The effect of exercise-induced shear stress on the endothelium in health and disease

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The candidate confirms that the work submitted is her own and that appropriate credit has been given where reference has been made to the work of others.

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Abstract

Cardiovascular disease is the leading cause of mortality worldwide, and one of the underlying conditions that causes this is atherosclerosis. Exercise could be a potential therapy in preventing or reversing atherosclerosis by increasing shear stress to areas susceptible to developing plaques. The aim of this thesis was to assess exercise-induced changes to shear stress patterns and magnitudes in arteries vulnerable to atherosclerosis and how that impacts the endothelial phenotype in vivo and in vitro. Using cardiac MRI and supine cycling exercise, tWSS in the thoracic aorta increased 10% in healthy participants and 15% in mitral regurgitation patients from rest. Shear stress was assessed by ultrasound in the common femoral artery of healthy participants during continuous and interval supine cycling exercise. Antegrade shear stress was higher in the interval protocol compared to the continuous ($P<0.05$), however there was no difference in FMD pre- to acutely post-exercise for either protocol ($P>0.05$). The in vivo shear stress from the common femoral artery for each exercise protocol was applied in vitro to HCAEC and the gene expression analysed by qRT-PCR. There was no difference in gene expression between each protocol however eNOS expression increased from 2-24 hours post-exercise ($P<0.05$), and MCP-1 and ICAM-1 both decreased from 2-24 hours post-exercise ($P<0.05$). The results of this thesis have shown that shear stress can be increased in areas of arteries that are susceptible to developing atherosclerosis and this provides a positive effect on endothelial phenotype by increasing anti-inflammatory gene expression, and decreasing inflammatory gene expression over time.

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List of Abbreviations

AoPP – aortic pulse pressure

ApoE – apolipoprotein E

AU – arbitrary units

AUC – area under the curve

aWSS – axial wall shear stress

BCA – brachiocephalic artery

BP – blood pressure

Ca^{2+} - calcium

CaM - calmodulin

CAV - caveolin

CCA – common carotid artery

cDNA – complementary deoxyribose nucleic acid

CFA – common femoral artery

cGMP – cyclic guanosine monophosphate

CHD – coronary heart disease

CMR – cardiac magnetic resonance imaging

CO – cardiac output

CO2 – carbon dioxide

CON - continuous

CPET – cardiopulmonary exercise test

CTn1 – cardiac troponin1

CVD – cardiovascular disease

CW – cardiac work

cWSS – circumferential wall shear stress

DBP – diastolic blood pressure

DNA – deoxyribose nucleic acid

ECG - electrocardiogram

ECM – extracellular matrix

eNOS – endothelial nitric oxide synthase

ETa – endothelin receptor a

FMD – flow mediated dilation

GPCR – G protein coupled receptor

GTN – glyceryl trinitrate

GTP – guanosine triphosphate

Hb - haemoglobin

HCAEC – human coronary artery endothelial cells

HDL – high density lipoprotein

HIIT – high intensity interval training

HPRT – hypoxanthine phosphoribosyltransferase

HR – heart rate

HRR – heart rate reserve

HUVEC – human umbilical vein endothelial cells

ICAM – intercellular adhesion molecule

IL - interleukin

INT - interval

IP3 – inositol trisphosphate

KLF2/4 – kruppel-like factor

LDL – low density lipoprotein

L-NMMA – N^G-Monomethyl-L-arginine

LSA – left subclavian artery

LT – lactate threshold

LV – left ventricle

LVEDV – left ventricular end diastolic volume

LVEF – left ventricular ejection fraction

LVESV – left ventricular end systolic volume

LVSV – left ventricular stroke volume

MAP – mean arterial pressure

MCP-1 – monocyte chemoattractant protein 1

MEJ – myoendothelial junction

MHC – major histocompatibility complex

MR – mitral valve regurgitation

MRI – magnetic resonance imaging

mRNA – messenger ribosenucleic acid

NFkB – nuclear factor kappa b

NHS – national health service

NO – nitric oxide

NOS – nitric oxide synthase

NYHA – New York heart association

OSI – oscillatory shear index

PAR-Q – physical activity readiness questionnaire

PBS – phosphate-buffered saline

PECAM – platelet endothelial cell adhesion molecule

PI3K – phosphatidylinositol 3-kinase

PIP – phosphatidylinositol trisphosphate

PKC – protein kinase c

qRT-PCR – quantitative reverse transcription polymerase chain reaction

RER – respiratory exchange ratio

RIT – ramp incremental test

RNA – ribose nucleic acid

ROS – reactive oxygen species

SBP – systolic blood pressure

SSRE – shear stress response element

T2D – type 2 diabetes

TFE – turbo field echo

TPR – total peripheral resistance

tWSS – total wall shear stress

VCAM – vascular cell adhesion molecule

VCO2 – carbon dioxide production

VE-cadherin – vascular endothelial cadherin

VEGFR – vascular endothelial growth factor receptor

VO2 – oxygen consumption

VSMC – vascular smooth muscle cell

WR – work rate

WSS – wall shear stress

Additional outcomes of this PhD

Oral presentations

Future Physiology 2017 – exercise induced wall shear stress dynamics in the aortic arch

North east postgraduate conference 2017 – the effect of exercise on wall shear stress in the aortic arch

MRC student conference 2018 – the effect of exercise upon wall shear stress in the aortic arch

MCRC 2018 – effect of exercise on wall shear stress in the aortic arch

MCRC 2019 – manipulating shear rate patterns in the common femoral artery using interval and continuous exercise

Postgraduate symposium 2019 – manipulating shear stress patterns in the common femoral artery using continuous and interval exercise

Poster presentations

Biomechanics in Vascular Biology and Cardiovascular Disease 2018 – the effect of exercise upon wall shear stress in the aortic arch

Biomechanics in Vascular Biology and Cardiovascular Disease 2019 – manipulating shear rate patterns in the common femoral artery using acute continuous and interval exercise

ACSM annual meeting 2019 – using continuous and interval exercise to manipulate shear stress patterns in the common femoral artery

Postgrad symposium 2018 – the effect of wall shear stress on the aortic arch

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Chapter 1 - Introduction

Cardiovascular disease (CVD) is the leading cause of mortality worldwide, responsible for a third of deaths in America (Roger et al. 2011), 25% of all deaths in the UK, and 31% of all deaths globally in 2015 which equates to 17.3 million people (BHF 2017). These figures include deaths by chronic rheumatic heart diseases; coronary heart disease; hypertensive diseases; strokes; diseases of the arteries, arterioles and capillaries; and diseases of the veins and lymphatic vessels. Of these 17.3 million deaths 7.3 million were from coronary heart disease (CHD) and 6.2 million were from cerebrovascular disease (strokes), for which one of the underlying diseases that cause these events is atherosclerosis. Furthermore, physical inactivity is the fourth leading risk factor for mortality, and is directly attributable to approximately 3.2 million deaths worldwide. Combined with other factors such as poor diet, a lack of physical activity contributes to the development of atherosclerosis and subsequently to other CVDs.

Physical inactivity is the fourth highest risk factor of all-cause mortality worldwide, across all demographics (WorldHealthOrganization 2009). Physical activity improves cardiovascular function and physical fitness and is a strong prognostic marker of mortality in populations both with and without CVD, when assessed by aerobic capacity. Myers et al. showed, in a study of 6213 men, peak exercise capacity (measured in metabolic equivalents [METs]) was a stronger prognostic predictor of an increased risk of death

than clinical variables or established CVD risk factors (Myers et al. 2002). This is indicative of a protective role of a higher exercise capacity even in the presence of other risk factors. Furthermore, Myers et al also reported that risk of death from any cause in participants whose exercise capacity was less than 5 METs was approximately double that of participants whose exercise capacity was more than 8 METs. Exercise capacity can be improved and every 1 MET increase in exercise capacity was associated with a 12% improvement in survival. This study showed the presence of a graded inverse relationship between exercise capacity and all-cause mortality, and highlighted the prognostic capabilities of exercise testing.

Physical inactivity is recognised as one of the major traditional risk factors for CVD. However, only 60% of traditional risk factors such as body mass index, hypertension, inflammatory/haemostatic biomarkers, lipids, and diabetes explain the inverse relationship between physical activity and CVD (Mora et al. 2007). The remaining 40% of unexplained incidences of CVD could be attributed to haemodynamic changes to the structure and function of the vasculature, and exercise-induced changes may contribute to cardiovascular benefits such as improving endothelial function (Green et al. 2017). Endothelial dysfunction is an early indication of CVD, particularly atherosclerosis.

Being active and having a high aerobic fitness can reduce the risk of developing atherosclerosis (Fernstrom et al. 2017). However, the modality (e.g. continuous, interval, or resistance training) and intensity (low, moderate or high) of exercise that would confer the greatest beneficial effects in both

healthy and patient populations is still unclear. Furthermore, it is not known whether what is beneficial to the healthy population is necessarily the same as those with cardiovascular disease. The relationship between exercise and the endothelium is best characterised by exercise-induced increases in blood flow and thus frictional forces along the endothelial layer. Understanding how these frictional forces or shear stresses change through exercise types and intensities underpins the understanding of how to provide the best treatment options to patients, and the best preventative measures to healthy individuals.

The aim of this thesis is to understand how shear stress patterns and magnitudes are altered with exercise in arteries susceptible to developing atherosclerosis.

Chapter 2 – Literature review

2.1 Blood vessel wall

2.1.1 Function and anatomy

Muscular arteries are formed of multiple layers, which give them their unique structure. Arteries are comprised of three distinct layers: tunica adventitia, tunica media, and tunica intima.

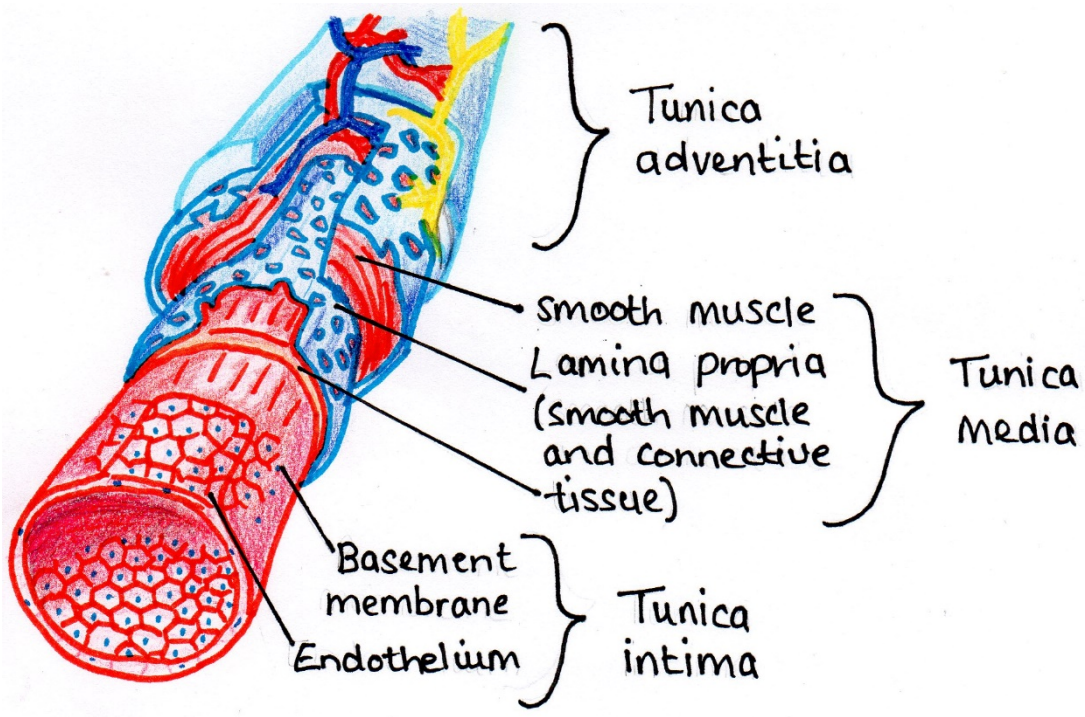


Figure 2. 1 Conduit artery structure
Conduit artery structure tunica adventitia, tunica media and tunica intima.

