ohori et al., 2012), circulating NO metabolite concentrations and reduces oxidative stress markers in chronic heart failure patients (Fujita et al., 2011). While a single session of whole-body heating offers protection from ischemia-reperfusion associated reductions in endothelial function (Brunt et al., 2016). Local heating (42 °C) is known to evoke cutaneous vasodilation, increase limb blood flow and shear stress without producing major systemic cardiovascular effects (Green et al., 2010). Moreover, local unilateral limb heating prevents physical inactivity (Teixeira et al., 2017) and hyperglycemia (Greyling et al., 2015) induced reductions in FMD. Given this, we hypothesized that acutely applied whole-body passive heat stress would cause an enhanced FMD secondary to an augmented anterograde shear rate and upregulated release of endothelial NO synthase and EDHF (Kinlay et al., 2001, Davis et al., 2006, Bellien et al., 2010, Green et al., 2010). This was only partly correct, in that anterograde shear was increased during whole-body passive heat stress, but rather than observing an increase in FMD, a decrease was found.

A poor FMD response under normothermic conditions is associated with increased future cardiovascular risk (40, 41, 55) and indicative of endothelial dysfunction. Thus, the reduced FMD during acute whole-body passive heat stress might be interpreted as a reduction in endothelial function. However, it is more likely that the reduction in FMD during acute whole-body passive heat stress was mediated by thermoregulatory-related radial artery vasodilation, which reduced the capacity for subsequent vasodilation during the radial FMD test. Indeed, the peak diameter

observed during FMD prior to Heat (i.e., Pre, 2.79 mm) was lower than that observed at baseline following heating (i.e., Post, 3.30 mm; Table 4.1). Moreover, the SR_{AUC} was diminished during the FMD following whole body heating (Heat trial; SR_{AUC} 16.4 vs. 7.50 $x10^3$ s^{-1} for Pre vs. Post, respectively), and when FMD was corrected for this attenuated SR_{AUC}, no difference in FMD was noted. An alternative explanation is that an elevated sympathetic vasoconstrictor tone resulting from acute whole-body heat stress reduced the FMD response in the current study. Some acutely applied sympatho-excitatory maneuvers have been shown to attenuate FMD (Hijmering et al., 2002). Indeed, reductions in FMD following strenuous dynamic exercise are reportedly prevented by alpha-adrenergic blockade suggestive of a sympathetically mediated reduction in FMD (Atkinson et al., 2015). Although chronic whole-body passive heat stress has been shown to decrease circulating norepinephrine concentrations in heart failure patients (Ohori et al., 2012), the extent and direction of any acute sympatho-excitatory adaptive changes here remains unclear.

The inflation of a cuff (to 75 mmHg) distal to the artery being examined is an established method of manipulating shear rate (Thijssen et al., 2009). In the present study, wrist cuff inflation abolished the heat-induced increase in mean and anterograde shear rate, but markedly increased retrograde shear (i.e., Heat + WC trial). Associated with this, and in stark contrast to the Heat trial, no increases in either radial artery diameter, velocity, blood flow or vascular conductance were observed. It should be noted that the wrist cuff was positioned distal to the portion of the radial artery being interrogated and therefore did not directly occlude flow to where the vessel was being imaged. Further, it seems unlikely that the wrist cuff inflation to 75 mmHg, which is lower than MAP and much lower than systolic BP, was sufficient to reduce downstream radial artery blood flow into the hand; yet this possibility cannot

be completed excluded (Junejo et al., 2019). Nonetheless, such an effect should not have severely compromised hand circulation as no participants reported altered sensation in the hands. Notably, while Heat diminished FMD, it was preserved during Heat + WC likely as a consequence of the greater SR_{AUC} during the FMD. This provides further support for the contention that the attention in FMD for the Heat trial was mediated by the reduction in shear stimulus and/or diameter and not a true change in endothelial vasodilator function. Heat and Heat + WC trials were well matched in so far as the evoked increases in core temperature, blood pressure and heart rate were not different, suggesting that a non-specific systemic factor was not involved. The between trial difference in FMD is likely explained by the wrist cuff preventing an increase in radial artery mean and anterograde shear rate, and thus no radial artery vasodilatation occurring. Therefore, with the radial artery at a baseline level in the Heat + WC trial, the FMD response was normal, despite core temperature being raised.

I hypothesized that whole-body passive heat stress would augment L-FMC, whereas it remained unchanged. Among the various suggested mechanisms underlying L-FMC is an endothelial contribution (Dawson et al., 2012). Indeed, L-FMC is attenuated by inhibition of endothelial derived hyperpolarizing factors, prostaglandins (Gori et al., 2008) and the endothelin receptor antagonist BQ-123 (Spieker et al., 2003). Notably, FMD was diminished with whole-body passive heat stress and it is well established that FMD is at least in part determined by endothelial dependent mechanisms. Given this, one might have expected L-FMC to change similarly, but despite the augmented baseline diameter this was not the case. A sympathetic mechanism has also been postulated to contribute to L-FMC, and whole-body passive heat stress is well known to increase sympathetic activity. Inflation of a

wrist cuff (i.e., Heat + WC trial) had no influence on L-FMC. This further supports the concept that manipulating shear rate, such that increases in anterograde shear rate are prevented and retrograde shear rate augmented, has a minimal effect on L-FMC. Elliott et al., (2018) observed an augmented L-FMC following dynamic exercise and among the potential mechanisms suggested was an increase in SNA. It might have been reasonable to expect that with the prevailing vasodilation, meaning more scope for vasoconstriction, along the increase in retrograde shear to attenuate endothelial function, a more pronounced L-FMC would have been exhibited. Further, it could also have been expected that the increased retrograde shear during Heat + WC would have worsened endothelial function (Thijssen et al., 2009) and attenuated L-FMC. However, this was also not observed.

4.5.1 Experimental considerations

Herein we assessed the radial artery and studies are required to verify these findings in other conduit vessels (e.g., coronary arteries). While the relationship between the brachial artery FMD and ACh infusion responses with bradykinin, ACh, adenosine and dobutamine infusion responses of the coronary vessels has been determined (Matsuo et al., 2004, Takase et al., 2005, Tarutani et al., 2005, Nardone et al., 2020), to the best of our knowledge such an investigation has not been carried out for any other peripheral conduit vessels. While some previously published investigations have examined the radial artery (e.g., Gori et al., 2008, Weissgerber et al., 2010, Dawson et al., 2012, Elliott et al., 2018), human studies of peripheral vascular function more commonly examine the brachial artery. Similar blood flow and shear patterns would be expected in both the radial and brachial arteries during

passive whole-body heat stress (Thijssen et al., 2008). A notable difference between the brachial and radial arteries relates to the propensity to observe an L-FMC response, with this being more commonly seen in the radial artery (Weissgerber et al., 2010).

Radial artery function was only assessed at a single time point following the whole-body passive heat stress intervention. As such we were unable to ascertain the time-course of the vascular response, and specifically determine how long the whole-body heat stress related decrement in FMD persisted for in the post-heat period, and if/when a conversion to an augmented FMD response occurred. It is a limitation that only healthy young men were studied. There are important sex-differences and ovarian hormone effects on vascular function (Hashimoto et al., 1995). Unfortunately, resource and logistical issues meant that we were unable to study young women at a standard phase of their menstrual cycle (e.g., early follicular phase) for the three separate experimental sessions that our study design necessitated, potentially over several months. Additional studies are required to ascertain whether sex-differences are present in our findings, and the extent to which they similarly manifest in patient populations in whom underlying impairments in vascular function are reported (e.g., healthy ageing, hypertension).

The use of a wrist cuff inflation to 75 mmHg is an established method to alter shear rate patterns, particularity during experimental conditions in which shear stress is elevated (e.g.Carter et al., 2013). Despite its utility, this model of shear rate manipulation simultaneously decreases anterograde and increases retrograde shear rates, respectively. As such, we are unable to state definitively whether the FMD response following Heat + WC is mediated by attenuated mean and anterograde shear, or is it driven by the large increase in retrograde shear, or a combination of

both. The wrist cuff and associated changes in shear pattern lead to a diminished blood flow response to whole-body heating. However, blood flow was not significantly reduced below baseline or time control values. We assume that forearm metabolic rate was not different between trials and as such do not expect differences in downstream tissue oxygen to have occurred and contributed to the vascular responses observed. We cannot discount the possibility that wrist cuff inflation may have evoked venous distension and a reflex increase in vasoconstrictor SNA (Haouzi et al., 1999, Mansur et al., 2019). The inclusion of assessments of SNA (Adlan et al., 2017) or blood based biomarkers of vascular function (Wilhelm et al., 2017) would have provided additional mechanistic insight and strengthened this study.

The study's sample size was determined through power calculations utilizing conservative estimates of the effect size. Nevertheless, the true effect size might have been smaller than expected, resulting in an underpowered study. Future research could gain advantages from larger sample sizes to enhance statistical power and enhance the applicability of the findings.

4.5.2 Conclusions

Collectively, these findings suggest that whole-body passive heat stress acutely elevates radial artery mean and anterograde shear rate, leading to a vasodilatation of the radial artery and a diminished FMD, but not L-FMC. Preventing these shear rate induced changes reduces radial artery vasodilation and the acutely diminished FMD. Therefore, shear rate modifications appear to underpin the conduit artery response to acute whole-body heat-stress, but further endothelial-dependent flow-mediated vasodilation is attenuated as the vasodilatory range limit is approached.

CHAPTER 5: IMPACT OF ACUTE DYNAMIC EXERCISE AND ARTERIAL SHEAR RATE MODIFICATION ON RADIAL ARTERY LOW-FLOW MEDIATED CONSTRICTION IN YOUNG MEN

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M.H.A contributions: M.H.A conceived and designed the research, performed the experiments, analysed the data, interpreted the data, prepared the figures, drafted the manuscript, edited the manuscript.

5.1 Abstract

Leg cycling exercise acutely augments radial artery low-flow mediated constriction (L-FMC). Herein, we sought to determine whether this is associated with exercise-induced changes in arterial shear rate (SR). Ten healthy and recreationally active young men (23 ± 2 years) participated in 30 min of incremental leg cycling exercise (50, 100, 150 Watts). Trials were repeated with (Exercise + WC) and without (Exercise) the use of a wrist cuff (75 mmHg) placed distal to the radial artery to increase local retrograde SR while reducing mean and anterograde SR. Radial artery characteristics were measured throughout the trial, and L-FMC and flow mediated dilatation (FMD) were assessed before and acutely (~10 min) after leg cycling. Exercise increased radial artery mean and anterograde SR, along with radial artery diameter, velocity, blood flow and conductance ($P<0.05$). Exercise + WC attenuated the exercise-induced increase in mean and anterograde SR ($P>0.05$) but also increased retrograde SR ($P<0.05$). In addition, increases in radial artery blood flow and diameter were reduced during Exercise + WC (Exercise + WC vs. Exercise, $P<0.05$). After Exercise, L-FMC was augmented (-4.4±1.4 vs. -13.1±1.6%, $P<0.05$), compared to no change in L-FMC after Exercise + WC (-5.2±2.0 vs. -3.0±1.6%, $P>0.05$). In contrast, no change in FMD was observed in either Exercise or Exercise + WC trials ($P>0.05$). These findings indicate that increases in L-FMC following exercise are abolished by the prevention of increases radial artery diameter, mean and anterograde SR, and by elevation of retrograde SR, during exercise in young men.

5.2 Introduction

The endothelium plays an essential role in maintaining vascular homeostasis (Birk et al., 2012, Green et al., 2017), while endothelial dysfunction is an early manifestation of atherosclerotic disease, arterial atheroma as well as other major cardiovascular diseases (Hambrecht et al., 2003, Watts et al., 2004). Flow mediated dilatation (FMD) is a non-invasive method widely used to assess endothelial function as it evokes an endothelial-dependent vasodilatory response to acute hyperemia (i.e., increased shear stress; mechanical interaction between the red blood cells and the blood vessel wall) in a conduit artery (e.g., brachial, femoral, radial) (Corretti et al., 2002a, Corretti et al., 2002b). In contrast to FMD, acute reductions in conduit artery blood flow (e.g., decreased shear stress) induced by a period of distal ischemia, evokes a low-flow mediated constriction (L-FMC) (Gori et al., 2008, Humphreys et al., 2014). Like FMD, L-FMC is impaired in individuals with cardiovascular disease risk factors and is suggested to provide information that is complimentary to that provided by FMD (Gori et al., 2008, Gori et al., 2012). However, while the influence of acute environmental (e.g., heat, hypoxia) and physiological (e.g., exercise, mental stress) stimuli on FMD has been widely studied (Crandall and Wilson, 2011, Dawson et al., 2012, Vianna et al., 2014, Tremblay et al., 2019), limited work has investigated how such interventions effect L-FMC and the underlying mechanisms (Alali et al., 2020).