The tunica adventitia is a connective tissue covering containing the extracellular matrix (ECM), progenitor cells, and fibroblasts. It has no obviously defined boundaries and its primary function is to hold the artery in place. In some of the larger arteries the adventitia contains the *vasa vasorum*, which

helps to sustain the tunica media. The tunica media houses vascular smooth muscle cells (VSMCs), which in larger conduit arteries are built up in multiple layers, interspersed with rings of elastic lamellae. This layer supplies the contractile power and mechanical strength of the artery, it is highly compliant and responds to increases in pressure without rupturing. These layers of smooth muscle are innervated by the sympathetic neurons of the autonomic nervous system and contract in response to stimulation to vasoconstrict. In elastic arteries, the tunica media contains a higher proportion of elastic fibres than muscular arteries and help to propel blood forward during ventricle relaxation. As the ventricles contract these arteries stretch and store mechanical energy and when the ventricle relaxes the arteries recoil and the potential energy is converted into kinetic energy as the blood is pushed further along the vasculature. Examples of this type of artery are the aorta and common iliac artery. Finally, the tunica intima is formed of a single layer of endothelial cells (ECs), known as the endothelium, on top of an extracellular matrix that links and facilitates communication between the tunica intima and the tunica media. This cross talk facilitates contraction and relaxation of the artery in response to mechanical and chemical stimuli.

2.1.2 Endothelium

The endothelium is comprised of a monolayer of ECs that line the inside of blood and lymphatic vessels. Endothelial cells have synthetic and metabolic functions by secreting an array of cellular mediators can maintain homeostasis throughout the endothelial network. These include: matrix products (fibronectin, laminin, collgen); anti-thrombotic factors (heparin, antithrombin, prostacyclin); pro-coagulant factors (thromboplastin, von Willebrand factor,

platelet activating factor); growth factors (colony stimulating factor, insulin-like growth factor, transforming growth factor); vasodilating factors (nitric oxide, prostacyclin); vasoconstricting factors (endothelin, free radicals, angiotensin converting enzyme); lipid metabolism (LDL-receptor, lipoprotein lipase); and inflammatory mediators (IL-1,6,8, MHC II). In the vascular endothelium, the cells typically align in the direction of flow forming a protective barrier between the blood and the VSMCs that lie beneath the endothelium. Not only does the endothelium have a protective role, it maintains vascular integrity, and also serves as a primary contact for cell signalling between blood vessel layers for both chemical and mechanical stimuli. By releasing vasoactive substances such as nitric oxide (NO) the endothelium also plays a role in vascular tone in addition to; modulating immune responses, controlling angiogenesis and cell proliferation by producing growth factors, and the endothelium maintains an anti-thrombotic status by secreting anti-coagulant molecules.

In maintaining vascular tone there are two molecules of note that have powerful vasoactive properties but with opposing effects. These are nitric oxide, a vasodilator, and endothelin, a vasoconstrictor. Production of NO by endothelial cells keeps the vasculature vasodilated. The catalysing enzyme to produce NO, endothelial nitric oxide synthase (eNOS), is found in endothelial cells in its type II and type III forms, which are cytokine-inducible and endothelial constitutive, respectively. The opposing force of the vasodilator NO is the vasoconstrictor endothelin. There are three types of endothelin however the only one endogenous to endothelial cells is endothelin-1. By stimu-

lating ET_A receptors on VSMCs, endothelin-1 employs its vasoconstrictor effects on blood vessels. Endothelin-1 also stimulates cell proliferation and increases gene expression of genes such as platelet-derived growth factor and collagenase.

The basic anatomy of an endothelial cell is quite simple in nature, it consists of apical, basal, and junctional membranes. The apical membrane faces out towards the lumen of the vessel and comes into contact with the blood. On the surface of the apical membrane are a number of mechanosensors that respond to changes in blood flow. Examples of mechanoreceptors are GPCRs, caveolae, G proteins, the glycocalyx, tyrosine kinase receptors, the endothelial cytoskeleton, and ion channels. The junctional membranes is where the endothelial cells bind to each other via tight junctions. The presence of tight junctions creates a barrier that prevents the entry of certain molecules to the VSMCs underneath, like a ‘fence’, and protects the endothelial cell’s polarity. In some cases in endothelial cells the tight junctions act more like a ‘gate’ and selectively regulate the passage of water, macromolecules, and ions. Tight junctions are often a target for bacteria and viruses and can lead to diseases affecting the vascular system such as oedema. The basal membrane contains integrins and is in contact with the extracellular matrix. Integrins are heterodimeric alpha/beta chains that are involved in the cell-extracellular matrix interaction and have been shown to be capable of converting a mechanical stimulus into a biochemical signal. Under normal conditions, the extracellular matrix consists mostly of laminin, proteoglycans, nidogen, and collagen IV. When a tissue is wounded however, the proteins in the extracellular matrix include thrombospondin, fibrinogen, and fibronectin to aid migration and growth that promotes wound healing. Fibronectin

has also been observed in the extracellular matrix at atheroprone regions of arteries.

2.2 Shear stress

2.2.1 What is shear stress

Shear stress is the frictional force of the blood against the endothelium as it flows through the vasculature. Mathematically, shear stress is made up of two components: axial wall shear stress (aWSS), which is the forward and backward component of shear stress; and circumferential wall shear stress (cWSS) which is the pressure exerted outward on the vessel.

Blood behaves much like any liquid flowing through a cylindrical tube in that it acts like a series of thin concentric shells known as laminae that slide past each other during flow. Shear stress is caused by the tugging of each laminar layer upon the adjacent layers, which causes them to slide along the blood vessel and exert a force upon the vessel wall. Blood flow velocity and the viscosity of blood directly impact this force. As blood flow velocity increases shear stress also increases. There are a number of factors involved in blood viscosity including haematocrit, temperature, gender, and disease state. These factors need to be taken into consideration when calculating shear stress. Endothelial shear stress is calculated by:

(i) shear rate: shear rate = $\Delta v/\Delta d$ (v = velocity, d = diameter of lumen) and

(ii) endothelial shear stress = shear rate x viscosity of blood (figure 2.2) (Wentzel et al. 2012). The lamina are always in direct contact with the endothelium which enables ECs to sense any changes in shear as a result of altered blood flow velocity in the vessel.

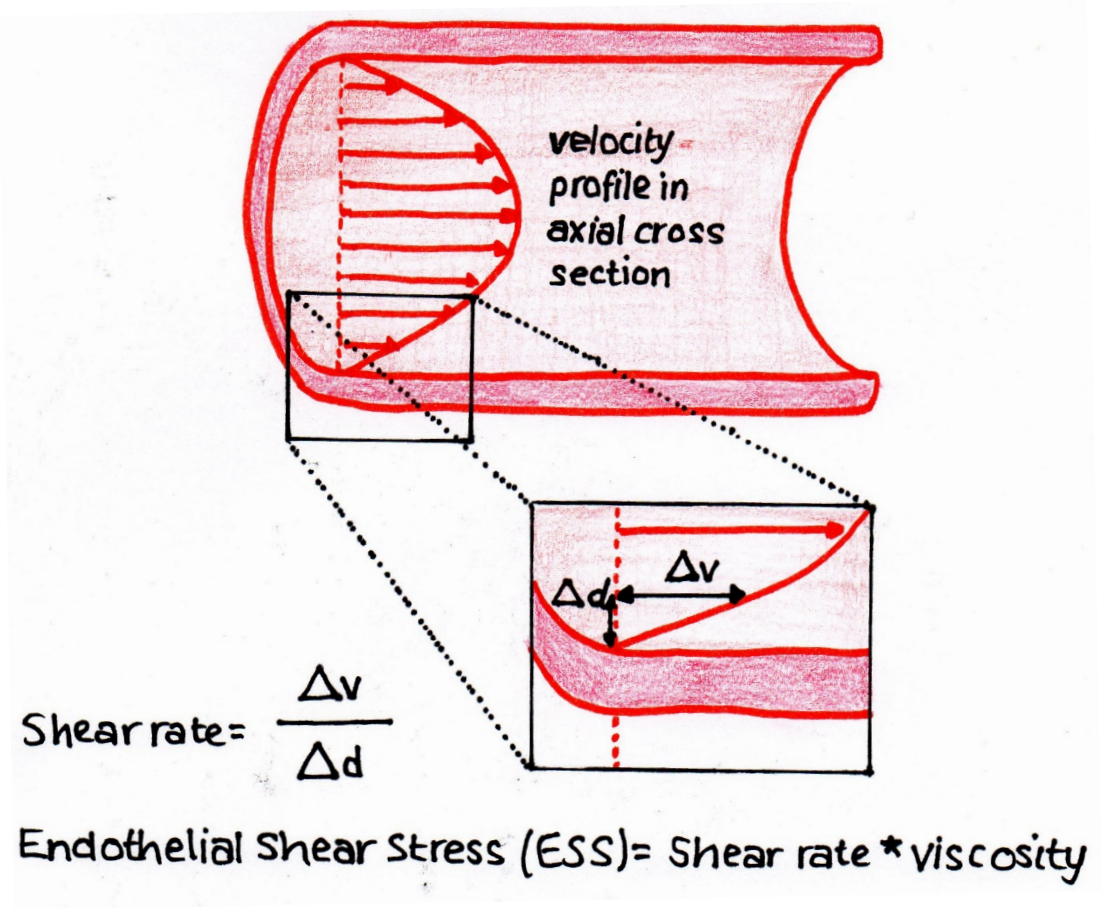


Figure 2. 2 Shear stress calculation

Shear stress exerted by the blood is determined by the shear rate (velocity gradient) multiplied by blood viscosity (Slager et al. 2005).

2.2.2 How the endothelium senses shear stress

Each endothelial cell plays host to mechanosensors on the apical, junctional and basal membranes and each generates cell signals responding to different stimuli. On the apical membrane there is the glycocalyx, caveolae, re-

ceptors such as Piezo1. On the junctional membrane is the mechanosensory complex that is comprised of VCAM, ICAM, and PECAM receptors. Along the basal membrane integrins sense changes in shear stress.

Mechanosensitive receptors respond to changes in shear stress and initiate signalling cascades to generate an appropriate response. On the apical membrane the mechanosensitive receptors include primary cilia, the glycocalyx, heterotrimeric G proteins, GPCRs, ion channels, caveolae, and Tie receptors (Hahn and Schwartz 2009). For example, under high shear stress with a laminar flow the G proteins on the apical membrane will initiate the signalling cascade that will generate NO and cause dilation of the artery. On the junctional membrane, the mechanosensory complex consisting of PECAM-1, VE-cadherin, VEGFR2 and VEGFR3 detect shear stress and consequently activate pathways that lead to the vasodilation or vasoconstriction of the blood vessel (Tzima et al. 2005; Coon et al. 2015). At the basal membrane, integrins detect shear and link the EC to the extracellular matrix.

2.2.3 Endothelial responses to shear stress

Shear stress itself can be presented in different forms that are associated with different EC phenotypes such as: high laminar shear stress which confers an anti-inflammatory and atheroprotective phenotype; and low shear stress with oscillations are associated with an inflammatory pro-atherogenic phenotype. High laminar shear stress is found in straight sections of the

vasculature, whereas low and oscillatory shear stress is located in areas of curvature and at bifurcations.

2.2.3.1 The anti-inflammatory response to shear stress

In the high laminar shear stress areas, ECs are elongated and aligned in the direction of flow with very few gaps between the cells, providing a robust barrier for the cell layers beneath. In a healthy individual, the ECs that make up the endothelium are aligned in the direction of flow so that no monocytes or macrophages can infiltrate between the endothelium and the VSMC layers. The healthy endothelium is also exposed to high laminar shear stress and can produce the nitric oxide (NO) required to vasodilate the blood vessel when required. Effective vasodilation and vasoconstriction are vital components of the healthy endothelium. One of the mechanisms in which blood vessels can vasodilate is through shear stress. In brief: mechanosensors on the apical membrane of the EC respond to high shear stress, this generates the production of NO which is the messenger signal sent to the VSMCs that ultimately causes the vessel to vasodilate. NO is the key signalling molecule when it comes to endothelial and vascular health and is often a key biological marker in *in vitro* experiments for showing a healthy endothelial cell.

2.2.3.2 The nitric oxide signalling cascade

The healthy endothelium has an anti-inflammatory phenotype with high levels of NO bioavailability characterised by large quantities of eNOS within the cell. The enzyme eNOS is one of the precursors to NO production. NO is important to arteries as it regulates vasodilation, as described above. If an

artery is unable to vasodilate this is an early indicator for CVD and atherosclerosis.