Exercise training improves cardiovascular outcomes and more specifically enhances endothelial function (Watts et al., 2004, Fletcher et al., 2013). Such beneficial effects on the endothelium may be a result of exercise-induced increases in anterograde shear stress (Laughlin et al., 2008, Tinken et al., 2010, Birk et al., 2013). We have previously observed that L-FMC is enhanced immediately (~10 min)

following a bout of acute dynamic exercise (Elliott et al., 2018), but it is not known whether this is attributable to an acute enhancement of endothelial function, secondary to changes in the pattern of shear stress (Dawson et al., 2012). Although we have previously observed that whole-body heating elevated radial artery shear rate, diameter, and blood flow but did not significantly change L-FMC (Chapter 4; Alali et al., 2020). Notably, dynamic exercise with a large muscle mass acutely decreases the conduit artery FMD response for ~1 hour, although this is not universally observed (Dawson et al., 2013, Elliott et al., 2018). This post-exercise attenuation of the FMD response may be a consequence of increased sympathetic vasoconstrictor tone (Padilla et al., 2010, Thijssen et al., 2014, Atkinson et al., 2015). Thus it is possible that enhanced L-FMC immediately (~10 min) following a bout of acute dynamic exercise (Elliott et al., 2018) is attributable to an increased sympathetic vasoconstrictor tone, rather than a change in the pattern of local blood flow.

Given this background, the aim of this investigation was to determine whether acute exercise-induced increases in L-FMC in young healthy individuals occurs because of a change in the pattern of blood flow that upregulates endothelial function. To achieve this, the influence of a bout of dynamic leg cycling exercise on radial artery blood flow pattern, FMD and L-FMC was investigated. Exercise trials were performed both with and without the experimental induction of a reduced mean SR and increase in retrograde SR in the radial artery by inflating a pneumatic cuff to 75 mmHg placed distal to the site of investigation (Thijssen et al., 2009, Carter et al., 2013, Thijssen et al., 2014). We hypothesized that wrist cuff inflation to reduce mean SR and augment retrograde SR during leg cycling exercise would attenuate radial artery L-FMC in young men. Investigations were focused on the post-exercise period because of its

association with cardiovascular risk (e.g., coronary vasospasm/ischemia, cardiac arrhythmias) (Albert et al., 2000, Akutsu et al., 2002, Goodman et al., 2016).

5.3 Methods

5.3.1 Ethical Approval

All study procedures were approved by the University of Birmingham, Science Technology Engineering and Mathematics Ethical Review Committee (approval number; ERN_18-0523). Written informed consent was obtained from all participants and studies conformed to the most recent revision of the *Declaration of Helsinki*, apart from registration in a database.

5.3.2 Participant characteristics

Eleven healthy young men (age: 23 ± 2 years; height: 175.5 ± 2.4cm; weight; 71 ± 3 kg) were recruited to undertake three separate experimental trials. The following inclusion criteria, as assessed by pre-participation general health and physical activity questionnaires, were used for the participants: 1) normotensive (e.g., resting blood pressure <140/90) and free from pulmonary, cardiovascular and metabolic disease; 2) engaging in regular exercise training (≥ 3 days per week) and no physical limitations; 3) non-smokers and medication free. Prior to experimental trials participants were requested to abide to the following guidance: no food or beverages ≥6 hours, no alcohol or caffeine for ≥12 hours, no polyphenol rich food/beverages for ≥18 hours, no vigorous exercise for ≥48 hours and no vitamin supplements for ≥72 hours. Ten participants completed the experiment, and one participant withdrew from the study after the first trial for personal reasons.

5.3.3 Experimental measures

Heart rate (HR) was assessed using a standard lead II surface electrocardiogram, and an automated sphygmomanometer was used to obtain systolic and diastolic blood pressure (BP) from the left brachial artery (Tango+, SunTech Medical Instruments, Raleigh, NC, USA). The right forearm was supported at heart level, while radial artery diameter and blood velocity were obtained using duplex Doppler ultrasound (Terason uSmart 3300, Teratech Corporation, Burlington, MA, USA). More specifically, the radial artery was imaged using a 4-15 MHz multi-frequency linear-array probe (Terason uSmart 15L4) that was held stable using and an adjustable holder and positioned 10-15 cm distal to the medial epicondyle. B-mode imaging and pulse-wave mode were used to gather radial artery diameter and peak blood velocity, respectively. Measurements were taken in line with recent technical recommendations (Thijssen et al., 2019). Automated edge detection and wall tracking algorithms software was used to record and save radial artery images (Cardiovascular Suite Version 3.4.1, FMD Studio, Pisa, Italy).

5.3.4 Experimental Protocol

Participants attended the laboratory on four separate occasions: one familiarisation session and three experimental trials. At the familiarisation session the equipment and the experimental procedures were demonstrated. Each experimental trial was carried out on a separate day with all three trials occurring within 14 days. All experimental trials were performed at the same time of day to avoid the potential confounding influences of diurnal variations in the measured variables (e.g.,

endothelial function) (Facer-Childs et al., 2019). Trials were conducted in temperature-controlled laboratory that was maintained at 23°C. At each experimental session radial artery function (e.g., L-FMC and FMD) was assessed twice, both before and after either 1) 30 min of leg cycling exercise intervention (Exercise), 2) 30 min of leg cycling exercise intervention while a cuff placed around the right wrist was inflated to 75 mmHg in order to modify the blood flow and SR patterns of right radial artery (Exercise + WC), or 3) 30 min of quiet rest (Time Control). The order of the trials was randomised by the simple allocation concealment technique.

Each experimental trial commenced with the participants being seated on a repurposed car seat that was attached to a sturdy frame on which a cycle ergometer (Angio, Lode, Amsterdam, the Netherlands) was also mounted, and being instrumented for the measurement of HR, BP and radial artery hemodynamics. Participants remained in this upright seated position for the duration of each experimental trial. An inflatable cuff was placed around the right wrist and used for both the blood flow occlusion phase of the L-FMC and FMD assessment and to manipulate blood flow pattern during the Exercise + WC trial. Participants then rested for 20 min after which baseline measures of HR, BP and radial artery variables were obtained. Radial artery function (L-FMC, FMD) was then assessed. This consisted of baseline (1 min), wrist cuff inflation to ≥220 mmHg (5 min), and recovery/cuff deflation (3 min) periods. Following this, for the Exercise and Exercise + WC trial, participants then undertook a 30 min bout of leg cycling on a semi-recumbent cycle ergometer. The workload was initially set at 50 W and thereafter increased by 50 W every 10 min (50 W, 100 W and 150 W), during which cycling cadence was maintained at 60 rpm. Following this, participants recovered for 10 min and radial artery function was reassessed. During the Exercise + WC trial, the wrist cuff was inflated to 75 mmHg

immediately following baseline and remained inflated throughout trial. In Time Control trial, the leg cycling was replaced with a quiet seated rest period and radial artery function assessed pre- and post-intervention as for Exercise and Exercise + WC.

5.3.5 Data analysis

Mean arterial pressure (MAP) was calculated as: diastolic BP (mmHg) + [0.33 + (HR x 0.0012)] x [systolic BP (mmHg) − diastolic BP (mmHg)] (Razminia et al., 2004). Blood flow (ml/min) was calculated from radial artery diameter and mean blood velocity as: mean blood velocity (cm/s) x π x radius (cm) ^{2} x 60. Vascular conductance was calculated as radial arterial blood flow (ml/min) divided by MAP (mmHg). Arterial wall shear rate (SR, s^{-1}) was estimated, without consideration of the blood viscosity, as: 4 x mean blood velocity (cm/s) divided by diameter (cm). Anterograde and retrograde SR were calculated using positive and negative blood velocities, respectively.

Steady-state measurements of HR, BP and radial artery variables were recorded at baseline and every 5 min throughout the Exercise, Exercise + WC and Time Control trials. At each 5 min time point, the radial artery was assessed for 60 s and the highest quality continuous 20 s section then analyzed. L-FMC was calculated as the change from average baseline (1 min) diameter to the average diameter of the last 30 s of cuff occlusion period (Gori et al., 2008). FMD was determined from the change in baseline diameter to the maximal diameter observed in the post-occlusion period (Gori et al., 2008). Responses of L-FMC and FMD are presented as relative (%) and absolute (mm). Total vessel reactivity (TVR, %) was calculated using the following equation (Rakobowchuk et al., 2012):

$$\frac{\text{FMD diameter (mm)} - \text{LFMC diameter(mm)}}{\text{Baseline average diameter (mm)}} \text{ x } 100$$

The shear rate area under the curve (SR_{auc}) and time to peak diameter was calculated from cuff release up to the point of maximum artery dilation. A simple ratio normalization of L-FMC against change in mean SR (difference between baseline SR and SR during L-FMC measurement period; L-FMC-to- Δ mean SR ratio, au) and ratio of FMD against SR_{auc} (FMD-to-SR_{auc} ratio, au) were calculated to evaluate role of shear stress in mediating constriction and dilation (Padilla et al., 2008, Alali et al., 2020).

Recent guidelines suggest taking into consideration whether allometric scaling is required when analyzing FMD (Atkinson et al., 2013). Need for accurate scaling can be determined by obtaining the slope and the upper 95% confidence intervals (CI) of the relationship between natural log-transformed baseline and nadir/peak diameters (not to equal and less than a value of 1). Allometric scaling for baseline diameters was not conducted in current study as the slope of the relationship between log-transformed baseline and nadir/peak diameters were not significantly different from 1 (all slopes 0.93 - 0.79 and all upper 95% CI 1.81 – 0.98).

The percentage of age-predicted maximal heart rate (HR_{max}) reached during each exercise workload (50, 100, 150 W) was calculated as; heart rate/HR_{max} x 100, where HR_{max} was calculated as 208-0.7 x age (Tanaka et al., 2001).

5.3.6 Statistical Analysis

Two-way repeated measures ANOVA were used to locate differences in hemodynamic responses, radial artery characteristics and blood flow with respect to time (baseline, 5, 10, 15, 20, 25, and 30 min), trial (Exercise, Exercise + WC, Time

Control) and their interaction (time x trial). Two-way repeated measures ANOVA were also used to examine the main effects of time (Pre vs. Post), trial (Exercise, Exercise + WC, Time Control) and their interaction (time x trial), for radial artery characteristics and functional responses (e.g., L-FMC and FMD). Analysis of covariance ANCOVA was used to investigate the FMD response for the corrected shear rate stimulus (SR_{AUC}-corrected-FMD%), by using SR_{AUC} as a covariate (Thijssen et al., 2019). Significant main effects and interactions were investigated *post hoc* using Tukey's tests. The level of significance for all tests at $P < 0.05$ was recognized as being statistically significant. All data are presented as mean (SD) unless stated otherwise. Repeated measures statistical analyses were performed using GraphPad Prism 8 (GraphPad Software, San Diego, CA) and ANCOVA analysis was performed with SPSS v. 21 (SPSS, Chicago, IL).

The minimum sample size was determined retrospectively on the basis of a priori power analysis, considering radial artery baseline diameter (within-participant and within-day repeat measure) as the primary efficacy variable. The power analysis was conducted using the G* Power version 3.1 (Buchner, Erdfelder, Faul and Lang, 2014) with an alpha level of 0.05, power = 0.80, and an estimated medium effect size of d= 0.67. Data for calculating the effect size were obtained from a similar previous study and used the mean and standard deviation differences for the control and intervention conditions as well as the between condition correlation of 0.94 (Elliott et al., 2018). These calculations suggested the need for a minimum sample size of 20 participants.

5.4 Results

5.4.1 HR and BP

HR, systolic BP and MAP increased progressively (all, $P<0.05$) and to a similar extent (all $P<0.05$) from baseline in both the Exercise and Exercise + WC trials but were unchanged ($P>0.99$) during the Time Control trial (Figure 5.1). Diastolic BP did not differ from baseline in any trial ($P\geq0.26$). The 50-, 100- and 150-Watt workloads corresponded to 56±8, 66±9 and 81±11 $\%HR_{max}$ for Exercise trial and 54±8, 65±10 and 81±10 $\%HR_{max}$ for Exercise + WC trial, respectively.

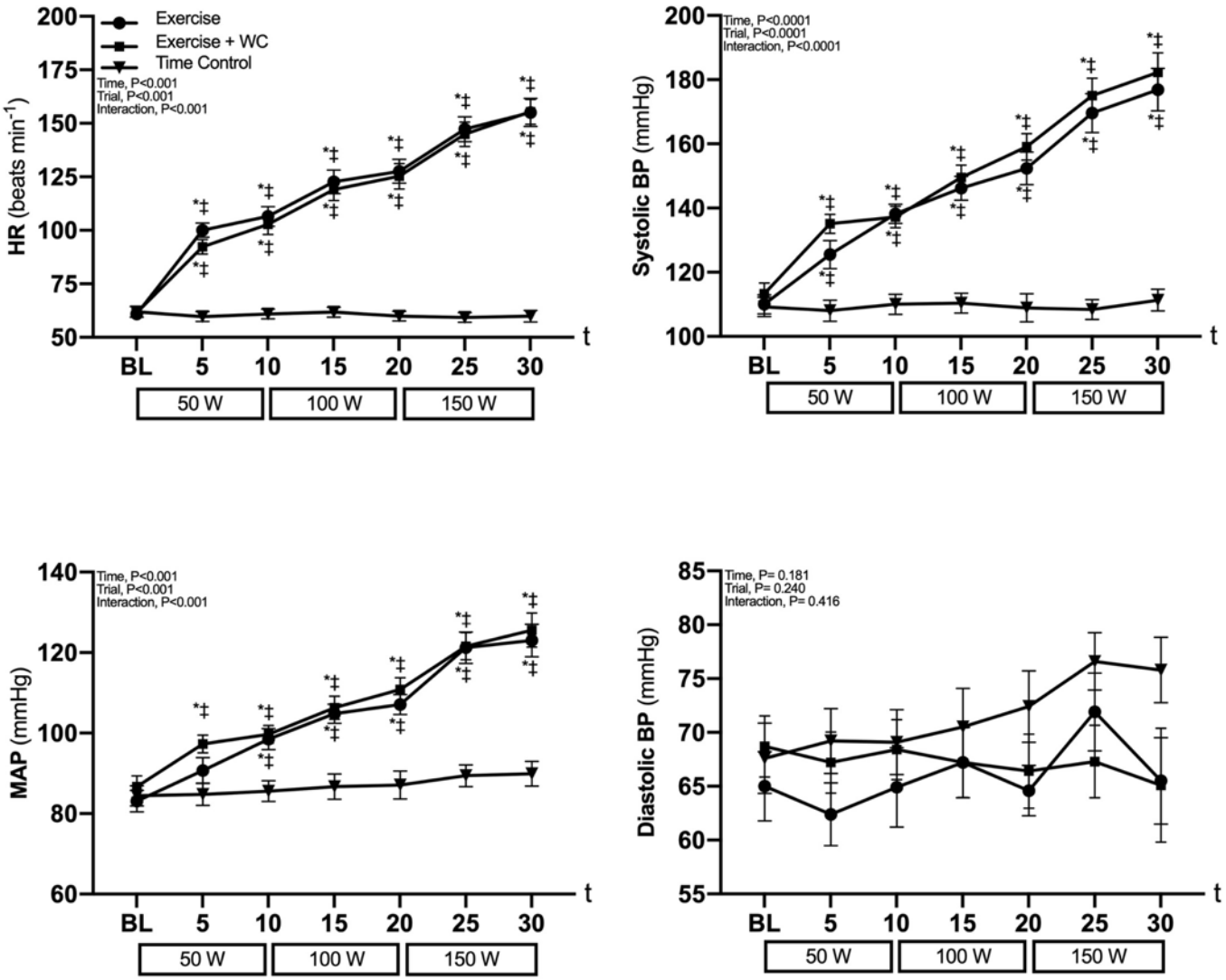

Figure 5.1 Cardiovascular responses

Heart rate (HR), systolic blood pressure (systolic BP), diastolic blood pressure (Diastolic BP) and mean arterial pressure (MAP) responses to leg cycling exercise (Exercise), leg cycling exercise with wrist cuff (Exercise + WC) and Time Control trials. Values are mean ± SE. * P < 0.05 vs. baseline (BL); † P < 0.05 vs. Exercise + WC; ‡ P < 0.05 vs. Time Control.

5.4.2 Radial artery characteristics

Baseline mean, anterograde and retrograde SR did not differ between trials (Figure 5.2). Mean and anterograde SR progressively increased during Exercise ($P<0.05$). In contrast during Exercise + WC, mean SR initially decreased ($P<0.05$, baseline vs 5 min) before returning to baseline ($P\geq0.90$, baseline vs. 25 and 30 min), and anterograde SR slightly increased ($P<0.05$ vs. Time Control at 30 min). During Exercise, retrograde SR transiently increased ($P<0.05$, baseline vs. 5 min) before returning to baseline ($P\geq0.45$, baseline vs. 10 min onwards), whereas during Exercise + WC retrograde SR was markedly increased ($P<0.01$, baseline vs. 5 min onwards). In the Time Control trial, mean, anterograde and retrograde SR were unchanged (all, $P\geq0.63$) from baseline.

Radial artery diameter, blood flow, velocity and vascular conductance were not different between all trials at baseline (all $P\geq0.41$, Figure 5.3). During Exercise, radial artery diameter, blood flow, velocity and vascular conductance all progressively increased (all, $P<0.05$ vs. Time Control at 25 and 30 min). In contrast, diameter, blood flow, velocity, and vascular conductance were not significantly different from baseline during Exercise + WC and Time Control (all $P\geq0.06$).

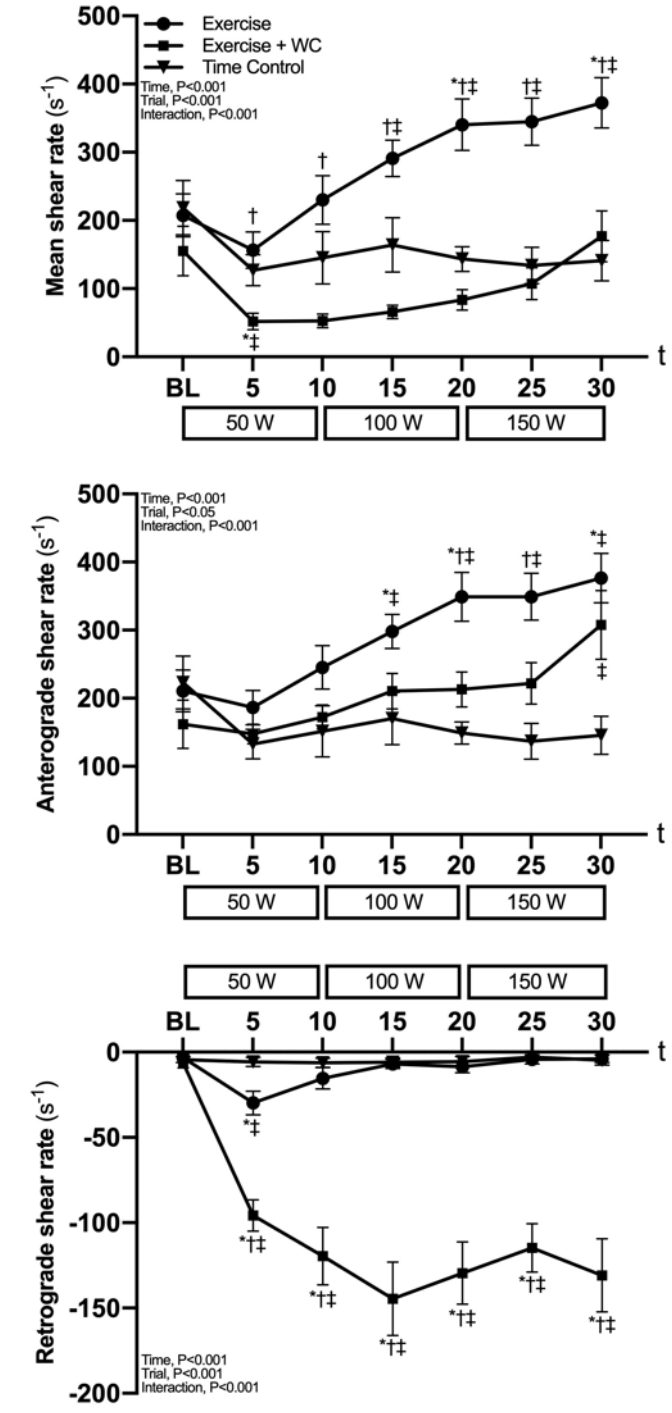

Figure 5.2 Radial artery blood flow pattern

Mean, anterograde and retrograde shear rate during leg cycling exercise (Exercise), leg cycling exercise with wrist cuff (Exercise + WC) and Time Control trials. Values are

the mean ± SE. * P < 0.05 vs. baseline (BL); † P < 0.05 vs. Exercise + WC; ‡ P < 0.05 vs. Time Control.

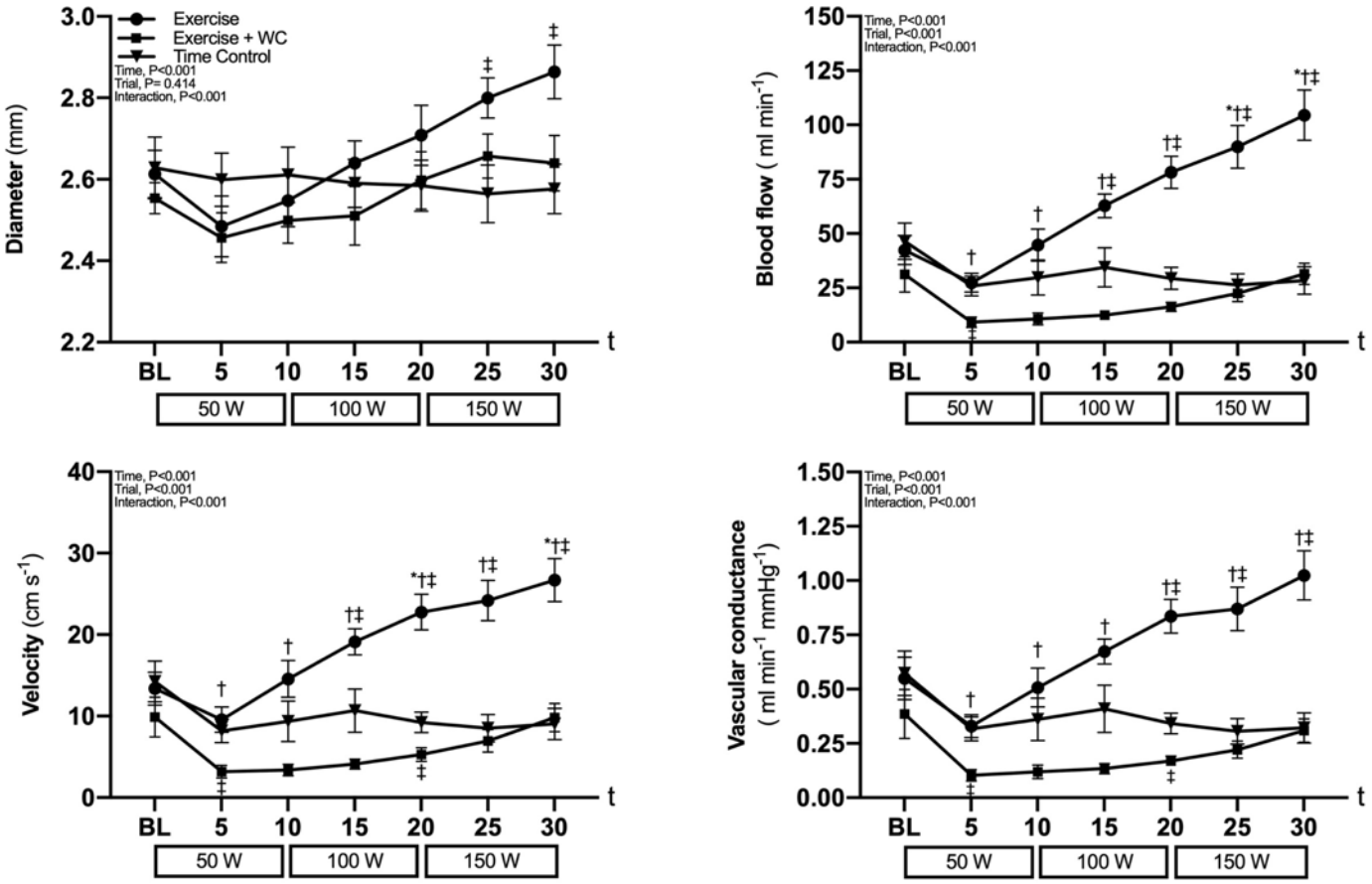

Figure 5.3 Radial artery characteristics

Radial artery blood flow, diameter, velocity and vascular conductance during leg cycling exercise (Exercise), leg cycling exercise with wrist cuff (Exercise + WC) and Time Control trials. Values are the mean ± SE. * P < 0.05 vs. baseline (BL); † P < 0.05 vs. Exercise + WC; ‡ P < 0.05 vs. Time Control.

5.4.3 Radial artery function responses

Radial artery characteristics before and after the Time Control, Exercise and Exercise + WC trials are presented in Table 5.1. FMD, L-FMC and TVR % responses were not different between trials at baseline ($P \geq 0.53$, Figure 5.4). The L-FMC % response increased significantly following Exercise ($P<0.05$ vs Pre; $P<0.05$ vs Exercise + WC; $P<0.05$ vs Time Control). The time x Trial interaction for FMD ($P=0.074$) and TVR ($P=0.053$) failed to reach the threshold for statistical significance, however tendencies for a reduction in FMD and an increase in TVR was noted following the Exercise trial.