One of the most physiologically important pathways that governs the function of the endothelium in both health and disease is the NO pathway. Initiated by shear stress, this signalling cascade increases the bioavailability of NO in the EC and signals to the VSMC in order to dilate the artery. Activation of the membrane receptors stimulates phosphorylation of PI3K in the cytoplasm that catalyses the phosphorylation of PIP2 to PIP3, stimulating the phosphorylation of PKC and Akt. Simultaneously, PIP2 is hydrolysed to IP3, which increases the concentration of cellular Ca^{2+} and binds to CaM. Phosphorylated PKC and Akt in turn phosphorylate CAV bound eNOS, and eNOS respectively, to form eNOS/CaM complex and stimulate the production of NO. NO then transfers across to the VSMC via myoendothelial junctions (MEJs), which is thought to be facilitated by haemoglobin α (Hbα) (Straub et al. 2012). However, this has recently been challenged by Parikh *et al.* (2017) who used a mathematical model to investigate the NO-dependent signalling in artery walls and came to the conclusion that while Hbα is potentially a regulator of NO signalling, it is dependent upon having significant concentrations being co-localised with eNOS in the MEJ and whether this response is translatable, giving a physiologically relevant response *in vivo* (Parikh, Kapela, and Tsoukias 2017). In the VSMC, NO acts upon guanylyl cyclase and GTP, activating cGMP, which stimulates protein kinase G and dephosphorylates the myosin light chain causing the relaxation of the VSMC and the subsequent dilation of the artery. Any aberrant signal-

ling in this pathway, for example eNOS inhibition, would lead to a decrease in NO bioavailability and the subsequent build-up of reactive oxygen species (ROS) leading to inflammation of the EC and potentially cell death.

2.2.3.3 The pro-inflammatory response to shear stress

In areas of low and oscillatory shear stress, ECs are misshapen and do not have a uniform alignment. This is due to the disturbed flow in the regions of low shear stress, which do not allow the cells to align in any one direction as it is constantly changing. As a result of this, there are often numerous gaps between the cells which compromises the protective nature of the endothelium as monocytes and macrophages are then able to infiltrate between the endothelium and VSMC layers and can begin to form atheromatous plaques.

Endothelial dysfunction is one of the defining criteria for the assessment of atherosclerosis as it is an early indicator of atherosclerotic risk factors and one of the criteria in the definition of plaque vulnerability. The assessment of endothelial function (or dysfunction) is an option in the determination of thromobogenesis, risk associated plaque vulnerability, and combined risk factor impact on atherogenesis in humans. In conditions of low shear stress or oscillatory shear stress, a pro-inflammatory response is generated. Inflammation of the endothelium occurs by the activation and translocation of NFκB from the cytoplasm to the nucleus and transcribing inflammatory proteins such as IL-1, IL-6, VCAM and ICAM. Furthermore, the inflammatory phenotype inhibits the production of NO due to a lack of eNOS being activated. This, in turn, reduces the dilatory function of the artery as NO is the

critical signalling molecule from the EC to VSMC, which in a healthy endothelium induces dilation.

Low and disturbed flows appear to lead to the onset of atherosclerosis. Disturbed flow, inducing retrograde shear, has a pro-inflammatory characteristic as the shear is acting in the opposite direction to the alignment of ECs which triggers an inflammatory response, as described above (Hahn and Schwartz 2009). Alignment of cells in the endothelium is important as any misalignment creates gaps in the endothelium in which low density lipoproteins (LDLs), macrophages and monocytes could enter these gaps between ECs and flood the space between the endothelium and VSMC layer, and trigger the pathway for atherogenesis (Wang et al. 2013; Mohamied et al. 2015). Inflammation if unregulated can lead to the progression of the atherosclerosis pathway and formation of an atheroma. High, laminar and anterograde shear is associated with an anti-inflammatory phenotype in blood vessels whereas low, oscillatory and retrograde shear is associated with a pro-inflammatory phenotype (Hopkins 2013)

2.3 Shear stress and atherosclerosis

2.3.1 Development of atherosclerosis

The development of an atherosclerotic plaque occurs in very distinct stages: LDL accumulation in the intima; oxidation of LDLs by macrophages and smooth muscle cells; recruitment of circulating monocytes by cytokines and transmigration by binding to EC adhesion molecules; foam cell formation by the monocytes having contact with the LDLs; smooth muscle migration to

the intimal layer; smooth muscle proliferation, and the formation of a necrotic core.

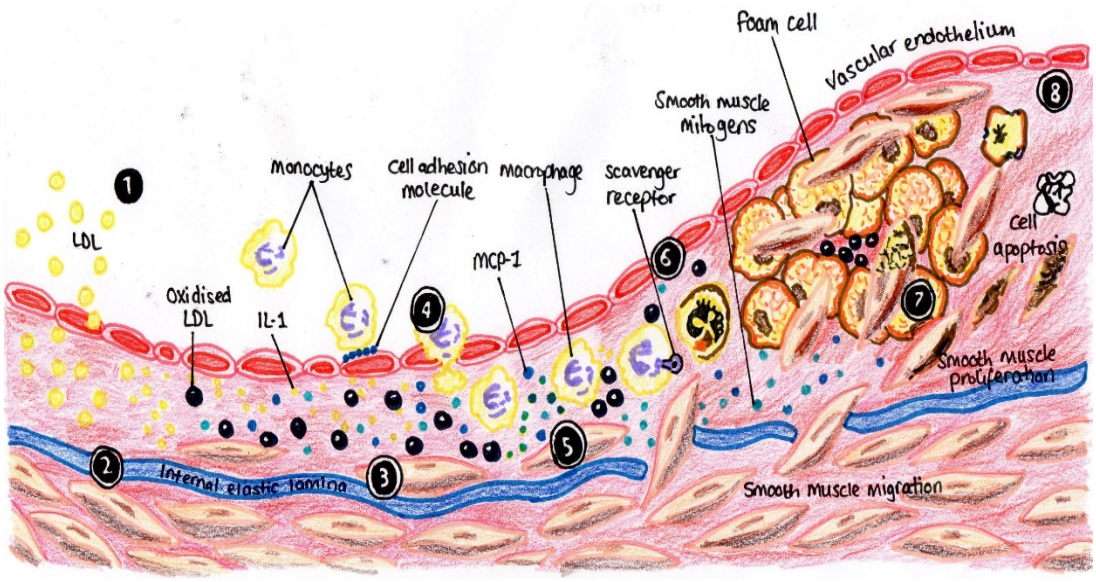


Figure 2. 3 The stages of atherosclerosis

The stages of atherosclerosis. LDLs are oxidised by macrophages and VSMCs turning macrophages into foam cells, growth factors and cytokines are released by endothelium and attract monocytes, foam cell accumulation and VSMC proliferation results in the formation and growth of a plaque (Faxon et al. 2004).

As discussed above, the continued presence of low and oscillatory shear means cells are not aligned and compromises the tight junctions connecting the endothelial cells together that allows for macromolecules to gain access beneath the endothelium. It was first shown back in the 1960s that it was these areas of low and oscillatory shear stress that atherosclerotic plaques were identified to be localised to areas that experienced flow separation which was at bifurcations, areas of curvature, and cross-sectional expansion (Spain 1966). It was Caro et al. (1969) who first revealed that atherosclerotic lesions were located upstream of flow separators in areas of low wall shear

stress (Caro, Fitz-Gerald, and Schroter 1969). To follow up on this original study, Caro and Nerem (1973) studied cholesterol uptake by perfusing the common carotid arteries of dogs and found that lipid uptake in arteries couldn't be correlated with fluid phase mass transport rates leading to the conclusion that blood flow on arterial walls influenced cholesterol transport (Caro and Nerem 1973). This was further corroborated by other groups, and Ku et al. also showed that low wall shear stress was not the only indicator of plaque development but there was a reversal of flow, which when assembled together creates the low oscillatory shear stress pattern that is commonly described today as disturbed flow (Ku et al. 1985).

Whilst the 60s brought about this initial surge of inspiration about atherosclerotic plaque development sites and the haemodynamics behind it, it wasn't until the 80s that the involvement of the endothelium began to be elucidated. Nerem (Nerem, Levesque, and Cornhill 1981) and Dewey (Dewey et al. 1981), in their respective groups both described how fluid flow directly affected the endothelium, which included discovering that endothelial cells align in the direction of flow. The shear stress renaissance of the 80s continued when both Frangos et al. (1985) and Grabowski et al. (1985) noticed that when shear stress was applied to endothelial cells, there was a sharp increase in prostacyclin production, an antithrombotic protein (Frangos et al. 1985) (Grabowski, Jaffe, and Weksler 1985). Then, into the 90s it was noted by both Shen et al. (1992) and Mo et al. (1991) in their respective groups, that shear stress stimulates the release of Ca^{2+} intracellularly and subsequently showed it acts as a signalling molecule (Mo, Eskin, and Schilling

1991; Shen et al. 1992). The final revelation that built the foundation of our current understanding of shear stress and atherosclerosis came from Kuchan et al. (1994) and Korenaga et al. (1994) who determined that shear stress was responsible for the continual release of NO from the endothelium (Kuchan, Jo, and Frangos 1994) (Kuchan and Frangos 1994) (Korenaga et al. 1994). This body of studies when assessed together gave a clear indication that the phenotype of endothelial cells is directly affected by shear stress.

One of the early stages of atherosclerosis development is an increase in the permeability of the endothelium. An initial application of shear stress to endothelial cells increases permeability but over time permeability decreases. This biphasic response of endothelial permeability to shear stress was as a result of an increase in endothelial NO production, which reduces permeability of the endothelium (Kurose et al. 1993) (Baldwin, Thurston, and al Naemi 1998) (Hillsley and Tarbell 2002). The sustained expression of NO and low permeability is characteristically seen in areas of the vasculature that are exposed to a relatively constant and regular pattern of laminar shear stress and as such, are protected against atherosclerotic plaque formation as LDL cholesterol is not able to penetrate the endothelial barrier. In areas that are prone to the development of atherosclerosis, endothelial permeability is increased because of degradation of the extracellular matrix, by matrix metalloproteinases, as shown by Son et al. (2013) in an atherosclerotic mouse model (Son et al. 2013). Not only does a degraded extracellular matrix lead to greater endothelial permeability, it also increases stiffening of the intimal

layer of the artery, which further increases the permeability of the endothelium (Huynh et al. 2011) .

2.3.2 Shear characteristics on plaques

Shear stress continues to play an important part in atherosclerosis not just at the early stages, but also throughout the progression of the disease. As the plaque develops, it begins to protrude into the lumen of the artery and begins to change the nature of the blood flow around the plaque site. Proximal to the plaque high shear stress is present and at the neck of the lesion. The highest shear stresses are found where the blood vessel is at its narrowest. Immediately distal to the plaque there is low and oscillatory shear stress, which in turn over time can lead to the spread of existing plaques or the formation of new plaques and increase the plaque burden. This is summarised in figure 2.4.