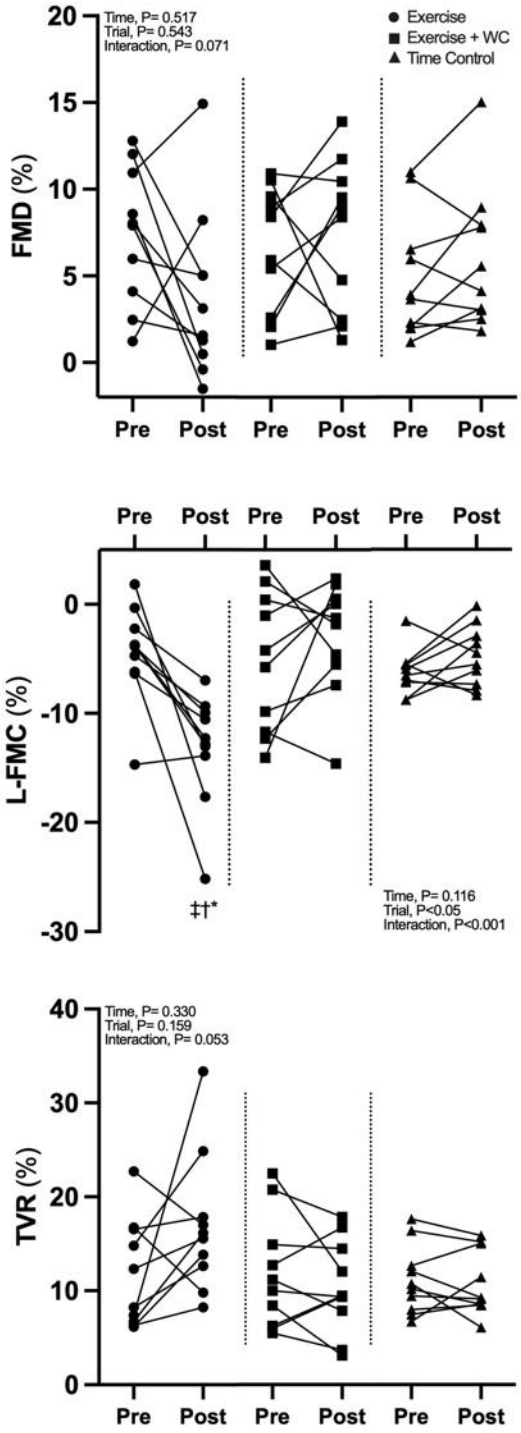

Figure 5.4 Radial artery function

Radial artery flow mediated dilatation (FMD), low-flow mediated constriction (L-FMC), and total vascular range (TVR) during leg cycling exercise (Exercise), leg cycling exercise with wrist cuff (Exercise + WC) and Time Control trials. Values are the

individuals data. * $P < 0.05$ vs. baseline (BL); † $P < 0.05$ vs. Exercise + WC; ‡ $P < 0.05$ vs. Time Control.

Table 5.1 Radial artery characteristics before [Pre] and after [Post] the Time Control, Exercise and Exercise + WC trials.

	Time Control		Exercise		Exercise + WC		P values		
	Pre	**Post**	**Pre**	**Post**	**Pre**	**Post**	**Trial**	**Time**	**Interaction**
Baseline									
Diameter (mm)	2.63 (0.24)	2.57 (0.18)	2.61 (0.18)	2.80 (0.25) *‡	2.55 (0.12)	2.66 (0.18)	**0.341**	**0.004**	**0.002**
Velocity (cm/s)	14.25(7.95)	7.72(4.40) *	13.37 (6.40)	20.45(7.44) *†‡	9.89 (7.70)	4.21 (1.87)	**<0.001**	**0.253**	**0.001**
Blood flow (ml/min)	46.45(26.35)	24.01(13.39)	42.35(21.21)	75.91(28.68) *†‡	31.20 (25.75)	14.46 (7.92)	**<0.001**	**0.733**	**<0.001**
Mean shear rate (s^{-1})	218.71 (126.18)	121.55 (70.84)	207.45 (99.18)	295.16 (115.24)†‡	155.34 (114.68)	62.72 (25.26) *	**0.002**	**0.122**	**0.002**
L-FMC									
Nadir Diameter (mm)	2.46 (0.22)	2.44 (0.16)	2.50 (0.23)	2.44(0.29)	2.42 (0.20)	2.57 (0.17) *	**0.894**	**0.389**	**0.012**
Δ Diameter (mm)	-0.17 (0.06)	-0.13 (0.07)	-0.11 (0.12)	-0.36(0.13)*†‡	-0.13 (0.17)	-0.09 (0.14)	**0.012**	**0.072**	**<0.001**
Mean shear rate (s^{-1})	18.41 (7.14)	20.40 (10.15)	22.13 (5.21)	29.54 (9.03)*†‡	19.33 (5.769)	19.20 (6.913)	**0.053**	**0.033**	**0.088**
Δ Mean shear rate (s^{-1})	200.31 (123.20)	101.15 (63.45)	185.32 (98.82)	265.61 (113.32)†‡	136.01 (112.80)	43.53 (24.75)*	**0.002**	**0.093**	**0.002**
L-FMC-to- Δ mean SR ratio (au)	-0.043 (0.032)	-0.067(0.050)	-0.009(0.052)	-0.066 (0.064)	-0.050 (0.113)	-0.061 (0.215)	**0.892**	**0.117**	**0.592**
FMD									
Peak Diameter (mm)	2.75 (0.23)	2.72 (0.20)	2.81(0.24)	2.91 (0.28)	2.72 (0.18)	2.85(0.14) *	**0.426**	**0.028**	**0.065**
Δ Diameter (mm)	0.12 (0.09)	0.15 (0.10)	0.20 (0.10)	0.11 (0.14)	0.17 (0.10)	0.19 (0.11)	**0.595**	**0.549**	**0.085**
Time to peak diameter (s)	101.10 (51.810)	82.60 (31.22)	85.50 (44.47)	108.90 (35.38)	97.50 (46.84)	73.50 (47.24)	**0.717**	**0.559**	**0.164**
SR_{AUC} ($x10^3$ s^{-1})	26.39 (23.31)	27.10 (13.48)	17.50 (6.66)	37.50 (35.66)	17.20 (8.43)	27.61(20.29)	**0.733**	**0.044**	**0.291**
FMD-to-SR_{AUC} ratio (au)	0.35 (0.37)	0.29 (0.25)	0.47 (0.31)	0.24 (0.35)	0.51 (0.39)	0.43 (0.41)	**0.388**	**0.194**	**0.728**
SR_{AUC}-corrected-FMD (%)	4.92 (4.06)	6.01 (4.06)	7.09 (4.12)	4.24 (4.18)	6.19 (4.12)	7.41 (4.06)	**0.543**	**0.868**	**0.215**

Values are means [SD]. L-FMC, low-flow mediated constriction; FMD, flow mediated dilatation; SR_{AUC}, shear rate area under curve. P values represent 2-way repeated ANOVA results (Trial; Time Control, Exercise and Exercise + WC: Time; Pre and Post: Interaction, Trial x Time). P value for SR_{AUC}-corrected-FMD (%) represent ANCOVA results. * $P < 0.05$ vs. Pre; † $P < 0.05$ vs. Exercise + WC; ‡ $P < 0.05$ vs. Time Control.

5.5 DISCUSSION

The primary objective of the present study was to determine whether augmented radial artery L-FMC following acute dynamic leg exercise is attributable to a change in local SR. Accordingly, the application of a cuff placed distal to radial artery (Exercise + WC) and inflated to 75 mmHg during leg cycling exercise was observed to attenuate exercise-induced increases in radial artery diameter, mean and anterograde SR, while significantly augmenting retrograde SR. In accordance with our hypothesis, this change in local SR prevented the exercise-induced increase in radial artery L-FMC in young men.

We have previously observed that leg cycling exercise acutely augments L-FMC in healthy adults (Elliott et al., 2018). The endothelium is one of the proposed mechanisms responsible for this L-FMC response to exercise (Dawson et al., 2012). Although, L-FMC is reportedly not altered by infusing NO synthase inhibitor (Gori et al., 2008), the inhibition of other endothelial derived vasodilatory substances (e.g. prostaglandins and endothelial-derived hyperpolarizing factors) does attenuate L-FMC, suggesting that it is partly endothelium mediated (Gori et al., 2008). Additional support for an endothelial contribution to radial artery L-FMC is provided by the observation that it is blunted by endothelial denudation induced by the radial artery catheterization procedure (Dawson et al., 2012). As observed by Elliott et al. (2018), and several others prior to this (Tinken et al., 2010, Birk et al., 2012), leg cycling exercise increases arm blood flow and anterograde SR, which is known to upregulate the release of endothelium-derived vasodilatory substances. This may explain the acutely increased radial L-FMC in the immediate post-exercise period (Elliott et al., 2018). Interestingly, acute whole-body heat stress was also observed to increase

radial artery diameter, blood flow and anterograde SR during, however, no significant change in L-FMC was noted (Chapter 4; Alali et al., 2020). However, there are important differences in the hemodynamic, thermoregulatory, and cellular signalling responses to acute exercise and whole-body heating (Cullen et al., 2020) that may explain the divergent L-FMC results.

In order to determine the endothelial contribution to the augmented post-exercise L-FMC response, we examined the effect of radial artery SR manipulation during leg cycling exercise. As expected, the application of a cuff inflated to 75 mmHg and placed distal to the radial artery prevented normal increases in mean and anterograde SR, whereas retrograde SR was observed to be markedly augmented. This is a well-recognized approach to modify local SR patterns during experimental manipulations (Thijssen et al., 2009, Tinken et al., 2009, Johnson et al., 2012). *In vivo* and *in vitro* studies of endothelial function have shown that this type of oscillatory shear stress, characterized by a high level of retrograde SR, causes endothelial dysfunction (e.g., increases proatherogenic genes, decreases FMD response) (Ziegler et al., 1998, Thijssen et al., 2009). At a cellular level, an increase in retrograde SR markedly reduces endothelial NO synthase (Ziegler et al., 1998). Given this, the reduction in L-FMC caused by Exercise + WC might be explained by a reduction in endothelial function due to the attenuated anterograde and mean SR, and/or the increased retrograde SR. However, other factors, including secondary changes in the prevailing arterial diameter, should also be considered.

Notably in the Exercise trial, the post-Exercise radial artery diameter significantly increased from pre-Exercise (pre: 2.61 ± 0.18 mm vs. post: 2.80 ± 0.25 mm), while the reduction in shear rate during L-FMC assessment was augmented (pre: Δ185.32 ± 98.82 s^{-1} vs. post: Δ265.61 ± 113.32 s^{-1}). Conversely, in the Exercise + WC

trial radial artery diameter did not change pre – to – post exercise and in fact the change in mean shear rate was much lower post-exercise (Δ43.53 ± 24.75 s^{-1}) as compared to pre (Δ136.01 ± 112.80 s^{-1}). As such, the L-FMC-to-Δ mean SR ratio was not different among trials. This indicates that differences in the prevailing radial artery diameter at the time of L-FMC and the change in shear rate caused by the occlusion cuff inflation used in the assessment of L-FMC may also be important contributing factors to the responses observed.

The beneficial effects of exercise on FMD appear to be mediated by the associated periodic increases in conduit artery blood flow (Watts et al., 2004, Fletcher et al., 2013) and more specifically the increase in anterograde SR that is known to upregulate endothelial NO synthase expression (Laughlin et al., 2008). Several studies have observed that FMD is acutely attenuated in the post-Exercise period (Hwang et al., 2012, Dawson et al., 2013). Importantly, Atkinson et al. (2015) showed that the attenuation of FMD following acute high-intensity dynamic exercise was prevented by administration of an oral alpha-adrenergic antagonist. This suggests that increases in SNA cause the acutely reduced FMD post-Exercise, a conclusion supported by earlier observations that other acute sympatho-excitatory manoeuvres are also associated with reduced FMD (Hijmering et al., 2002). In agreement with Elliott et al. (2018), who used the same incremental exercise protocol as the present study, no significant reduction in FMD was noted following Exercise. However, there was a tendency for a significant interaction between time and trial (P=0.071) and a numerically lower FMD post-Exercise (3.8± %) versus pre-Exercise (7.4±4%, Figure 5.4). Interestingly, FMD was not different following Exercise + WC despite the wrist cuff manipulation significantly augmented retrograde SR. Thijssen et al. (2009)

previously reported that brachial artery FMD was attenuated by increased retrograde SR induced by cuff inflation (e.g., 25, 50, 75 mmHg), while anterograde SR was unchanged. In addition, Johnson et al. (2012) reported that a wrist cuff (inflated to 60 mmHg) increased retrograde SR and attenuated brachial FMD following supine leg cycling exercise (90 W, 20 min). A potential explanation for the conflicting findings of these studies (Atkinson et al., 2009, Thijssen et al., 2009, Johnson et al., 2012) and those of the present study, may be that we observed a small but significant increase in anterograde SR during Exercise + WC (at 30 min time point) that may have counteracted the negative effects of retrograde SR on endothelial function. Nevertheless, the observed failure of Exercise + WC to diminish FMD in our study does not support the view that the attenuated L-FMC response at this time is attributable to a reduction in endothelial function or diameter.