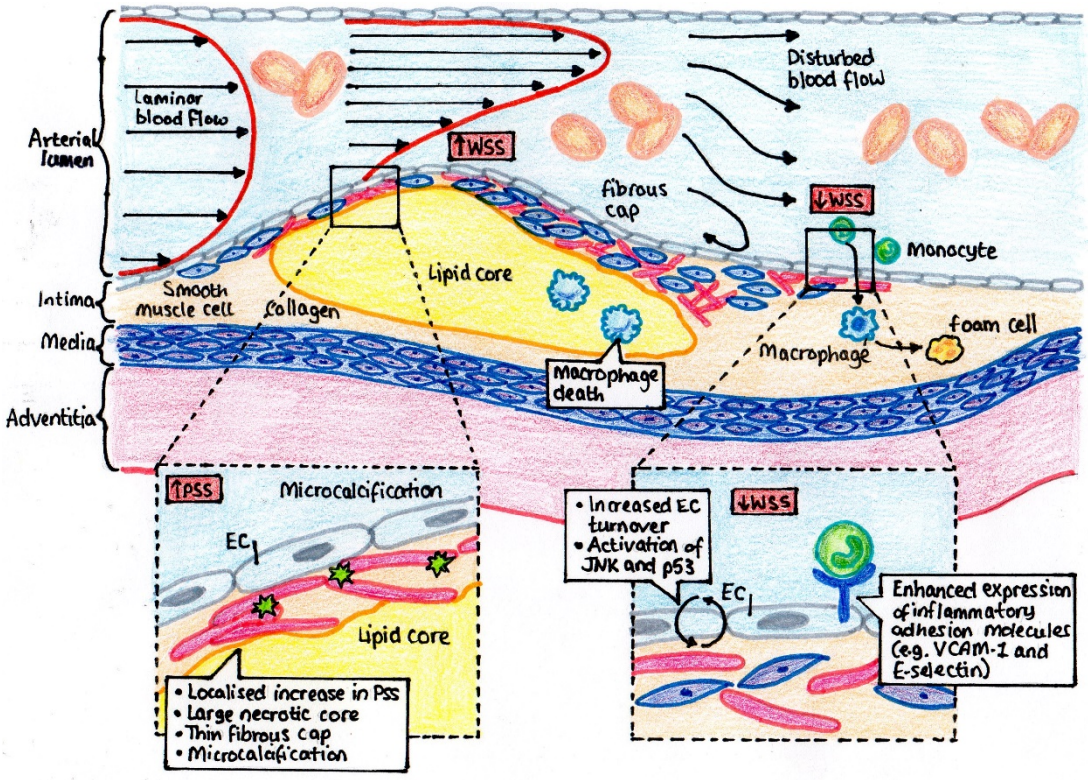


Figure 2. 4 Biomechanical forces in atherosclerosis

Biomechanical forces in atherosclerosis depicting the change in blood flow in the blood vessel in the presence of an atherosclerotic plaque and the subsequent effect on the surrounding endothelium. High WSS increase PSS before the plaque, low and oscillatory WSS enhances inflammation downstream of the plaque (Brown et al. 2016).

Research conducted by Costopoulos et al. (2019) has recently shown the relationship between plaque structural stress and wall shear stress on the progression or regression of atherosclerotic plaques. They determined that in areas of plaque progression high plaque structural stress was associated with larger increases in the necrotic core and smaller increases in fibrous tissue, which leads to the plaque having a more vulnerable phenotype (Costopoulos et al. 2019). The more vulnerable plaque phenotype was even observed in areas of regression where there were greater decreases in fibrous tissue than the necrotic core. Wall shear stress was associated with plaque growth. Low wall shear stress was associated with a larger increase in plaque burden and plaque area, as well as smaller decreases in areas of

regression than high wall shear stress. When these results from this study are taken together it has shown that in areas of plaque regression, lower wall shear stress elicited a smaller decrease in plaque burden suggesting that high wall shear stress may be more beneficial in reducing plaque burden.

Shear stress is also involved in plaque rupture. Whilst low and oscillatory shear stress makes plaques progress, it has been shown that the rupturing of thin-cap fibroatheromas (the most vulnerable plaques that are most prone to rupture) occurs most often in areas where wall shear stress is increased. A study by Richardson et al. (1989) indicated that most plaque ruptures took place on the lateral shoulder of plaques (63%) rather than in the midcap region (37%) however, a study conducted 10 years later by Burke et al. (1999) found that the ruptures from people who had died during exertion were located mostly at the midcap region, which was in contrast to people who had died at rest who exhibited plaque rupture mostly in the lateral cap shoulders (Richardson, Davies, and Born 1989) (Burke et al. 1999). Taken together these studies indicate that there may be different mechanisms in action at different locations on the plaque. Indeed it was suggested that repetitive cyclic tensile stress over a long period of time was responsible for lateral plaque shoulder rupture however, there has been no evidence provided for this mechanism to be correct. Furthermore, tensile stress that exists below the threshold for fracture stress may actually be responsible for strengthening the plaque as cyclic strain has been shown to cause smooth muscle cells to proliferate and stimulate extracellular matrix production. High shear stress

has been suggested to stimulate the endothelium to cause fibrous cap thinning, an effect that may be enhanced at the cap shoulders by the dual effect of high shear stress and cyclic strain that preserves the endothelium, and leads to the suppression of VSMC proliferation. This was confirmed in an in vivo study by Fukumoto et al. (2008) who showed, using 3D intravascular ultrasound, that a localised elevation in shear stress proximal to the plaque was related to plaque rupture in the coronary artery, also suggesting that this tool may be useful in predicting rupture sites (Fukumoto et al. 2008). These findings have been further corroborated by Gijsen et al. (2008) who also used intravascular ultrasound to show that distal to the plaque are areas of low shear stress and strain and that the plaque is more stable in these areas, whereas proximally is an area of high shear stress and high strain, which is suggestive of weaker wall material and may be an area more prone to rupture (Gijsen et al. 2008).

In terms of effective treatment there are few currently available. Stents could be used to stabilise plaques that are particularly vulnerable to correct differences in shear stress surrounding them. In practice, however, this is not necessarily a viable option as unstable plaques are often not limited to just one area and would require extensive invasive treatments to correct, which may not be beneficial to the patient. Endothelial function may be improved by pharmacological interventions but as discussed previously, this may do more harm than good and cause further weakening in already unstable plaques. Finally, exercise could potentially be an intervention for patients with atherosclerosis but this comes with a hefty health warning for patients

who have vulnerable plaques and are unused to physical activity in their day-to-day lives, and should be advised against strenuous exertion. Instead, a more gradual approach to increases in habitual activity has been suggested involving regular endurance exercise to gain the benefit of the cardioprotective effects of exercise, which lower blood pressure, increase HDL levels, and improve endothelial function (Leon 2005). This health warning however may only apply for those with severe atherosclerosis and highly unstable plaques, the effects of more strenuous exercise on early stages of atherosclerosis in terms of plaque stability, progression or regression, and the mechanisms behind these effects are not well understood and present the opportunity for further research.

2.4 Shear stress and exercise

2.4.1 How exercise changes shear stress

Shear stress can be altered in the arteries by exercise. The mechanism for this, on the surface, is relatively simple; exercise increases heart rate, which increases blood flow velocity, thus increasing shear stress. Specifically, in the active regions, blood flow and shear stress increase once exercise begins in order to meet an increase in metabolic demand (Thijssen, Steendijk, and Hopman 2009) (Green et al. 2005). Exercising the lower limbs engages a large volume of muscle, which increases cardiac output and blood pressure, is concomitant with a rise in blood flow as a consequence of resistance vessels downstream dilating alongside escalations in central driving pressure causing a change in blood flow not just to the artery supplying the work-

ing muscle, but systemically (Thijssen, Steendijk, and Hopman 2009). Not all exercises result in this level of change to the vasculature. In contrast, handgrip exercises only induces small changes in cardiac output and blood pressure however induces a large hyperaemic response, which is indicative that the resistance vessels downstream vasodilating is the greatest factor for the increase of blood flow during handgrip exercises (Green et al. 2005). What both of these types of exercise do show is that blood flow and shear stress both increase in active regions in an exercise intensity dependent manner. However whether there is any difference in shear stress for the same intensity but between continuous and interval exercise remains as yet unknown.

2.4.2 Shear stress and endothelial function in sedentary lifestyles

In an increasingly sedentary population, more research is being undertaken to understand the effect of shear stress on endothelial function for prolonged periods of rest, and whether exercise can alter these effects. Firstly, Trinity et al., (2014) assessed the effect of age and body position upon femoral artery shear rate and found that older participants had reduced mean shear rate in both the seated (young: 32±4 sec^{-1}; old: 17±3 sec^{-1}; $P<0.05$) and supine (young: 42±6 sec^{-1}; old: 18±3 sec^{-1}; $P<0.05$) positions compared to the young group, which was driven by an attenuation in anterograde shear stress (Trinity et al. 2014). Additionally, when NOS was inhibited in the young participants by an infusion of N^{G}-Monomethyl-L-arginine (L-NMMA), anterograde shear rate was reduced and the age-related differences in shear rate were abolished in both the supine (young: 341±36 sec^{-1}; old: 355±25 sec^{-1}; $P>0.05$) and seated (young: 274±27 sec^{-1}; old: 322±23; $P>0.05$) positions leading to the conclusion that older people have a lower

mean shear rate due to a reduced anterograde shear rate, which is caused by a diminished bioavailability of NO (Trinity et al. 2014). This reduction in shear rate seen with age is thought to be by large the reason that atherosclerosis propensity increases with age, particularly within the lower limbs (Ross et al. 1984) (Stary et al. 1995) (Kroger et al. 1999).

In a study assessing the effects of prolonged sitting Restaino et al., (2015) measured brachial and popliteal blood flow, and FMD, and showed that whilst shear rate decreased in the brachial (pre-sit: 324.3±33.5 s^{-1}; post-sit: 176.2±37.1 s^{-1}; $P<0.05$) and popliteal (pre-sit: 59.7±8 s^{-1}; post-sit: 28.1±3 s^{-1}; $P<0.05$) arteries, only popliteal FMD was blunted after 6 hours of sitting with a ~2.5x decrease in FMD% ($P<0.05$) (Restaino et al. 2015). Popliteal FMD was however restored after a short 10-minute walk to pre-sit values ($P<0.05$). This study adds to the body of evidence detailing the increased risk of lower limbs to atherosclerosis (Aboyans et al. 2011) (Li et al. 2014) and contributes with the knowledge that whilst shear rate and FMD are impaired with prolonged sitting, a short bout of exercise can return the artery back to its baseline state.

Lastly, in order to prevent endothelial dysfunction that occurs from prolonged sitting Morishima et al., (2017) assessed the effect of exercise prior to sitting upon endothelial dysfunction (Morishima et al. 2017). They found that in agreement with Restaino et al., prolonged sitting caused impairment to popliteal FMD (pre-sit: 4.0±0.6%; post-sit: 1.2±0.6%; $P<0.05$) but this was prevented by a bout of cycling exercise prior to the sitting period (pre-sit:

4.0±0.6%; post-sit: 3.5±0.6%; P>0.05) (Morishima et al. 2017). Standing was found to preserve endothelial function, as FMD did not alter pre- to post-intervention (pre-stand: 4.1±0.5%; post-stand: 4.2±05%; P>0.05) (Morishima et al. 2017). These three studies when taken together show that prolonged periods of sitting cause impairment in lower limb endothelial function and older people are more susceptible to lower shear rates than younger people in this position and thus could have a greater negative effect on endothelial function. As the exercise intervention from the Morishima study proved effective, this could mean that people who are in more sedentary jobs would benefit from exercising before starting work rather than after in order to maintain endothelial function.

2.4.3 Endothelial function in habitually active people

Shear stress is a driving factor for endothelial function and has been well studied both at rest and during exercise in a variety of interventions and different age ranges. Endothelial function, measured by FMD, is an assessment of cardiovascular health and is related to cardiovascular risk. It has been shown that physically active individuals have a greater FMD than their sedentary counterparts, and FMD declines with age in sedentary populations. DeSouza *et al.* showed that endurance-trained men do not have a reduced endothelium-dependent vasodilation in response to acetylcholine that is usually associated with age compared to the younger group because, at the highest dose of acetylcholine (16 ug/100 mL tissue per minute) forearm blood flow was nearly identical to that of the younger group (older: 17.3±1.3 mL/100 mL tissue per minute; younger: 17.7±1.4 mL/100 mL tissue per minute; P>0.05) (DeSouza et al. 2000). Furthermore, previously sedentary

middle-aged and older men that start to do regular aerobic exercise can recover the age-associated loss in endothelium-dependent vasodilation by an increase in forearm blood flow in response to acetylcholine (pre-training: 12.3±1.3 mL/100 mL tissue per minute; post-training: 15.9±1.5 mL tissue per minute; $P<0.01$), and these changes are not significantly different from the endurance trained older men and younger men ($P>0.05$) (DeSouza et al. 2000). This appears to be very promising in the improvement of endothelial function by aerobic exercise however, one limitation to this particular study is that it was only conducted in men, as women have a different physiology the effect may not be so pronounced. Franzoni et al., (2005) assessed the FMD in young athletes, young sedentary, older athletes, and older sedentary groups and found that FMD was significantly ($P<0.01$) lower in the older sedentary group (2.3%±1.0%) when compared to the other groups (older athletes: 5.3%±3.2%; sedentary young: 5.4%±2.0%; young athletes: 6.1%±3.2%), and the older athletes showed no significant difference in FMD compared to either young group ($P>0.05$) (Franzoni et al. 2005). These results suggest that regular exercise preserves endothelial function in older individuals, however only men were enrolled to participate in this particular study.