5.5.1 Methodological considerations

Our investigations were confined to an assessment of the radial artery and additional studies are needed to validate these results in other conduit arteries. We acknowledge that the brachial artery is frequently assessed in human studies of peripheral vascular function, although it is not uncommon for the radial artery to be interrogated. Furthermore, the radial artery is used as a graft in coronary bypass surgeries (Song et al., 2012), where it may be susceptible to functional vasospasm (He and Taggart, 2016), further supporting the relevance of its study. Importantly, we observed radial artery blood flow, diameter and SR patterns during Exercise that are comparable to those previously documented for the brachial artery (Padilla et al., 2011, Birk et al., 2013). The L-FMC response is reported to be more commonly observed in radial artery than brachial artery (Weissgerber et al., 2010). Additional

mechanistic insights would have been provided had a measurement of sympathetic nervous system activation been included in the present study. We can only assume that it was equivalent in the Exercise and Exercise + WC conditions, but in support of this neither HR nor BP were not different between the Exercise and Exercise + WC conditions. In the present study, only healthy young men were recruited which is a limitation. Both sex- and ovarian hormone concentrations can modify vascular function (Hashimoto et al., 1995). Regrettably, in the current study we were not sufficiently resourced to study young women at a standard phase (e.g., early follicular stage) or multiple phases of their menstrual cycle for the three separate trials. The present results cannot be extrapolated to women and further studies are needed to examine whether the findings of the present study are applicable to women. The exercise mode employed was incremental dynamic exercise (e.g., 50, 100, 150 Watts), which was well tolerated by participants and permitted the simultaneous acquisition of high-quality radial artery images. In addition, the leg cycling exercise modality and workloads employed are representative of those suggested for establishing and preserving cardiorespiratory fitness.

Assessments of radial artery function (i.e., L-FMC) were made at rest and immediately (~10 min) following acute dynamic exercise. The broader significance of this time period is that it is associated with an increased cardiovascular risk (e.g., coronary vasospasm/ischemia, cardiac arrhythmias) (Albert et al., 2000, Akutsu et al., 2002, Thompson et al., 2007, Franklin et al., 2020). There are known parallels between the vasodilatory responses of the peripheral conduit and coronary arteries (Anderson et al. 1995; Takase et al. 1998). As such our findings from the radial artery support the concept (Thijssen et al., 2009, Carter et al., 2013, Thijssen et al., 2014) that in healthy individuals increases in mean and anterograde SR facilitate local

vasodilatation, hyperaemia and functional capacity, thus making an important contribution to oxygen delivery via the coronary vasculature. Several studies have reported a temporal variation in FMD following exercise (Birk et al., 2013), but unfortunately only a single time point was assessed in this study and therefore further investigation are required to determine the time course of the L-FMC response post-exercise.

The sample size of this study was determined based on power calculations using conservative effect size estimates. However, the actual effect size may have been smaller than anticipated, which may have contributed to an underpowered study. Future studies may benefit from larger sample sizes to increase statistical power and improve the generalizability of the results.

5.5.2 Conclusions

These findings confirm that dynamic leg cycling exercise acutely increases radial artery mean and anterograde SR, leading to radial artery vasodilatation and an augmented L-FMC in young men. The application of a cuff placed distal to the radial artery and inflated during leg cycling exercise attenuated exercise-induced increases in mean and anterograde SR, significantly augmented retrograde SR, and prevented the vasodilatation of the radial artery during exercise. Importantly, these hemodynamic alterations acutely attenuated L-FMC. Collectively, these observations suggest that SR modifications explain the radial artery responses to acute leg cycling and the ensuing augmentation of L-FMC. Such findings are consistent with the view that lower limb exercise evokes a pattern of blood flow through the upper limb conduit arteries

that modifies endothelial function such that local vasodilatation, hyperaemia and functional capacity (in terms of L-FMC) are acutely augmented.

CHAPTER 6: IMPACT OF ACUTE SYMPATHETIC ACTIVATION ON RADIAL ARTERY LOW-FLOW MEDIATED CONSTRICTION IN YOUNG MEN

6.1 Abstract

Lower body negative pressure (LBNP) is known to enhance SNA and is associated with a reduction in blood flow/shear rate of conduit artery (e.g., brachial), thus acutely attenuating flow mediated dilation (FMD) (Hijmering et al., 2002). Herein, we sought to determine how acute sympathoexcitatory (via LBNP) and sympathoinhibitory [via lower body positive pressure (LBPP)] manoeuvers influences radial artery low-flow mediated constriction (L-FMC), and the role of sympathetic factors in mediating L-FMC response. Ten young healthy men (23 ± 2 years) underwent LBNP (-20 mmHg) and LBPP (+10 mmHg). Trials were randomised and separated by at least 24 hours. Radial artery characteristics and function (e.g., FMD and L-FMC) were assessed prior to and upon reaching 10 minutes of stabilisation of both manoeuvers (LBNP and LBPP). LBNP (-20 mmHg) markedly reduced radial artery mean shear rate, blood flow and velocity (Pre vs During, $P<0.05$), and consequently attenuated FMD % (Time, $P<0.05$). Unexpectedly, similar results of radial artery characteristics and FMD % were also noted during LBPP (+10 mmHg) (Time, $P<0.05$). In contrast, L-FMC was not different in either trial ($P>0.05$). In summary, acute LBNP, which was anticipated to enhance SNA, markedly reduced radial artery shear rate and thus attenuates FMD, but unchanged L-FMC. Acute LBPP, which was anticipated to supress SNA, altered radial artery characteristics and FMD as observed in LBNP yet radial artery L-FMC remained unchanged. However, SNA in both LBNP and LBPP was not measured.

6.2 Introduction

It is widely recognized that endothelial dysfunction is a clear indication of preclinical and clinical atherosclerosis (Rajendran et al., 2013). Epidemiologic studies have shown that impaired endothelial function is also associated with major cardiovascular diseases risk factors (Neunteufl et al., 1997, Hambrecht et al., 2003, Watts et al., 2004). Furthermore, several studies reported that both invasive and non-invasive techniques can successfully detect reductions in endothelial responses during the early manifestation of cardiovascular disease states (Neunteufl et al., 1997, Shechter et al., 2014). One such non-invasive technique is flow mediated dilation (FMD), which has been widely adopted to evaluate conduit arteries (e.g., brachial, femoral, radial) endothelial function as it evokes an endothelial vasodilatory response to acute increase in blood flow and shear stress (Corretti et al., 2002a, Thijssen et al., 2011, Thijssen et al., 2014). Utilizing this method, various studies have shown that a reduction in FMD response are related to cardiovascular risk factors (Matsuo et al., 2004, Tarutani et al., 2005, Shechter et al., 2014). However, low-flow mediated constriction (L-FMC), where endothelial vasoconstrictor response to an acute blood flow reduction in conduit artery created by a period of ischemia, is a less widely recognized vascular function measure (Gori et al., 2008). Like FMD, L-FMC is also reported to be negatively affected in individuals with cardiovascular diseases risk factors (Gori et al., 2012). However, unlike FMD, which is known to be endothelial dependent (nitric oxide (NO) dependent), L-FMC is not altered following administration of nitric oxide synthase antagonism (Gori et al., 2008). Therefore, the mechanisms underlying L-FMC are not completely understood but have been suggested to be

mediated differently from that in the FMD response (Gori et al., 2010, Elliott et al., 2018).

Although the endothelium plays a major role in the vasoconstriction regulatory mechanism, sympathetic nerve activity (SNA) also acts as an extrinsic vasoconstriction regulator in vasculature (Hijmering et al., 2002). Indeed, it has been suggested that SNA has a role in meditating L-FMC (Gori et al., 2008), though this has not been explored yet. Acute whole-body heat stress (increasing core temperature by +1 °C) is known to enhance SNA (Crandall and Wilson, 2015) but does not significantly change radial artery L-FMC (Chapter 4; Alali et al., 2020). However, 30 minutes of acute dynamic cycling exercise, which is also recognized as an enhanced SNA period (Atkinson et al., 2015), significantly increased radial artery L-FMC (Chapter 5; Alali et al., 2022). Parallel to this, Elliott et al. (2018) also observed that following an acute session of cycling exercise, significantly increased radial artery L-FMC, anterograde and retrograde shear rate. Despite the fact that SNA enhancement is reported earlier in both heat stress and exercise protocols (Crandall and Wilson, 2011, Atkinson et al., 2015), the divergence of radial artery L-FMC responses in both interventions (Chapter 4 and Chapter 5) is justified as there are significant differences in the thermoregulatory, hemodynamic, and cellular signalling responses to acute heat stress and exercise (Cullen et al., 2020). Accordingly, the possibility of non-endothelial mediators such as SNA linked with L-FMC augmentation cannot be discounted.

Moreover, SNA activation, independent of heat stress and exercise, via baroreceptor unloading using lower body negative pressure (LBNP) contributed to reductions in endothelial function (e.g. FMD) (Hijmering et al., 2002, Thijssen et al., 2014). Previous findings concluded that acute SNA stimulation is not associated in deregulate NO bioavailability (Engelke et al., 1997). Thijssen et al. (2014) linked the

reduction in FMD response following acute stimulation of SNA via LBNP to changes in an arterial shear pattern specifically due to increases in retrograde shear. However, this was not the case with the radial artery L-FMC response during and/or following acute retrograde shear rate augmentation (Elliott et al., 2018, Alali et al., 2022). Collectively, the impact of SNA on radial artery L-FMC remains unclear.

Given this background, the aim of this research was to examine the effect of acute SNA (sympathoexcitatory and sympathoinhibitory manoeuvres) on radial artery characteristics and L-FMC response. To achieve this, the influence of SNA activation via baroreceptor unloading utilised by LBNP and lower body positive pressure (LBPP) on radial artery characteristics, and vascular function (FMD and L-FMC) were explored. The application of LBPP (+10 mmHg) has been shown previously to suppress SNA (Fu et al., 1998). Accordingly, we hypothesised that; 1) LBNP would increase radial artery retrograde shear rate and L-FMC response and attenuate FMD, and 2) LBPP would decrease retrograde shear rate and attenuate L-FMC response, and augment FMD.

6.3 Methods

6.3.1 Ethics approval

The study was approved by the University of Birmingham, Science Technology Engineering and Mathematics Ethical Review Committee (Approval number: ERN_18-0523).

6.3.2 Participant characteristics

Ten healthy young men with a mean age of 23 ± 2 years, height 175.5 ± 2.4 cm, and weight of 71 ± 3 kg (mean ± SD) were recruited. All participants were medication-free, nonhypertensive (resting blood pressure <140/90) and free of pulmonary, cardiovascular, metabolic and neurological disease. Prior to experimental conditions participants were requested to follow these directions: no food or beverages ≥ six hours, no alcohol or caffeine for ≥ twelve hours, no polyphenol rich food/beverages for ≥ eighteen hours, no vigorous exercise for ≥ forty-eight hours and no vitamin supplements for ≥ seventy-two hours.

6.3.3 Experimental measures

Systolic and diastolic blood pressure (BP) was assessed from the left brachial artery using an automated sphygmomanometer (Tango+, SunTech Medical Instruments, Raleigh, NC, USA). Whereas heart rate (HR) was measured using a standard lead II surface electrocardiogram. Transthoracic fluid volume was measured using 4 electrodes impedance plethysmography (UFI; Morro Bay, CA) to determine blood volume changes before and after LBNP and LBPP trials. Duplex Doppler ultrasound (Terason uSmart 3300, Teratech Corporation, Burlington, MA, USA) was

used to obtain right radial artery diameter and blood velocity while the right arm was supported at heart level. More specifically, a 4-15 MHz multi frequency linear array probe was used to image radial artery. The probe was positioned using an adjustable probe holder and placed 10-15 cm distal to the medial epicondyle. Additionally, ultrasound B-mode imaging and pulse-wave mode were used to obtain radial artery diameter and blood velocity, respectively. Measurements were taken in compliance with recent technical guidance (Thijssen et al. 2019). Radial artery images were recorded as video file using automated edge detection and wall tracking algorithms software (Cardiovascular Suite Version 3.4.1, FMD Studio, Pisa, Italy). An inflatable cuff placed around the right wrist was used for the assessment of radial artery function (L-FMC and FMD). Lower body pressure was measured inside a customised chamber via a pressure transducer (Cardiorater CR7, Cardica Records Ltd, London, UK).

6.3.4 Experimental protocol

Participants visited the laboratory on three separate occasions, one familiarisation session and two experimental trials. All trials were conducted in a temperature-regulated laboratory (between 22-24 °C) and at the same time of the day. Participants first attended a familiarization session prior to experimental trials, in which study procedure and the equipment were described and applied. Following this, participants returned for two separate experimental visits to explore the impact of sympatho-excitatory and sympatho-inhibitory manoeuvres evoked using LBNP and LBPP on radial artery endothelial function, respectively. These trials were separated by a minimum of twenty-four hours and completed within fourteen days. In both experimental trials, participants were asked to enter their lower body into a customised

wooden pressure chamber to the level of the iliac crest. Following this all instruments were applied, and participants rested for a 20 minute period before baseline measurements were obtained. Experimental measures outlined above (heart rate, systolic and diastolic blood pressure, thoracic impedance and radial artery vascular function) were then recorded. The assessment of radial artery function (L-FMC and FMD) consisted of 1 minute baseline, followed by 5 minutes wrist cuff inflation to ≥220 mmHg, and 3 minutes recovery period. For the LBNP intervention, the chamber pressure was initially started at -5 mmHg and increased by -5 mmHg every 5 minutes until the chamber pressure reached -20 mmHg. This was followed by a 10 minute stabilisation period before LBNP vascular function measurements (FMD and L-FMC) were taken. During the LBPP intervention, the pressure inside the chamber was increased to +10 mmHg, followed by a 10 minute stabilisation period (to match the LBNP trial) before vascular function measurements (FMD and L-FMC) were retaken. The order of LBNP and LBPP trials was randomised by a coin toss.

6.3.5 Data analysis

Mean arterial pressure (MAP) was calculated using the following equation: diastolic BP (mmHg) + [0.33 + (HR x 0.0012)] x [systolic BP (mmHg) − diastolic BP (mmHg)] (Razminia et al., 2004). Blood flow (ml/min) was calculated using radial artery diameter and mean blood velocity variables as: mean blood velocity (cm/s) x π x radius (cm) ^{2} x 60. Radial artery wall shear rate (SR, s^{-1}) was calculated as: 4 x mean blood velocity (cm/s) divided by diameter (cm). Anterograde SR was calculated using positive blood velocity, whereas negative blood velocity was used for obtaining retrograde SR.

Measurements of HR, BP and radial artery function assessment (L-FMC and FMD) were undertaken at baseline and immediately following the stabilisation period described above. L-FMC was calculated as the variation from average baseline diameter (1 min) to the last 30 s of wrist cuff occlusion period (5 min)(Gori et al., 2008). FMD was calculated as the variation in baseline diameter (1 min) to the maximal diameter of recovery period (3min) (Gori et al., 2008). Outcomes of L-FMC and FMD are presented as relative (%) and absolute (mm). Total vessel reactivity (TVR) was defined as the difference between the maximal diameter during the recovery period (3 min) and the average diameter of the last 30 s of wrist cuff occlusion period (5 min) divided by the average baseline diameter and presented as relative (%) (Rakobowchuk et al., 2012). Time to peak diameter and the shear rate area under the curve (SR_{auc}) was calculated from the recovery period (3 min) of radial artery function assessment, specifically from the point of cuff release to the point of maximum radial artery dilation. Simple ratio normalization of L-FMC response against the change in mean SR (the variation between baseline SR and SR during L-FMC measurement stage; L-FMC-to-Δ mean SR ratio, au) and ratio of FMD response against SR_{auc} (FMD-to-SR_{auc} ratio, au) were calculated (Padilla et al., 2008).

6.3.6 Statistical analysis

Baseline measures (Pre) were 1 minute averages taken after the 20 minute resting period but prior to any LBNP/LBPP stimulus. During intervention measures were 1 minute averages taken during the LBNP/LBPP stimulus had reached its target level and following a 10 minute stabilisation period.

Two-way repeated measures ANOVAs were used to investigate the main effect of time (Pre vs. During intervention), trial (LBNP and LBPP) and their interaction, on HR, systolic BP, diastolic BP, thoracic impedance, radial artery characteristics and function (L-FMC, FMD and TVR). Significant main effects and interactions were assessed *post hoc* using Students t-tests with Bonferroni adjustment. $P<0.05$ was determined as being statistically significant. Data presented as mean ± standard deviation unless stated otherwise.

The minimum sample size was determined retrospectively on the basis of a priori power analysis, considering radial artery baseline diameter (within-participant and within-day repeat measure) as the primary efficacy variable. The power analysis was conducted using the G* Power version 3.1 (Buchner, Erdfelder, Faul and Lang, 2014) with an alpha level of 0.05, power = 0.80, and an estimated medium effect size of d= 0.67. Data for calculating the effect size were obtained from a similar previous study and used the mean and standard deviation differences for the control and intervention conditions as well as the between condition correlation of 0.94 (Elliott et al., 2018). These calculations suggested the need for a minimum sample size of 20 participants.

6.4 Results

6.4.1 Hemodynamic characteristics

Hemodynamic characteristics Pre and During the LBNP and LBPP trials are presented in Table 6.1. HR, systolic BP, diastolic BP, MAP and impedance did not differ with time or between LBNP and LBPP trials (all, $P \geq 0.06$). There was a tendency for elevation in diastolic BP at Post in both LBNP and LBPP trials (Trial, $P<0.07$). Additionally, a tendency for elevation was also observed in MAP time main effects ($P<0.06$).

Table 6.1 Hemodynamic characteristics before [Pre] and [During] the LBNP and LBPP.

	LBNP		LBPP		P values		
	Pre	**During**	**Pre**	**During**	**Trial**	**Time**	**Interaction**
Heart rate (bpm)	58 ± 7	61 ± 8	58 ± 7	57 ± 8	**0.34**	**0.56**	**0.34**
Systolic BP (mmHg)	125 ± 7	125 ± 9	124 ± 6	129 ± 7	**0.52**	**0.33**	**0.25**
Diastolic BP (mmHg)	68± 8	73 ± 6	65 ± 7	68 ± 6	**0.07**	**0.09**	**0.57**
MAP (mmHg)	87 ± 7	90 ± 6	85 ± 5	88 ± 4	**0.18**	**0.06**	**0.98**
Impedance (W)	4 ± 0.5	4 ± 0.5	4 ± 1.0	4 ± 1.2	**0.97**	**0.78**	**0.64**

Values are means ± standard deviation. P values represent 2-way repeated ANOVA results (Trial; LBNP and LBPP: Time; Pre and Post: Interaction, Trial x Time).

6.4.2 Radial artery characteristics

Mean, anterograde and retrograde SR did not differ between LBNP and LBPP trials (Figure 6.1). However, there was a tendency (Time, $P<0.07$) for a reduction in anterograde SR and mean SR, and an increase in retrograde SR at Post in both LBNP and LBPP trials.

Radial artery diameter, blood flow, and velocity were not different between LBNP and LBPP trials ($P>0.47$, Table 6.2). However, radial artery velocity and blood flow were significantly decreased at During in both trials ($P< 0.05$).

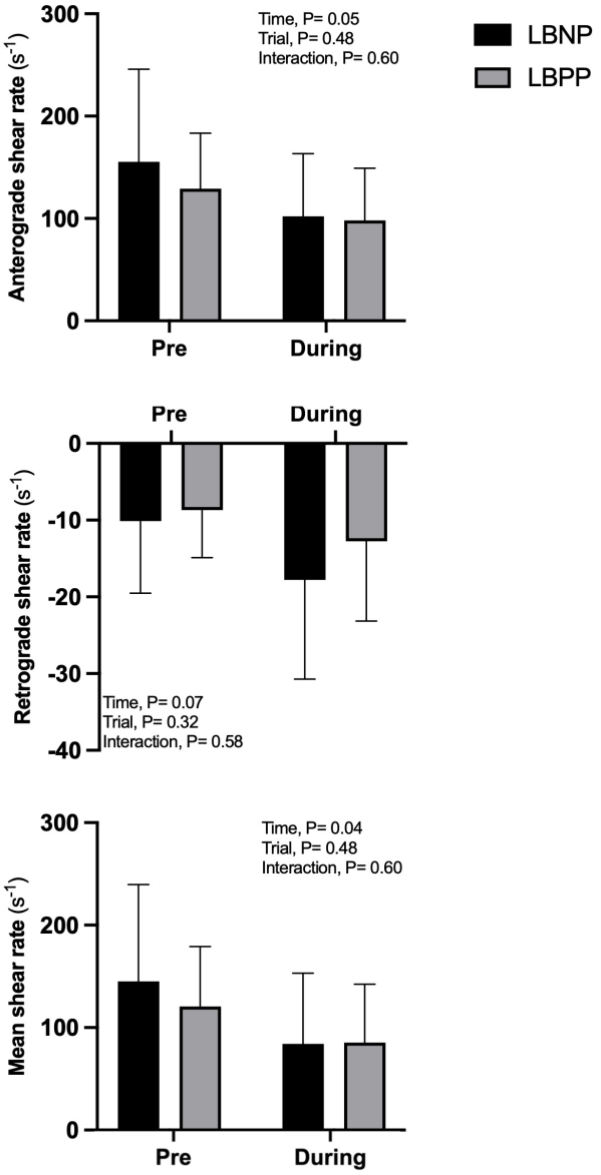

Figure 6.1 Anterograde, retrograde and mean shear rate Pre and During lower body negative pressure (LBNP) and lower body positive pressure (LBPP).

Values are the mean ± SE.

6.4.3 Radial artery function responses

Radial artery functional responses before and after LBNP and LBPP trials are presented in Table 6.2. FMD, L-FMC and TVR % were not different between trials ($P>0.79$, Figure 6.2). There was no change in the L-FMC % response across or between trials ($P>0.42$). The FMD % response decreased significantly following both trials (Time, $P<0.05$). There was a tendency (Time, $P=0.05$) for TVR % to decrease during both trials.

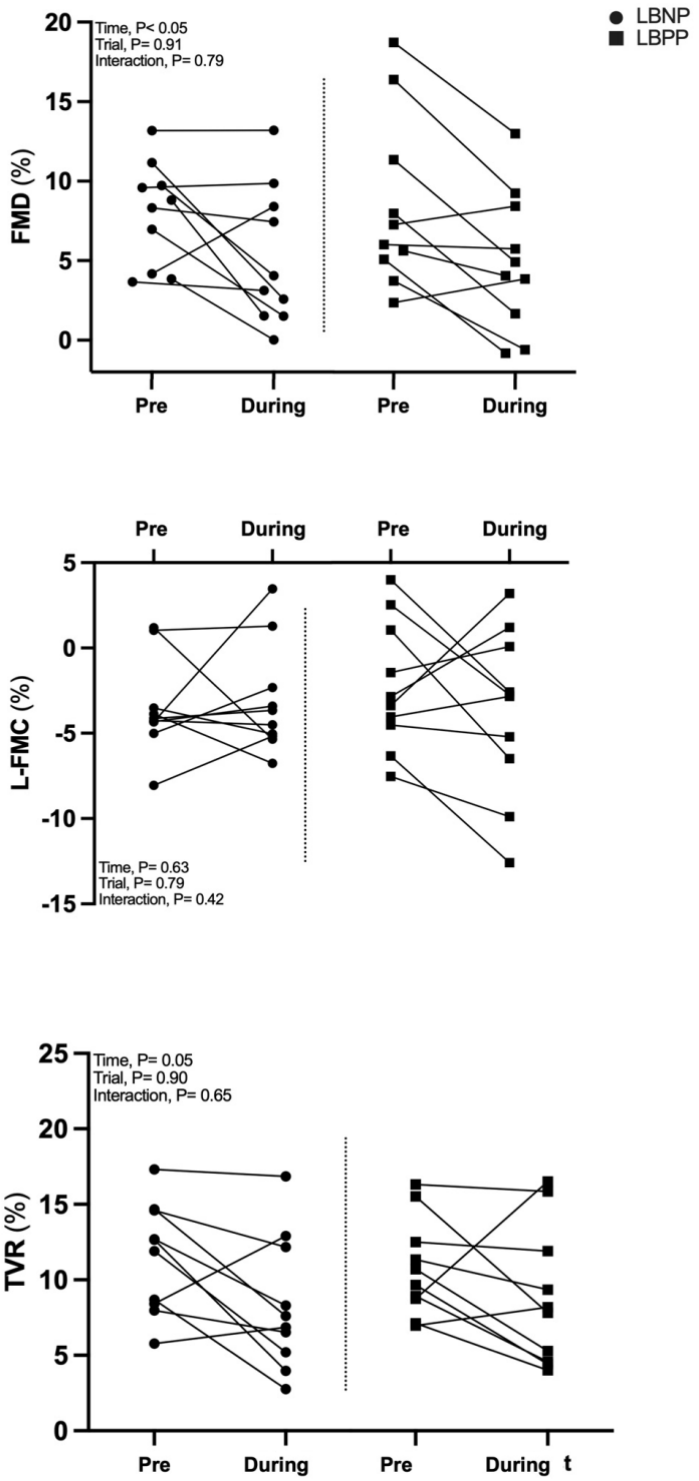

Figure 6.2 Radial artery flow mediated dilation (FMD), low-flow mediated constriction (L-FMC), and total vascular reactivity (TVR) Pre and During lower body negative pressure (LBNP) and lower body positive pressure (LBPP).

Table 6.2 Radial artery characteristics before [Pre] and [During] the LBNP and LBPP.