2.4.4 Exercise as an intervention to improve endothelial function

As it has been established that lifelong exercise can be protective against endothelial function, one question remains: can an exercise intervention improve endothelial function to become more protective? In acute exercise, Dawson et al., (2008) showed that after a marathon brachial FMD was preserved compared to before the event (~0% change in FMD; $P>0.05$) where-

as FMD in the superficial femoral artery was significantly lower than pre-event (~3% decrease; P=0.04), however there was no difference in shear rate, which suggests that the impaired FMD values of the superficial femoral artery were not due to any haemodynamic changes (Dawson et al. 2008). Additionally, cardiac troponin I (cTnI) measured by venous blood sampling was measured pre- and post-race and was detectable post-race in 12 out of the 13 runners. 7 of these were above 0.05 ug/L, which was the threshold for determining acute myocardial infarction at the time of publication, however with new assays developed the 99th percentile concentration (the threshold of acute myocardial infarction diagnosis) of troponin is decreasing as the assays become more sensitive (Tate 2008) so the number of runners with elevated troponin levels by today's standards may be increased. The post-race cTnI levels however, were not significantly correlated to left ventricular or femoral or brachial FMD.

In a training study, Black et al., (2009) found that at baseline older fit, older sedentary and younger fit men showed no significant difference (P>0.05) in brachial FMD whereas younger women had a greater brachial FMD than older sedentary women (P<0.05) but not older fit women (P>0.05) (Black et al. 2009). After a 24-week exercise intervention study exercise training improved endothelial function (FMD-to-GTN ratio) in older sedentary women (P<0.05) but this was not evident in men (P>0.05) suggesting that whilst men and women experience declines in endothelial function with age the effect is more pronounced in women (Celermajer et al. 1994) and implementing an exercise regime can help to alleviate this dysfunction, which is sup-

ported in the literature by other studies that have assessed cardiopulmonary fitness in relation to endothelial dysfunction (Rywik et al. 1999; Hagmar et al. 2006). In a similar study Pierce et al., (2011) conducted an 8-week exercise intervention in middle aged/older men and women and found, in contrast to Black et al., (2009) that brachial artery FMD significantly increased in middle aged/older men by more than 50% (pre-training: 4.6±0.6%; post-training: 7.1±0.6%; $P<0.01$) but showed no change in the postmenopausal women (pre-training: 5.1±0.8%; post-training: 5.4±0.7%; $P>0.05$) (Pierce et al. 2011). It is worth noting that the duration of this training study was 24 weeks long compared to the Pierce *et al.* study of eight weeks suggesting that a longer period of exercise training may be required in order to see significant improvements to vasodilation in males. However, it may also be the case that no significant improvements have been seen due to vascular remodelling that can happen as early as two weeks into a training programme, after which, the responses return back to baseline in subsequent weeks (Birk et al. 2012; Tinken et al. 2008). As neither study assessed brachial artery FMD at two weeks, or other regular intervals, they may well have missed the period of adaptation.

In another training study, Tinken et al., (2010) assessed the effect of shear stress induced by hand grip exercise on brachial artery FMD in a non-cuffed and cuffed arm every two weeks over an 8-week period. They found that brachial artery FMD was significantly higher at weeks 2, 4 and 6 compared to baseline ($P<0.05$) but at week 8 the FMD values returned close to baseline levels in the non-cuffed arm (Tinken et al. 2010). There were however

no changes to brachial artery FMD in the cuffed arm throughout the 8-week intervention (P>0.05), which reinforces the idea that endothelial function is only improved in the presence of an increase in shear stress. The cuffed arm limited blood flow to the limb thus reducing any changes to shear stress that were occurring as a result of the handgrip exercise. This seems to support the findings from Dawson et al., (2008) study of marathon runners acutely post-exercise, as there were no improvements to superficial femoral artery FMD due to a lack of increase in shear stress in the artery observed. Taken together, whilst these results have shown that there are clearly disagreements in the data as to whether habitual exercise is more beneficial in men or women, they have also shown that there are no negative effects on endothelial function in increasing physical activity. Furthermore, these studies are indicating that the driving force behind improvements in endothelial function is a result of increased shear stress.

2.4.5 The systemic effect of exercise upon shear stress and endothelial function

The effect of exercise upon endothelial function from mostly a prolonged intervention point of view has been discussed but what are the systemic effects of exercise to the non-working limbs? In 2006 Tanaka et al., studied the effects of blood flow and shear stress to non-working limbs (i.e. the arms are the non-working limbs during leg cycling exercise, and the legs are the non-working limbs during hand cycling exercise) during incremental exercise. This study showed that during leg exercise brachial blood flow increased 4-fold from rest to 100 W (rest: 19±6 mL/min; 100 W: 77±16 mL/min; P<0.05) and in arm exercise femoral blood flow increased 3.5-fold

from rest to 30 W (rest: 286±62 mL/min; 30 W: 982±252 mL/min; P<0.05) (Tanaka et al. 2006). Endothelial function by FMD was not assessed but what this study showed was that exercise does seem to have a systemic effect on calculated shear stress, which was measured as a function of blood flow and follows the same patterns of increase over the duration of exercise. A later study by Padilla et al., (2011) assessed the mechanics of how lower limb exercise affected the brachial artery diameter and shear rate, and found that at the onset of exercise brachial diameter decreased initially (-3.9±1.2% at 5 min; P<0.05) but increased over time during the remainder of the exercise bout (+15.1±1.6% at 60 min; P<0.05) (Padilla et al. 2011). This diameter change was accompanied by a 2.5-fold increase in mean shear rate from rest to the end of exercise, and was able to be replicated by forearm heating over the same time period (60 minutes) suggesting that in the absence of exercise, heating the limb of interest can reproduce the same vasodilatory response (P>0.05) (Padilla et al. 2011). This study provided insight into understanding how vasodilation is driven by shear rate however the effect upon endothelial function was not measured so it cannot from this study be determined if this was a positive effect. Birk et al., (2012) assessed brachial artery adaptation to lower limb exercise and were looking specifically at the role of shear stress as the driving force of arterial changes. During an 8-week cycling training study comparing brachial artery responses to shear stress in a non-cuffed and cuffed arm they measured FMD to an ischemic stimulus, an ischemic handgrip exercise stimulus, and administration of an endothelium-independent donor (GTN). They found that FMD increased in the non-cuffed arm at week 2 but returned to baseline levels over the rest of the 8-week study (0 weeks: 5.8±4.1; 2 weeks: 8.6±3.8; 4 weeks: 7.4±3.5; 8

weeks: 6.0±2.3; P=0.04) there were no changes in FMD in the cuffed arm (Birk et al. 2012). There were also no changes in response to either ischemic handgrip exercise or a dose of GTN in both the non-cuffed and cuffed arms of the 8-week study. This suggests that shear stress is at least partly responsible for the transient increases in upper limb vascular function and adaptation in healthy young humans and supports the existing evidence of endothelial adaptation in non-cuffed arms locally in handgrip exercise (Tinken et al. 2010) and forearm heating (Green et al. 2010) (Naylor et al. 2011). Finally, Lyall et al., (2019) assessed the acute effect of different types of interval (short interval, long interval, long interval 70) compared to continuous leg cycling exercise upon brachial artery shear rate during exercise and endothelial function immediately post exercise. They found that continuous exercise had a greater amount of total, mean and maximum anterograde shear rate (total: $14x10^5±4x10^5$ s^{-1}; mean: 1044±297 s^{-1}; maximum: 1892±408 s^{-1}) than long interval exercise (total: $10x10^5±3x10^5$ s^{-1}; mean: 803±251 s^{-1}; maximum: 1403±441 s^{-1}; $P<0.05$) and continuous mean and maximum anterograde shear rate was greater than short interval (mean: 859±265 s^{-1}; maximum: 1584±430 s^{-1}; $P<0.05$) (Lyall et al. 2019). Total, mean and maximum retrograde shear rate did not differ between the protocols ($P>0.05$). However, when FMD was assessed despite the differences in shear rate patterns, there was an increase in absolute (+0.14±0.01 mm; $P<0.05$) and relative (+3.36±0.48%; $P<0.001$) FMD acutely after pre- to post-exercise in all protocols in the brachial artery but no difference between protocols, which the authors postulate to be due to an increase in shear rate stimulus pre-to post-exercise, in agreement with existing literature. All of these studies taken together show that increases in shear stress can be

identified systemically to the non-working limbs, and that these shear stress changes do not have a negative effect on endothelial function and that it may even be a positive change in endothelial function.

2.4.6 The impact of continuous and interval exercise on shear stress and endothelial function in cardiovascular disease patients

Regular aerobic exercise has been known to reduce morbidity and mortality in both the healthy general population as well as in patients diagnosed with coronary artery disease, and has been strongly evidenced by studies dating back from as early as the 1980s (Leon 1987) (Ekelund et al. 1988). Interval training was first described in the scientific literature by Reindell and Roskamm (1959), and has been adopted by athletes from many sports as a staple training style. Although the 1959 paper was the first recorded entry into a scientific journal, athletes and trainers had used interval training since the 1910s, but no one had published these training programmes in journals. Interval training is a particularly attractive style of exercise as it is time effective and improvements to aerobic fitness can be seen in as few as two weeks (Burgomaster et al. 2005). The study by Burgomaster et al. (2005) examined the effect of 6 session of sprint interval training over two weeks on cycle endurance capacity and showed 100% increase from pre- to post-intervention (pre: 26±5 min; post: 51±11 min; $P<0.05$) Whilst interval training has been used among athletes for around a century, the application of this training style to disease populations as a potential therapy is a relatively new concept.

As heart rate fluctuates with work rate, it would follow that shear rate should follow a similar pattern in that there would be greater levels of both antero-grade and retrograde shear during the work intervals and lower levels of shear during the rest. However, until recently it was not known what, if any, benefits there were to the endothelium from interval training and if it could be utilised in a pathological population. Francois *et al.* (2016) investigated the effect of resistance- and cardio-interval exercise on patients with type 2 dia-betes (T2D) as well as trained and untrained normoglycaemic participants. The cardio-based interval exercise took place on a cycle ergometer and was a 1:1 ratio of 1-minute intervals at 85% Wpeak followed by 1-minute recov-ery at 15% Wpeak. The resistance-based interval exercise was a circuit of seven 1-minute intervals of leg resistance exercise interspersed with 1-minute recovery. For the T2D patients FMD was higher immediately post-exercise for the resistance group compared to the control group (control: 5.1±1.6; resistance: 8.6±5.8; P=0.03) and shear rate was significantly higher in cardio-interval and resistance exercise groups compared to control (P>0.05) (Francois et al. 2016). This study shows that both resistance-interval exercise and cardio-interval exercise are effective in improving endo-thelial function and in contrast to existing literature did not show an impair-ment immediately post-exercise (Johnson, Padilla, and Wallace 2012; Dawson et al. 2013; Atkinson et al. 2015) suggesting that interval training, whether by resistance or cardio-intervals, is beneficial to patients with T2D.

A number of other studies in various groups of patients have been carried out to compare the effectiveness of high-intensity interval training (HIIT) ver-

sus continuous training. In a continuous vs interval study of type 2 diabetics, after a 12-week intervention FMD increased for both continuous (pre: 4.8±1.6%; post: 6.1±1.8%; $P<0.05$) and interval groups (pre: 5.4±1.1%; post: 7.4±0.9%; $P<0.05$) with the interval group having a greater improvement in FMD than the continuous group ($P<0.05$), again showing that interval exercise is effective in improving the endothelial function of patients with T2D (Mitranun et al. 2014). Hypertensive patients were enrolled into a 12-week training study comparing continuous and interval exercise (Molmen-Hansen et al. 2012). At the end of the intervention it was found that endothelial function as measured by FMD was only improved in the interval group (pre: 6.49±3.71%; post: 10.66±5%; $P<0.01$) and not the continuous (pre: 6.5±5.01%; post: 7.11±5.1%; $P>0.05$), which adds to the pre-existing knowledge that regular exercise improves the endothelial function of hypertensive patients (Higashi et al. 1999), but contradicts that study in showing no improvement in the continuous exercise group. Heart failure patients underwent a 12-week training study and were assigned to either a continuous exercise or interval exercise intervention group (Wisloff et al. 2007). This study showed that whilst both continuous and interval training showed a significant improvement in endothelial function compared to the control group post-training ($P<0.01$), the interval exercise group also had a significantly higher FMD post-training than the continuous exercise group ($P<0.01$). Furthermore, the interval exercise group showed a greater improvement in exercise capacity than the continuous exercise group following the intervention (46% vs 14%, respectively; $P<0.001$). All of these studies showed that interval training was more effective than continuous training for improving FMD in

patient populations presenting with different CVD pathologies, indicating that interval exercise is safe and effective in improving endothelial function.

Further to improvements in endothelial function, exercise training also improves antioxidant status, which is a significant predictor of FMD, as well as the availability of NO (Tjonna et al. 2008; Franzoni et al. 2005). A study by Franzoni et al. (2005) showed that lifelong exercise preserves total oxyradical scavenging capacity in plasma against peroxyl radicals and hydroxyl radicals compared to an older sedentary population ($P<0.001$) and are at levels comparable to that of a young sedentary group ($P>0.05$). Furthermore, plasma malondialdehyde was elevated in the older sedentary group compared to older atheletes, young athletes and young sedentary ($P<0.001$) showing that antioxidant capacity is diminished in with sedentary aging. In a study by Tjonna et al. (2008) that assessed endothelial function and the bioavailability of NO in healthy participants in continuous or interval exercise, they found that after a 16-week exercise intervention, FMD increased by 9% in the interval group ($P<0.001$) and 5% in the continuous group ($P<0.001$) compared to baseline with the interval exercise having a greater effect than the continuous ($P<0.001$). Additionally, they observed that the availability of plasma NO increased in the interval group from baseline (36±3%; $P<0.05$) but not in the continuous group ($P>0.05$) and a significant difference between the two groups ($P>0.05$). Furthermore oxidised LDL, which negatively regulates NO bioavailability was reduced by 17% ($P<0.001$) in the interval group but not reduced in the continuous group. These data indicate that exercise preserves antioxidant capacity with age, improves bioavailability of

NO, and reduces oxidised LDL all of which are contributors to atherosclerosis development.

What still remains unknown is what the best form of exercise is for those patients with pre-existing atheromatous plaques. As plaques protrude directly into the lumen of the vessel, they become susceptible to higher shear rates at the neck of the plaque as the lumen space becomes reduced particularly in curved vessels and at bifurcations. Plaques can become unstable when the neck is exposed to high shear stresses and this may lead to plaque ruptures and obstruction of arteries further downstream and cause a myocardial infarction (Slager et al. 2005). A recent study has shown that lifelong endurance masters athletes are more likely to have a high coronary artery calcium score >300 AU or plaques in the coronary artery in comparison to sedentary males with a similar risk profile (Merghani et al. 2017). However, as pointed out by the authors, due to the stability in nature of the plaques in the masters athletes this could reduce the risk of myocardial infarction as a result of a plaque rupturing.

Current guidelines use continuous endurance training as the preferred type of exercise by several professional societies including, the American College of Sports Medicine, the American Heart Association (minimum 3 days/week, 20-60 minute session, including 10-15 minute warm-up and cool-down at 40-80% heart rate reserve), and the European Union (moderate to vigorous exercise 3-5 days/week for 30-60 minutes/day to total a minimum of 150 minutes/week), (Haskell et al. 2007; Perk et al. 2012). Due to the simplicity

of continuous endurance training, anyone who has the physical capability to perform exercise should able to carry out continuous endurance training in some form with few instructions needed (Adams et al. 2017). However, if interval training is proven more effective in treating patients with or at risk of cardiovascular disease then a policy change may be required in order to have the greatest benefit to the general population.

2.4.7 Summary

The literature has illustrated that exercise has a positive effect of increasing shear stress and endothelial function whether that is by exercise intervention or acute exercise and the changes can be seen systemically and through locally in relation to the exercising limb. However, the vast majority of these studies have conducted their shear stress and FMD analysis in the brachial artery. The brachial artery has been used, as it is easier to access than many arteries and does not require any invasive treatments in order to detect changes in shear stress and endothelial function. On the downside it is a straight artery with no curves or bifurcations and is not susceptible to atherosclerosis development. In order to create a complete understanding on the impact of exercise on shear stress and endothelial function, arteries susceptible to plaque development need to be studied for example, the common femoral artery and the thoracic aorta. This thesis aims to understand the effect of continuous and interval exercise upon shear stress and endothelial function in arteries susceptible to atherosclerosis in vivo and apply this to an in vitro model to determine the mechanisms by which exercise effects the endothelium.

2.5 Hypothesis and aims

2.5.1 Hypothesis

Exercise-induced changes in shear stress lead to an increase in shear stress in atherosusceptible regions of arteries, and improve endothelial function at a cellular level.

2.5.2 Aims

i) assess the shear stress patterns and magnitudes of the thoracic aorta in response to supine cycling exercise in healthy participants and patients with severe mitral valve regurgitation.

ii) determine whether high-intensity interval or continuous exercise gives a greater shear stress response, changes the patterns of shear stress, and confers a positive change in endothelial function in the common femoral artery.

iii) measure gene expression over time in human coronary artery endothelial cells in response to shear stress patterns and magnitudes acquired from the common femoral artery during exercise.

Chapter 3 – the effect of exercise intensity on aortic shear stress patterns in healthy participants and patients with severe mitral valve regurgitation

3.1 Introduction

Atherosclerosis is a disease that is prevalent in the curvature and bifurcations of blood vessels. One such blood vessel that is susceptible to atherosclerotic plaques is the aorta, as it has one large curve as well as three bifurcations to the brachiocephalic artery (BCA), left common carotid artery (CCA), and left subclavian artery (LSA). The main site of plaque formation is the inner curvature of the aortic arch; this is due to the low and oscillatory shear stress in this area contributing to the formation of atherosclerotic plaques. Presence of atherosclerotic plaques in the aortic arch is strongly associated with strokes and peripheral emboli; therefore it is a site of significant interest in not only atherosclerosis but in other vascular diseases (Tunick, Perez, and Kronzon 1991) (Amarenco et al. 1994) (Jones and Donnan 1995). What is as yet unknown is whether exercise can increase the shear stress in this low shear region to a level that could ameliorate the progression of these plaques.

Furthermore, it has not yet been uncovered as to whether having a pathology upstream of the aorta, in terms of blood flow, can have an effect on aortic shear stress compared to a healthy population, and if exercise has a further effect on aortic shear stress. For this question patients with severe mitral re-

gurgitation (MR) were recruited to explore this possibility. MR is the second most common valvular disease in Europe and is often characterised by the degeneration of the valve leading to prolapse of either one or both of the leaflets. Furthermore, patients are often diagnosed by an increase in chamber sizes, increase in systolic pulmonary pressure, atrial fibrillation, and left ventricular impairment (O'Rourke and Crawford 1984) (Gaasch and Meyer 2018). What is particularly interesting is blood flow patterns in the left ventricle of MR patients are much different to those in a healthy individual. In a healthy left ventricle, the main path of the blood flows down towards the apex of the ventricle then back up to the aorta, with a smaller vortex swirling in the opposite direction underneath the mitral valve (Al-Wakeel et al. 2015). However, in MR patients, blood flow into the left ventricle is more distorted, as there are multiple vortices present in the chamber, with no smooth path of blood flow towards the aorta as can be seen in healthy individuals (Al-Wakeel et al. 2015). This may be due to the characteristics of the blood flow jet from the left atrium into the left ventricle. In MR patients the jet is hemispheric in shape and a bigger area typically means worse MR. What remains to be understood is whether the disturbed flow in the left ventricle is conserved into the aorta.

The aims of this study were to i) determine shear stress patterns along the aorta when transitioning from rest to exercise in a healthy population, ii) determine shear stress patterns along the aorta when transitioning from rest to exercise in MR patients, and iii) compare these shear stress patterns between the two groups.

3.2 Methods

3.2.1 Participants

Both healthy participants and patients diagnosed with severe mitral valve regurgitation were recruited for this study, but were recruited via separate mechanisms as detailed below.

3.2.1.1 Healthy participants

A total of 10 healthy young participants free of any history of cardiovascular disease, respiratory disease, or current musculoskeletal injuries were recruited for this study. Each was given a participant information sheet to read before commencement of the study and gave their written informed consent. Participants were told that they were free to withdraw from the study at any time without reason. Ethics was granted from the University of Leeds Faculty of Biological Sciences ethics committee in accordance with the Declaration of Helsinki (BIOSCI 14-005).

3.2.1.2 MR patients

12 patients with moderate-severe mitral valve regurgitation as diagnosed by an echocardiogram were recruited for this study. Patients were included on the basis that they had a degenerative mitral valve, NYHA class I, a LVEF >55 %, and a previous cardiopulmonary exercise test (CPET) that had been undertaken within the NHS. Each was given a participant information sheet to read before commencement of the study and gave their written informed consent. Participants were told that they were free to withdraw from the

study at any time without reason. Ethics was granted from a local NHS ethics committee (Yorkshire & The Humber-Leeds West 12/YH/0551) in accordance with the Declaration of Helsinki.

3.2.3 Experimental protocol

All participants attended the Leeds General Infirmary to participate in supine cycling exercise within the MRI. Participants exercised at a work rate equivalent to HRs associated with light and moderate intensity exercise. These HRs were determined from the previously conducted CPET for patients and RIT for healthy participants.

3.2.4 Determination of exercise work rate

3.2.4.1 Healthy participants – ramp incremental test

Healthy participants were invited to the University of Leeds exercise physiology laboratory where they each completed a ramp incremental test on a supine cycle ergometer (Lode BV, Groningen, The Netherlands). The laboratory ergometer was designed to exactly replicate the ergometer used in the MRI in order to achieve a work rate that could be directly translated to exercise in the MRI scanner. This included using a shorter crank length on the pedals to ensure the participant's knees did not exceed the simulated height of the bore of the scanner. A 12-lead ECG was fitted to each participant to monitor heart rate (HR) and observe any abnormalities that may occur during maximal exercise and cause the test to be terminated prematurely. Once the ECG was positioned, participants lay supine on the couch and their feet were strapped in the pedals of the cycle ergometer. The position of the er-

gometer was adjusted so that participants could comfortably cycle without over-extending their knees, whilst remaining within the simulated height of the bore of the MRI scanner. Breath-by-breath gas exchange was measured at rest, during, and after exercise using MedGraphics Ultima (MGC Diagnostics, USA). Participants breathed through a mouthpiece and wore a nose clip to prevent any nasal breathing. Before the test started the gas analysers were calibrated against known concentrations of oxygen and carbon dioxide in a pre-mixed gas cylinder, and airflow was calibrated against a known volume (3L) at different speeds of inspiration and expiration.

The test began with ~2 minutes of rest followed by a warm up period of 2-3 minutes at 10 W, with a cadence of 50–60 rpm. Once oxygen uptake (VO2) and rest expiratory ratio (RER) (RER = 0.75-0.9) had stabilised, the ramp section of the test at a rate of 15 W/min began. Participants were instructed to cycle at a cadence of >50 rpm and continued to cycle until they reached volitional fatigue. Volitional fatigue was described as being unable to cycle above a cadence of 50 rpm despite strong verbal encouragement. Once this point was reached, participants entered an active recovery period where they cycled at a load of 10 W at their own pace until HR and VO2 returned back towards baseline levels.

3.2.4.2 MR patients – cardiopulmonary exercise test

MR patients were recruited based upon having pre-existing cardiopulmonary exercise tests (CPET) as part of the inclusion criteria. These, however, were

conducted on a treadmill using the Bruce protocol. The Bruce protocol involves patients exercising on a treadmill at a set speed and incline that increases every three minutes until the patient cannot physically run any further. A standard Bruce protocol works as follows:

Stage 1 = 1.7 mph at 10% incline
Stage 2 = 2.5 mph at 12% incline
Stage 3 = 3.4 mph at 14% incline
Stage 4 = 4.2 mph at 16% incline
Stage 5 = 5.0 mph at 18% incline
Stage 6 = 5.5 mph at 20% incline
Stage 7 = 6.0 mph at 22% incline
Stage 8 = 6.5 mph at 24% incline
Stage 9 = 7.0 mph at 26% incline

Heart rate was measured via ECG and monitored to assess for any abnormalities that might cause the test to be terminated prematurely. Breath-by-breath gas exchange was measured before, during and after exercise.