	LBNP (-20 mmHg)		LBPP (+10 mmHg)		P values		
	Pre	**During**	**Pre**	**During**	**Trial**	**Time**	**Interaction**
Baseline							
Diameter (mm)	2.57 ± 0.20	2.60 ± 0.27	2.50 ± 0.26	2.56 ± 0.26	**0.47**	**0.57**	**0.79**
Velocity (cm/s)	9.41 ± 6.25	5.25 ± 3.98	7.54 ± 3.70	5.42 ± 1.87	**0.56**	**0.04***	**0.48**
Blood flow (ml/min)	30.36 ± 22.24	15.73 ± 10.64	22.96 ± 13.20	16.89 ± 12.16	**0.52**	**0.04***	**0.38**
Mean shear rate (s^{-1})	145.25 ± 94.44	84.23 ± 68.91	120.51 ± 58.53	85.30 ± 25.26	**0.60**	**0.04***	**0.57**
L-FMC							
Nadir Diameter (mm)	2.48 ± 0.23	2.52 ± 0.26	2.44 ± 0.30	2.47 ± 0.34	**0.62**	**0.74**	**0.98**
Δ Diameter (mm)	-0.088 ± 0.071	-0.082 ± 0.083	-0.054 ± 0.088	-0.090 ± 0.122	**0.68**	**0.56**	**0.49**
Mean shear rate (s^{-1})	18.60 ± 16.50	24.41 ± 13.47	29.45 ± 13.94	30.78 ± 15.16	**0.03***	**0.69**	**0.51**
Δ Mean shear rate (s^{-1})	126.64 ± 86.11	59.82 ± 63.69	91.26 ± 51.36	54.52 ± 50.07	**0.22**	**0.03***	**0.66**
L-FMC-to- Δ mean SR ratio (au)	-0.039 ± 0.065	-0.295 ± 0.696	-0.051 ± 0.077	-0.090 ± 0.118	**0.50**	**0.20**	**0.64**
FMD							
Peak Diameter (mm)	2.78 ± 0.24	2.73 ± 0.28	2.70 ± 0.29	2.69 ± 0.32	**0.52**	**0.73**	**0.84**
Δ Diameter (mm)	0.20 ± 0.09	0.13 ± 0.11	0.21 ± 0.12	0.13 ± 0.11	**0.98**	**0.03***	**0.95**
Time to peak diameter (s)	70 ± 40	75 ± 37	64 ± 57	74 ± 63	**0.84**	**0.64**	**0.86**
SR_{AUC} ($x10^3$ s^{-1})	18.6 ± 4.4	26.5± 17.4	19 ± 7.3	22.6 ± 9.0	**0.63**	**0.10**	**0.51**
FMD-to-SR_{AUC} ratio (au)	0.45 ± 0.19	0.26± 0.23	0.52± 0.46	0.28 ± 0.28	**0.66**	**0.03***	**0.80**

Values are means ± standard deviation. L-FMC, low-flow mediated constriction; FMD, flow mediated dilatation; SR_{AUC}, shear rate area under curve. P values represent 2-way repeated ANOVA results (Trial; LBNP and LBPP: Time; Pre and During: Interaction, Trial x Time). * $P < 0.05$.

6.5 Discussion

The objective of the present study was to characterize the influence of acute sympathoexcitatory and sympathoinhibitory manoeuvres (e.g., LBNP and LBPP) on radial artery vascular characteristics and function (FMD and L-FMC). We observed that during -20 mmHg LBNP radial artery mean shear rate, blood flow and velocity were significantly decreased. Additionally, and as expected, FMD was attenuated during LBNP. However, radial artery L-FMC was not altered. Surprisingly, a reduction in radial artery characteristics (mean shear rate, blood flow and velocity) and FMD were also observed during LBPP (control), like LBNP, no alteration in radial artery L-FMC was observed during 10 mmHg LBPP. Collectively, these findings suggest that both LBNP (-20 mmHg) and LBPP (+10 mmHg) stimuli modified radial artery blood flow, shear rate and velocity, and attenuate FMD, but do not change radial artery L-FMC.

The application of LBNP is well recognized method to stimulate SNA. Specifically, LBNP at -20 mmHg has previously been shown to increase muscle SNA in the radial nerve (Rea and Wallin, 1989, Hijmering et al., 2002). Additionally, several studies noted that LBNP at -20 mmHg can successfully elevate SNA and still without causing changes in hemodynamics such as heart rate, BP, and cardiac output (Rea and Wallin, 1989, Hijmering et al., 2002), which matches our observation in the present study, however, this observation is not universal (Ligtenberg et al., 1997). In clinical settings, muscle SNA in patients with endogenous sympathetic outflow conditions (e.g., renal failure) was reported to be comparable to that in healthy adults at -20

mmHg LBNP (Ligtenberg et al., 1997, Ligtenberg et al., 1999). Based on the above, LBNP at -20 mmHg is assumed in our study as a valid model to induce SNA activation.

Several studies highlighted the interaction between SNA and endothelial function. Indeed, the chronic elevation in SNA is an indication of the prognosis of cardiovascular diseases and also can be linked to endothelial dysfunction. In our study we observed that endothelial function represented by FMD was attenuated during -20 mmHg LBNP (mean and P value). FMD impairment was also noted by several studies following acute SNA activation in healthy adults (Hijmering et al., 2002, Thijssen et al., 2014). Although, NO is the primary vasoactive substance responsible for FMD response, the release of NO at the microvascular level was not attenuated during acute SNA activation (Engelke et al., 1997). Others speculated that the impaired FMD following acute SNA activation does not represent dysfunction in endothelial but, maybe due to the reduction of shear rate stimulus and/or increased vasoconstrictor tone (Hijmering et al., 2002, Thijssen et al., 2014).

In the present study, we observed that radial artery characteristics (blood flow, and mean SR) and FMD were attenuated during the LBNP trial (-20 mmHg) (Time, $P<0.05$). In addition to this, we observed a tendency for retrograde SR elevation during LBNP at -20 mmHg (Time, $P=0.07$). The reduction in SR along with FMD response during LBNP has been reported earlier (Thijssen et al., 2014). In this study, FMD was assessed at baseline and immediately during either LBNP or LBNP with SR manipulation via a partial cuff inflation method. In both trials mean SR was attenuated, retrograde SR was evoked, and consequently, FMD attenuated (Thijssen et al., 2014). However, the reduction of FMD during LBNP was reported more with the addition of SR manipulation (LBNP + cuff trial). Accordingly, we can assume that the reduction of

mean SR and tendency of retrograde SR elevation in our study may be associated with attenuating FMD response during the LBNP (-20 mmHg) trial.

While the L-FMC assessment is developed on the basis of evaluating the vasoconstriction response during a period of ischemia (Gori et al., 2008). The SNA activation is known to modify the basal tone towards more vasoconstriction activity (Hijmering et al., 2002). Therefore, we hypothesized that acute SNA activation via LBNP at -20 mmHg would augment radial artery L-FMC, whereas it remains unchanged. This suggests no direct association between enhancing vasoconstrictor tone activity through SNA activation and L-FMC response. Currently, the mechanistic information on conduit arteries L-FMC not well understood. L-FMC is not altered by infusing NO synthase inhibitor (Gori et al., 2008). On the other hand, in vivo studies pharmacologically inhibiting endothelin receptor antagonists, attenuated L-FMC response in conduit artery (Gori et al., 2008). These data support that L-FMC in the radial artery is at least partially mediated by the endothelium. However, in healthy adults, acute changes in shear rate pattern in the radial artery are characterized by an elevation in retrograde shear rate (via leg cycling exercise) have been previously shown to augment the L-FMC response (Elliott et al., 2018). At the cellular level, an increase in retrograde shear rate was found to evoke endothelial vasoconstrictor substances (e.g., ET-1) (Ziegler et al., 1998). We observed in our study that LBNP at -20 mmHg significantly decreased mean shear rate and there was an elevation in retrograde shear rate, with no change in L-FMC response. Given this, such an increase in ET-1 bioavailability induced by retrograde SR elevation would expect to mediate greater L-FMC response. However, this was not observed in the present study and further studies to address the role of shear rate on L-FMC are needed.

In contrast to LBNP, LBPP is a recognized method to suppress SNA. In our study, LBPP was conducted to differentiate between enhancing (via LBNP) and suppressing SNA on L-FMC response. The mechanism of suppressing SNA during LBPP via shifting body fluid (blood) from the lower body to the thoracic region, which leads to more loading on the baroreceptor and consequently inhibits efferent sympathetic activity (reduction in SNA). No significant change in hemodynamic and impedance in our study was observed to determine the amount of fluid shifting during LBPP at +10 mmHg. An earlier study noted the inhibition of SNA at +10 mmHg achieved without observing changes in HR, systolic BP, diastolic BP, MAP and total peripheral resistance (Fu et al., 1998). However, in our study radial artery blood flow, velocity, and mean shear rate attenuated acutely during LBPP at +10 mmHg. Additionally, FMD was also reduced with no change found in L-FMC during LBPP at +10 mmHg. No known study explored the impact of LBPP on L-FMC response before. Accordingly, these data suggest that suppressing SNA induced by LBPP at +10 mmHg has no association with L-FMC and further studies with higher grades of positive pressure (e.g. +30 mmHg) during LBPP are needed to explore the conduit artery responses during such a stimulus.

6.5.1 Experimental considerations

In the present study, the radial artery was only used to assess vascular characteristics and function, and additional studies are required in other conduit arteries to verify these outcomes. Although findings of characteristics and function in radial artery were earlier found comparable to that in brachial artery (Thijssen et al.,

2008). L-FMC response is reported to be more commonly observed in radial than brachial artery in healthy adults (Weissgerber et al., 2010).

Radial artery functional assessment (FMD and L-FMC) was only measured at a single time point during LBNP and LBPP stimuli. Accordingly, we were unable to determine the association of time-course with vascular response during LBNP and LBPP intervention. Additional limitation, only healthy young men were examined. Due to the fact, sex differences variations and the effect of ovarian hormone on vascular function already reported (Hashimoto et al., 1995). Unfortunately, we were unable to recruit and assess young women at standard phase of their menstrual cycle for two sessions due to logistical and resource issues. However, additional investigations are needed to quantify whether sex-differences are present in our outcomes.

No measurement of sympathetic nervous system activation was included in the present study, and based on our findings and earlier studies outcomes, we can only assume that LBNP at -20 mmHg enhanced SNA and LBPP at +10 mmHg suppressed SNA.

The determination of the sample size in this study was obtained retrospectively upon power calculations employing estimates of medium effect size. Nevertheless, it is plausible that the observed effect size might have been smaller than expected, thus potentially leading to an underpowered study. Future investigations could gain benefits from employing larger sample sizes to enhance statistical power and improve the generalizability of the findings.

6.5.2 Conclusions

These findings confirm that LBNP acutely modified radial artery blood flow, velocity, SR, and attenuated vasodilation response. Acute FMD impairment during LBNP is suggested to be mediated by a reduction SR. However, such response in L-FMC was not noted. Collectively, these observations suggest that LBNP, which is known to modify SNA, attenuates radial artery FMD without causing changes in radial artery L-FMC.

CHAPTER 7: General Discussion and Future Directions

7.1 Overview

The research presented in this thesis aimed to examine the acute impact of different environmental stressors and shear stress on non-invasive markers of vascular function particularly L-FMC in healthy male individuals. More specifically, this thesis studied the acute influence of heat stress, leg cycling exercise and simulated hyper/hypotension (i.e., LBNP/LBPP) on radial artery L-FMC. While shear stress is an essential stimulus for normal endothelial function, this thesis work also tested, for the first time, the role of shear stress (e.g., shear pattern modification) along with the above-mentioned interventions on the L-FMC of the radial artery.

7.2 Major findings

The first experimental chapter (Chapter 4) sought to determine the influence of acute whole-body heat stress on radial artery characteristics and function (e.g., L-FMC) with and without change in local shear rate. To achieve this, L-FMC of the radial artery was assessed before and following 1 °C elevation of core temperature induced via temperature-regulated water at 48 °C being perfused through a tube line suit covering the whole body except for the measurement area. These trials were conducted both with and without partial wrist cuff inflation (75 mmHg) positioned distal to the radial artery to manipulate local SR. In eleven young healthy men, heat stress successfully augmented radial artery mean shear rate, blood flow, velocity and diameter. Such increases were prevented with the addition of partial wrist cuff inflation, but retrograde shear rate was noticeably increased. FMD was markedly attenuated following whole body heat stress, whereas there was no change in FMD when partial

cuff inflation was applied. However, radial artery L-FMC did not change following whole body heat stress in either cuff or no cuff trial. There are several interpretations for these findings: 1) during whole body heat stress along with partial cuff inflation (75 mmHg), increases in retrograde shear rate which is known to attenuate endothelial function has minimal influence on L-FMC; 2) suggesting that factors that are independent of endothelium may be responsible for preserving L-FMC response during both whole-body heat stress trials, such as SNA.