3.2.5 Exercise protocols

The healthy participants (median 8 days later) and patients were invited to Leeds General Infirmary cardiac MRI unit to carry out the in-exercise MRI scans. On giving their informed consent were given instructions and what to expect during the scans. Participants were laid on the table of the 1.5T MRI scanner (Philips, NL) and their feet strapped to the MRI-compatible cycle ergometer (Lode BV, Groningen, The Netherlands) attached to the table end (Figure 3.1). A 4-lead ECG was attached to their chest as the majority of scans were ECG gated and to monitor when they reached their target HR. To check that the participant would not hit their knees on the bore of the

magnet during cycling, they were entered into the bore and adjusted the position accordingly during a brief bout of unloaded pedalling. Following this, a 28-channel coil was placed on top of their torso and strapped to the participant using Velcro straps to ensure stability throughout exercise. A blood pressure cuff was attached to the participant's left arm and measurements were taken at rest and during each phase of exercise. Participants were instructed to reduce diaphragmatic breathing as much as possible as this can cause artefacts on the cine images. Baseline scans were taken at rest to establish anatomical positioning and resting values. These scans were then repeated at each subsequent work rate.

Once participants had been fitted with the ECG, 28-channel coil and were in a comfortable cycling position, they were left at rest for ~15 minutes whilst the resting scans and blood pressure were taken. When instructed, they began cycling and work was added at a rate of 20 W every two minutes until they achieved their target HR and WR. This was adjusted to ensure target HR was achieved. After a period of two minutes, where HR remained constant at the target, the scans commenced and blood pressure was measured again whilst the participant continued to cycle for ~15 minutes. On completion of the scans, participants were instructed to stop cycling and take the opportunity to have a two-minute rest before commencing the second work rate. Again, work was increased as described above until the target HR was met. When the scans had been completed, participants were told to stop cycling and were removed from the scanner.

3.2.5.1 Selection of exercise heart rates

The healthy participants were studied prior to the mitral patients as a feasibility assessment. Thus the light and moderate intensity work rates were preselected as 55% and 75% maximum HR, respectively. However, it became apparent that the resolution of the MRI scanner in terms of acquisition of aortic flow was poor above heart rates of 110-115 bpm. Consequently, the data presented for healthy participants in this chapter will only be presented for the light intensity exercise. The subsequent selection of exercise heart rates for the mitral patients revealed that 55% maximum heart rate pushed many above the resolution of the MRI scanner and induced blood pressure abnormalities. Thus the exercise intensities were adjusted to 30-39% heart rate reserve (HRR) and 40-49% HRR (light and moderate, respectively). As a result 10 healthy participants conducted exercise at 55% HR max and 10 MR patients conducted exercise at 30-39% and 40-49% HRR. An adjustment of the healthy participants HRs to HRR revealed 5 participants falling into the equivalent of 30-39% HRR. In this chapter the data for all participants is reported unless healthy vs patient comparisons are undertaken.

3.2.5.2 Healthy participants

Healthy participants performed continuous exercise at 55% max HR. The work rate each participant would perform is described as follows:

$$Target\ HR\ (bpm) = \ peak\ HR \times \%\ HR\ max$$

$$Work\ rate\ (W) = \frac{(t - s)}{60} \times r + l - d$$

Abbreviations: t, time target HR was reached; s, start of ramp; r, ramp rate; l, starting load; d, delay between VO2 and work.

Figure 3. 1 MRI exercise set-up
Participant cycling supine in the magnet during scanning

3.2.5.3 MR patients

MR patients performed continuous exercise at a work rate set at 30-39% heart rate reserve (HRR) based upon their previous CPET.

$$HRR = ((max\ HR - rest\ HR) \times \%\ HR) + rest\ HR$$

As the work rate for the patients was unable to be calculated from the available data, work rate was increased by 10-20 W every two minutes until they reached the target HR and stabilised for two minutes.

3.2.6 Assessment of ventricular volumes and shear stress patterns

The scan protocol for both the healthy participants and mitral patients was the same and included standard long axis views (horizontal and vertical long axis) and a short axis ventricular volume stack. Cine imaging was performed

using a free-breathing, multi-shot, respiratory-navigated, balanced steady-state free precession pulse sequence. A respiratory navigator was placed on the right hemi-diaphragm with a 5mm gating window and continuous gating level drift activated. A cylindrical MRI radiofrequency excitation pulse from which a 1-dimensional projection of the lung-liver interface was generated and was used to infer the breathing phase. Cartesian sampling was used, and the acquired k-space lines were only accepted for image reconstruction if the right hemi-diaphragm position was within the gating window during end-expiratory phase. Other scan parameters were as follows: typical field of view 320x320 mm, repetition time 2.8 msec, echo time 1.4 msec, flip angle 60°, temporal resolution 33msec, SENSE factor 2, multi-shot turbo field echo (TFE) factor 11, TFE acquisition duration 30.4 msec, phase percentage 50%, slice thickness 10 mm, 0mm gap, 30 phases, in-plane spatial resolution 2.4x2.4 mm, matrix 132x106. These scan parameters were used to capture LVESV, LVEDV, LVSV, CO, LVEF.

Cardiac work (CW) and total peripheral resistance (TPR) were calculated using the following formulae:

CW = CO x AoPP

TPR = MAP ÷ CO

CO – cardiac output; AoPP – aortic pulse pressure; MAP – mean arterial pressure

To assess shear stress patterns throughout the protocols, 4D flow scans were utilised to measure blood flow and were transformed into shear stress post processing. Scans were acquired as above however, in order to capture blood flow in the full aortic arch, the apex of the heart was clipped from the field of view.

3.2.7 Cardiac MRI analysis

Cardiac MRI (CMR) analyses were performed by Dr Pei Gee Chew and Dr Pankaj Garg, who have over 3 years of CMR experience each, using commercially available software for the heart (cmr[42], Circle Cardiovascular Imaging Inc., Calgary, Alberta, Canada) and aorta (CAAS, Pie Medical, The Netherlands). Left ventricular volumes and ejection fraction were calculated by manually tracing endocardial contours in end-diastole and end-systole on the short axis stack, as is standard. 4D flow scans were analysed in CAAS. Images were reconstructed to form a 3D aorta and were contoured to remove any artefacts. Wall shear stress was measured at eight planes of the aorta (Figure 3.2) at peak systole, these planes are further split into six segments to show the distribution of shear stress at each plane throughout the thoracic aorta. Wall shear stress is presented as total wall shear stress (tWSS), axial wall shear stress (aWSS) and circumferential wall shear stress (cWSS).

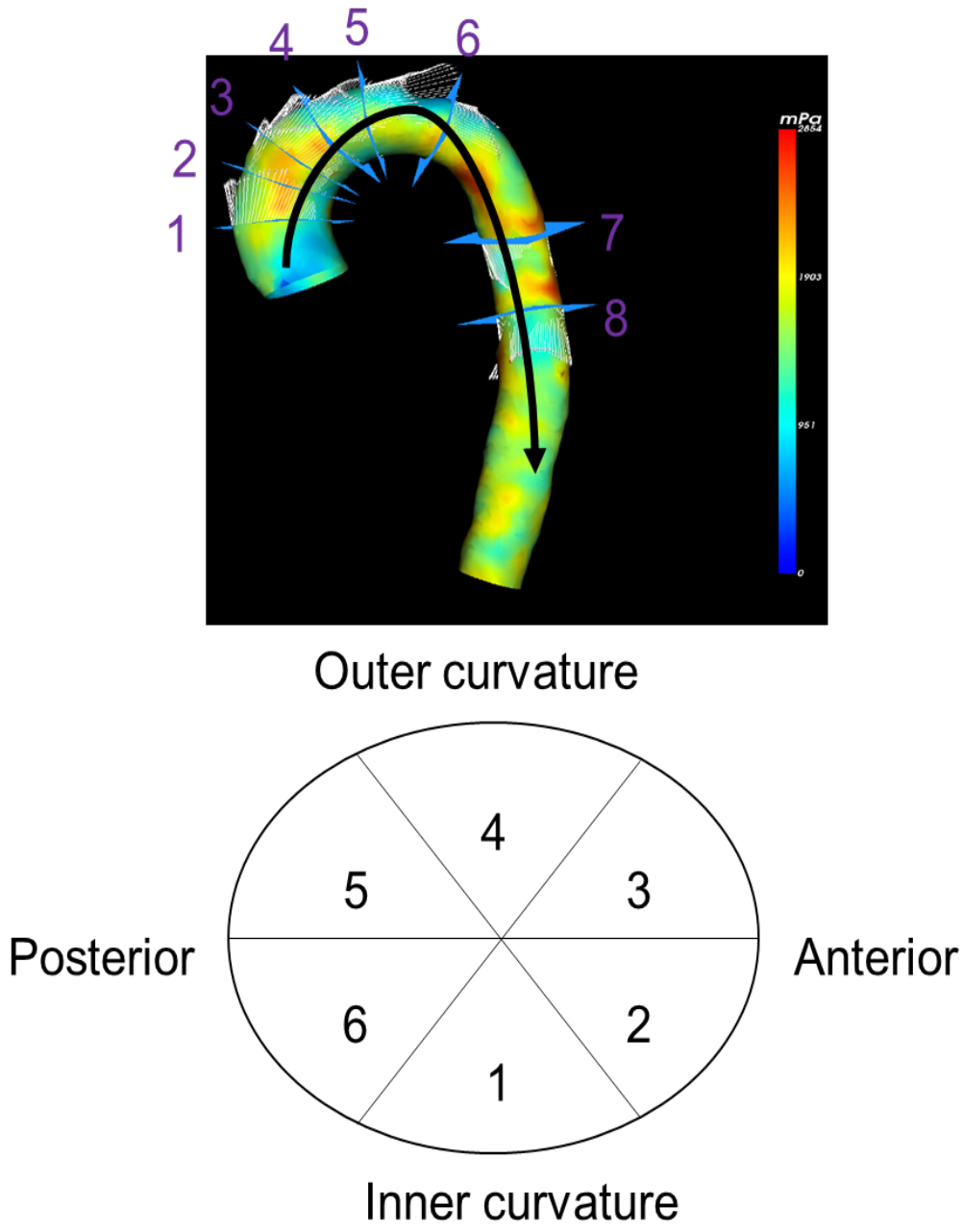


Figure 3. 2 Shear stress map of the aorta

Shear stress map of the aorta at rest showing the eight planes (1 at the ascending aorta above the aortic root, 8 in the descending thoracic aorta in line with the aortic valve) where shear stress is measured. Each plane is further divided into six segments (segment 1 at the bottom in the middle of the inner curvature, segment 4 at the top in the middle of the outer curvature).

3.2.7 Analysis of data

Data from the healthy participants RIT was analysed in Origin and used to calculate the HR and WR. For patients and participants all cardiac scans were analysed on Circle (cmr[42], Circle Cardiovascular Imaging Inc., Calgary, Alberta, Canada). 4D flow data were analysed in CAAS by splitting the aorta into eight planes, and then further divided into six segments to show the spatial differences in WSS across the aorta (Figure 3. 2).

Statistical analyses were conducted in SPSS using T tests to examine patient versus healthy patient differences and linear mixed model to examine differences in parameters between segments and planes (segments and planes as within subject and group or exercise mode (rest, exercise) as between subject variables), and have been stated where appropriate. Data are presented as mean ± SD unless otherwise stated, and data are accepted as statistically significant at $P<0.05$.

3.3 Results

3.3.1 Participant characteristics

Participant characteristics are described in Table 3.1. All healthy participants and MR patients that completed the protocol have been characterised, however only 10 healthy and 5 mitral participants had 4D flow scans at rest and exercise due to ECG failure, blood pressure abnormalities, and poor image resolution.

Table 3. 1 Participant characteristics for healthy participants and mitral patients.

	Healthy	MR
Sex (female)	10 (3)	10 (2)
Age (yrs)	25 ± 2	54 ± 13
Height (cm)	176.5 ± 7	177.1 ± 7
Body mass (kg)	72.7 ± 9.3	77.7 ± 11.7
Work rate (W)	28 ± 20	48 ± 29

3.3.2 Blood pressure and heart rate responses to exercise

Heart rate and blood pressure at rest and during exercise for both the healthy and MR participants are displayed in table 3.2.