Heat stress is known to enhance SNA as well as other factors such as thermoregulatory and hormonal responses, all of which likely have some influence on L-FMC results. In addition, acute dynamic exercise has been shown to improve radial artery L-FMC, with SNA among the various mechanisms suggested to elicit such changes (Elliott et al., 2018). Accordingly, the second experimental study (Chapter 5) explored the acute impact of leg cycling exercise and local shear manipulation on the L-FMC of the radial artery. The aim was to determine the impact of acute exercise and the change in the pattern of blood flow on radial artery L-FMC in young healthy men. To address this, radial artery characteristics were monitored throughout all trials and L-FMC was measured before and acutely after 30 minutes of incremental leg cycling exercise (50, 100, 150 Watts) and trials were conducted with and without the application of partial cuff inflation (75 mmHg) placed distal to the radial artery measurement location to manipulate local shear pattern characterized by an increase in retrograde SR. In ten healthy active young male participants, an acute bout of leg cycling exercise increased radial artery anterograde and mean shear rate, velocity, blood flow and L-FMC %. Such increases in radial variables and L-FMC were prevented (and retrograde SR markedly augmented) by partial wrist cuff inflation (75 mmHg). These findings suggest that the reduction in radial artery L-FMC caused by a

local change in SR might be justified as a reduction in endothelial function to the attenuated anterograde, mean shear rate and elevated retrograde shear rate.

Lastly, the previous experimental chapters heat (Chapter 4) and exercise (Chapter 5) are interventions known to enhance SNA. However, other factors such as hormonal and endothelial cannot be discounted. Accordingly, Chapter 6 aimed to determine the influence of acute sympatho-excitatory and inhibitory manoeuvres on radial artery L-FMC in 10 healthy young men. To address this, the SNA activation conducted via lower body negative pressure (-20 mmHg) and lower body positive pressure (+10 mmHg) on radial artery characteristics and function (L-FMC) were investigated. Radial artery characteristics and L-FMC were measured before and during both interventions. Blood flow, velocity and mean shear rate of radial artery reduced duirng lower body negative pressure (-20 mmHg). Surprisingly, during lower body positive pressure (+10 mmHg) radial artery blood flow, velocity and mean shear rate were also reduced ($P<0.05$). Interestingly, no change in radial L-FMC was observed during either trial ($P>0.05$). The reduction in radial artery blood flow and mean shear rate duirng LBNP (-20 mmHg) may suggest the contribution of SNA. However, whether the lower body positive pressure suppresses SNA at +10 mmHg is unknown as the SNA was not measured. Nevertheless, findings of this experiment suggest that acute sympatho-excitatory (-20 mmHg) and -inhibitory (+10 mmHg) manoeuvres failed to alter radial artery L-FMC.

Collectively, these results demonstrate that changes in baseline diameter, blood flow pattern and differences in the magnitude of anterograde SR and retrograde SR during different environmental stimuli (mentioned above) modifies radial artery vasoconstrictor response (e.g., L-FMC) in young males. Thus, L-FMC is likely to be at

least partly attributable to a change in the pattern of local blood flow and/or SR, rather than an increased sympathetic vasoconstrictor tone.

7.3 Methodological considerations and limitations

In the present thesis, only the radial artery was studied. Even though various investigations have assessed the radial artery function (Dawson et al., 2012, Elliott et al., 2018), human studies of peripheral vascular function are more commonly assessed in the brachial artery. Notably, blood flow, diameter and SR patterns are expected to be similar and comparable in both the radial and brachial artery (Thijssen et al., 2008). However, the L-FMC response is noted to be more commonly observed in radial than brachial artery (Weissgerber et al., 2010). Collectively, whether our findings from the radial artery can be influenced and/or altered in a similar way in other conduit arteries is still unknown.

In the present thesis, only healthy young men were recruited in all experimental studies which is a limitation. Due to the fact, that there are important sex differences and ovarian hormone concentrations can affect vascular function responses (Hashimoto et al., 1995). This study investigated whether changes in endothelial function occurred throughout the menstrual cycle in healthy females. The study involved 17 males and 17 females, with females tested during their menstrual follicular and luteal phases. Their findings indicated that FMD levels in females during the early follicular phase were similar to those in males, but increased during the late follicular and luteal phases of the cycle. However, there has been little research on whether L-FMC is influenced by the menstrual cycle. Rakobowchuk et al. (2012) observed that L-FMC remained unchanged in eight healthy females (33±10 years) throughout the

menstrual cycle (early follicular vs. ovulation vs. mid-late luteal). However, in this study the limited sample size, the age range of female participants and the estimation of menstrual cycle phase (via counting days since menses) determines the effect of changes in ovarian sex hormones remains unclear. Moreover, limited research has evaluated potential sex differences in L-FMC. One pervious study found that radial artery L-FMC was lower in males than females with varying levels of coronary artery disease (Gori et al., 2012). Hence, further research is necessary to understand biological sex differences and ovarian sex hormones effects on LFMC. Unfortunately, due to the limited time frame in conducting each experimental study of the current thesis and lack of logical resources we were incapable of studying young women at a standard phase specifically the early follicular phase for several separate experimental sessions. Accordingly, the present results of this thesis cannot be generalized to women.

Another limitation is that radial artery function was only evaluated at a single time point acutely either during (Chapter 4 and 6) or following (Chapter 5) the intervention in all experimental studies. Additional to this, although radial artery function was acutely measured during (heat stress and LBNP) or following (leg cycling exercise) all experimental trials in each study, however, the time point of measuring radial artery function post-intervention was different between the presented experimental studies. Consequently, we were unable to determine the time course of the vascular response and specifically the L-FMC response acutely post-intervention in our experimental studies.

In the present thesis I did not specifically wait for the restoration of baseline diameter and SR to pre-intervention values before the second L-FMC and FMD assessment (post-intervention) was undertaken in all experimental chapters (Chapter

4. 5 and 6). Specifically, to capture L-FMC and FMD responses acutely post-intervention in Chapter 4 (heat stress) and 5 (exercise) was initiated before radial artery diameter and SR returned to pre-intervention state. Consequently, post-intervention reductions in FMD response may reflect a diminished dilator reserve (Dawson et al., 2013). Given the lack of change and the improvements in L-FMC outlined in Chapter 4 and 5 respectively, differences in baseline diameters pre- and post-intervention might be considered a negative bias.

In Chapter 5, leg cycling exercise protocol was conducted as absolute incremental workloads (50, 100 and 150W). Accordingly, some participants could have been exercising at higher intensity than the others.

It is a limitation that the SNA was not measured in our experimental studies as the role of SNA in the experimental studies and/or mediating vascular responses in the present thesis was made based on assumptions.

7.4 Directions for future research

From the present thesis findings and the limitations mentioned above (section 7.3), several gaps in the literature are evident which require future research, such as:

Further investigations in other conduit arteries are necessary to validate the current findings and provide a more comprehensive understanding of vascular responses to environmental stimuli. Specifically, future studies could focus on other peripheral arteries, such as the femoral or brachial artery. Integration of different arteries responses may provide insight into how vascular function differs depending on the location within the cardiovascular system and subsequently, enhance our understanding of vascular function throughout the vascular tree.

Additionally, investigating vascular responses and function (e.g., L-FMC and FMD) in different patient populations, such as those with cardiovascular disease or diabetes, may provide valuable information on how these conditions affect vascular health. Furthermore, incorporating other measures of vascular function, such as arterial stiffness, could provide a more complete picture of vascular health. Overall, additional research in this area will contribute to our understanding of vascular responses and function (e.g., L-FMC and FMD) and may lead to the development of novel diagnostic tools and therapeutic interventions for cardiovascular disease.

Due to the influence ovarian sex hormones have on endothelium function and the dynamic nature of these hormone across the menstrual cycle (and indeed between women), females were excluded for the sample population used in this thesis. It is recognised that this decision, made at the beginning of this PhD, limits the external validity of this thesis' findings and as such is a significant limitation (Stanhewicz and Wong, 2020). Thus, further research is warranted to examine the extent to which our findings can be extended to women and to better understand potential sex-specific differences in vascular function, especially L-FMC. Additional research on how changes in ovarian sex hormones (across the menstrual cycle, with menopause or with oral contraception) would also provide valuable insights into the underlying mechanisms of vascular control. Such studies can help advance our understanding of the differences in physiological responses between males and females and ultimately contribute to developing more personalized and effective treatments for both sexes.

Standardisation of exercise intensity by calculating the relative percentage of maximum is an important factor to minimise differences between participants cardiorespiratory fitness.

Further investigations of L-FMC response at different time points post-interventions are required to determine the variation and/or the ideal time course of L-FMC response.

The inclusion of an assessment of SNA or norepinephrine will provide a better understanding of the mechanism mediating L-FMC response.

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APPENDICES

Appendix A Consent Form

Participant Code:.....

Consent Form

Study Title

Low-flow mediated constriction: environmental modifiers and underlying mechanisms

The information given on this form is **confidential.**

Please put your initials in the boxes if answer is yes

I have read and understood the participant information leaflet. ☐

I have had an opportunity to discuss the study and ask any questions to members of the research team either on the telephone or during today's visit. ☐

I am satisfied with the explanations provided. ☐

I understand that I am able to withdraw from the study

- at any time, up to 2 weeks following the final experimental visit. ☐
- without need to give a reason. ☐

I agree to the storage of my contact details and data collected for the purposes of conducting the study. I understand that all information about me will be kept in a confidential way and destroyed after a period of ten years in line with the University of Birmingham Code of Conduct for Research. ☐

I agree to adhere to the the pre-study guidelines ☐

1

Consent form (version 2)

Participant Code:……

Participant

I declare that the information given on this form is true to the best of my knowledge. The details of this study have been explained to me and I agree to take part in the study titled:
Low-flow mediated constriction: environmental modifiers and underlying mechanisms

Signature …… **Date** ……

Name (print) ……

Research Staff

I confirm that I have explained the nature of the study to the participant in terms that they understand, outlining both risks and benefits. I confirm that they have given their consent freely to partake in this study.

Signature …… **Date** ……

Name ……

Appendix B General health questionnaire

GHQ (Version 3)

The University of Birmingham

School of Sport, Exercise and Rehabilitation Sciences

General Health Questionnaire

Name: .....
Address: .....
.....
.....
Phone: .....

Name of the responsible investigator for the study:

.....

Please answer the following questions. If you have any doubts or difficulty with the questions, please ask the investigator for guidance. These questions are to determine whether the proposed study is appropriate for you. Your answers will be kept strictly confidential.

Well it is therefore important to consider that the most

1.	You are.....	Male	Female
2.	What is your exact date of birth? Day..... Month.....Year..... So your age is..... years		
3.	When did you last see your doctor? In the: Last week..... Last month..... Last six months..... Year..... More than a year.....		
4.	Are you currently taking any medication?	YES	NO
5.	Has your doctor ever advised you not to take vigorous exercise?	YES	NO
6.	Has your doctor ever said you have "heart trouble"?	YES	NO
7.	Has your doctor ever said you have high blood pressure?	YES	NO
8.	Have you ever taken medication for blood pressure or your heart?	YES	NO
9.	Do you feel pain in your chest when you undertake physical activity?	YES	NO

GHQ (Version 3)

10.	In the last month have you had pains in your chest when not doing any physical activity?	YES	NO
11.	Has your doctor (or anyone else) said that you have a raised blood cholesterol?	YES	NO
12.	Have you had a cold or feverish illness in the last month?	YES	NO
13.	Do you ever lose balance because of dizziness, or do you ever lose consciousness (i.e., fainted)? If so, please provided details:	YES	NO
14.	a) Do you suffer from back pain b) if so, does it ever prevent you from exercising?	YES YES	NO NO
15.	Do you suffer from asthma?	YES	NO
16.	Do you have any joint or bone problems which may be made worse by exercise?	YES	NO
17.	Has your doctor ever said you have diabetes?	YES	NO
18.	Have you ever had any previous heat related illness (heat rash, heat stroke, cramps, syncope)?	YES	NO
19.	Have you ever had viral hepatitis?	YES	NO
20.	If you are female, to your knowledge, are you pregnant?	YES	NO
21.	Do you have kidney or gut dysfunction (e.g., abnormalities with oesophageal or bowel structures, fistulas, obstructive conditions involving gastro-intestinal tract, or inflammatory bowel disease)?	YES	NO
22.	Do you know of any reason, not mentioned above, why you should not exercise?	YES	NO
23.	Are you accustomed to vigorous exercise (an hour or so a week)?	YES	NO
24.	Do you have any difficulties swallowing tablets?	YES	NO

I have completed the questionnaire to the best of my knowledge and any questions I had have been answered to my full satisfaction.

Signed: .....

Date: .....

Appendix C International Physical Activity Questionnaire

Participant Code:.....

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

   _____ **days per week**

   ☐ No vigorous physical activities → ***Skip to question 3***

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

   _____ **hours per day**

   _____ **minutes per day**

   ☐ Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

   _____ **days per week**

   ☐ No moderate physical activities → ***Skip to question 5***

SHORT LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised August 2002.

Participant Code:.....

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ **days per week**

☐ No walking → ***Skip to question 7***

6. How much time did you usually spend **walking** on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

This is the end of the questionnaire, thank you for participating.

SHORT LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised August 2002.