Table 3. 2 Heart rate and blood pressure for healthy participants and MR patients

	Healthy		MR	
	Rest	Exercise	Rest	Exercise
HR (bpm)	67 ± 13	93 ± 9*	74 ± 10	112 ± 5**
SBP (mmHg) [£]	118 ± 9	125 ± 11*	124 ± 13	154 ± 13**
DBP (mmHg)	67 ± 7	70 ± 9	79 ± 10	86 ± 10

N= 10 healthy, n=10 MR

** P<0.05 compared to healthy at rest*

*** P<0.05 compared to MR at rest*

[£] *P<0.05 mode*group interaction*

SBP – systolic blood pressure; DBP – diastolic blood pressure

There was no difference between the healthy participants and MR patients at rest for HR, SBP or DBP. Heart rate and SBP increased from rest to exercise, whereas DBP remained level in both the healthy participants and MR patients. The increase in HR and SBP was however greater in the MR patients than healthy participants (mode*group interaction, P<0.05).

3.3.3 Left ventricle structure and function at rest and in exercise

The left ventricle structure and function characteristic at rest and exercise are displayed in table 3.3.

Table 3. 3 Left ventricular structure and function for healthy and MR participants at rest and during exercise.

	Healthy		**MR**	
	Rest	Exercise	Rest	Exercise
LVESV (mL)	76 ± 17	69 ± 16	82 ± 13	74 ± 15
LVESVi (mL/m^2)	40 ± 8	37 ± 7	42 ± 8	38 ± 9
LVEDV (mL)	177 ± 29	175 ± 28	196 ± 31	206 ± 34
LVEDVi (mL/m^2)	90 ± 13	92 ± 14	101 ± 18	107 ± 21
LVEF (%)	57 ± 4	61 ± 5	58 ± 3	64 ± 5**
CO (mL) £	6793 ± 1134	10173 ± 2100*	8522 ± 1946*	14449 ± 2914**
LVSV (mL) £	100 ± 13	106 ± 16	114 ± 20	132 ± 24**
LVSVi (mL/m^2)	50 ± 7	55 ± 8*	59 ± 11*	68 ± 14**
MAP (mmHg)	84 ± 7	88 ± 9	95 ± 10	108 ± 10
TPR (mmHg.min/L)	13 ± 2	9 ± 1	12 ± 3	8 ± 2
SW	8443 ± 1236	9426 ± 2104	10879 ± 2459	14231 ± 2813
AoPP£ (mmHg)	51 ± 6	56 ± 6	43 ± 7	68 ± 12
CW£ (mmHg/mL)	576 ± 130	911 ± 270	815 ± 234	1562 ± 299

N=10 healthy, n=10 MR

** Denotes significant difference to healthy resting values at P<0.05*

*** Significant difference to MR resting values at P<0.05*

*£ P<0.05 mode*group interaction*

LVESV – left ventricular end systolic volume; LVESVi – left ventricular end systolic volume indexed to body surface area; LVEDV – left ventricular end diastolic volume; LVEDVi – left ventricular end diastolic volume indexed to body surface area; LVEF – left ventricular ejection fraction; CO – cardiac output; LVSV – left ventricular stroke volume; LVSVi – left ventricular stroke volume indexed to body surface area; MAP – mean arterial pressure; TPR – total peripheral resistance; SW – stroke work; AoPP – aortic pulse pressure; CW – cardiac work.

It can be seen in table 3.3 that CO and LVSVi increased with exercise for both healthy participants and MR patients ($P<0.05$). With the increased HR and LVSVi, this in turn translated into an increase in CO by 50% in healthy participants and 70% in MR patients. Additionally, MR patients had further increases in LVEF, LVSV, and LVSVi in response to exercise ($P<0.05$).

When comparing the two groups, each LV variable increased with exercise (mode effect, $P<0.05$) except LVEDV. However only CO, LVSV, and LVSVi differed between the groups and were greater in the MR patients (group effect, $P<0.05$). The increase in CO and LVSVi with exercise was greater in MR patients than healthy participants (mode*group interaction, $P<0.05$) but all other variables remained similar between the two groups.

Further analysis of the drivers of WSS in regards to LV structure and function characteristics (table 3.3) has indicated that for each of MAP, TPR, SW, AoPP, MAoP, and CW there was a significant effect of exercise where all showed an increase from rest to exercise except TPR, which decreased with exercise (mode effect, $P<0.05$). Furthermore these measures all differed between the healthy and MR participants (group effect, $P<0.05$) with the exception of TPR and AoPP where there was no difference between the two groups ($P>0.05$). Finally, the increases seen in exercise for AoPP and CW were greater in the MR patients than the healthy participants (mode*group interaction, $P<0.05$).

3.3.3 Healthy participants wall shear stress

3.3.3.1 Shear stress at rest and during exercise

Total, axial and circumferential wall shear stress averaged across the aortic arch are presented in figure 3.3. It can be seen that in response to exercise there was a 10% increase in WSS from resting conditions for tWSS ($P<0.05$), as well as its constitutive components aWSS ($P<0.05$), and cWSS ($P<0.05$), which increased by 8% and 35%, respectively.

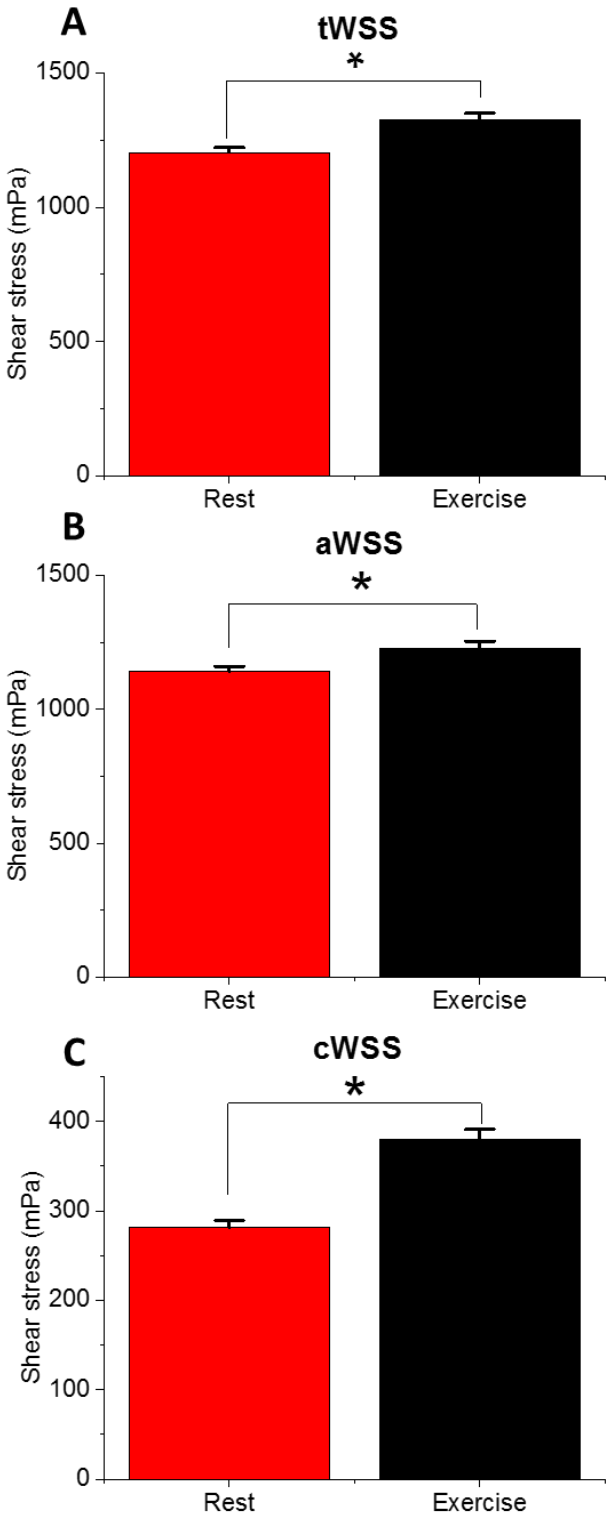
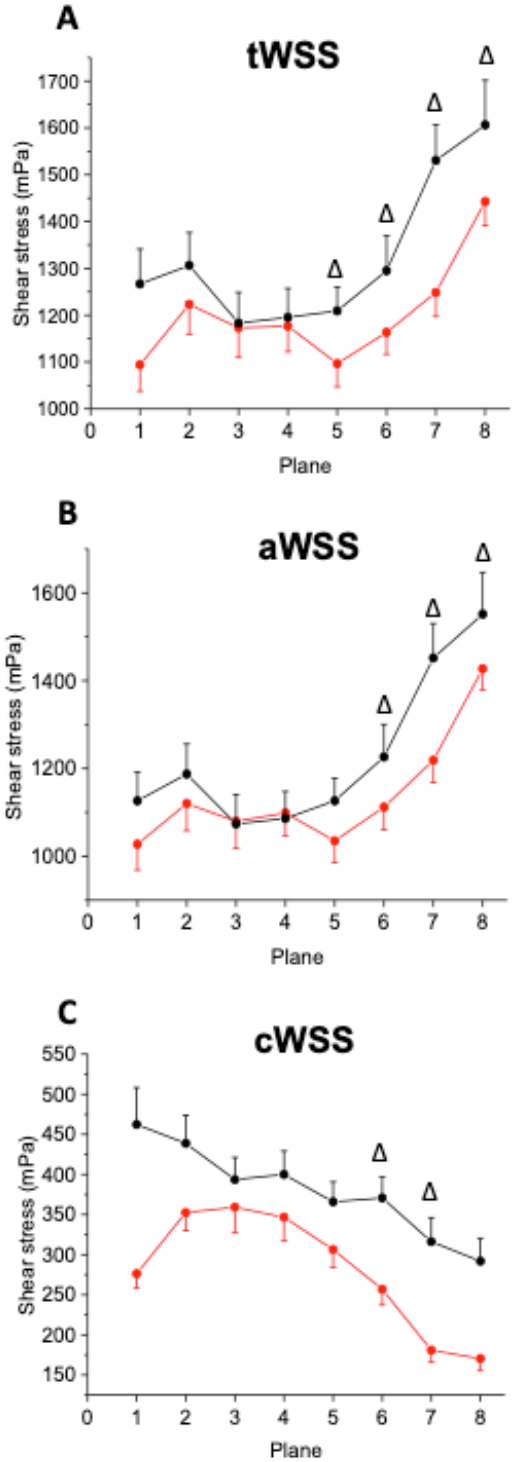


Figure 3. 3 Time-averaged WSS for healthy participants at rest and exercise

Time-averaged WSS for healthy participants at rest and exercise. A) tWSS is increased during exercise, B) aWSS is increased during exercise, C) cWSS is increased during exercise. N=10, * denotes significance at P<0.05. Data are presented as mean ± SE.

3.3.3.2 Impact of plane on WSS

WSS for tWSS, aWSS and cWSS at rest and exercise for each of the 8 reported aortic planes plane are shown in figure 3.4. tWSS differed across planes (plane effect: $P<0.05$), and a plane*mode interaction ($P<0.05$) indicated that the pattern of tWSS across planes differed from rest to exercise. Similarly, aWSS and cWSS showed an effect of plane ($P<0.05$) however, there was no plane*mode interaction ($P>0.05$) indicating that the pattern of aWSS and cWSS across the aortic arch did not change in response to exercise. At rest for tWSS and aWSS plane 1 had the lowest amount of shear stress and plane 8 had the highest with a general trend for an increase between these points. In exercise however, planes 3,4, and 5 had the lowest levels of shear stress with planes 3 and 4 being similar to resting levels. However, cWSS at rest was highest at planes 2, 3, and 4 and lowest at plane 8, but in exercise was highest at plane 1 and decreased gradually to plane 8 where cWSS was lowest.



Plane Effects *
P1 v P8
P3 v P8
P4 v P8
P5 v P7
P5 v P8
P6 v P8

Plane Effects *
P1 v P7
P1 v P8
P2 v P8
P3 v P8
P4 v P8
P5 v P7
P5 v P8
P6 v P8

Plane Effects *
P1 v P7
P1 v P8
P2 v P7
P2 v P8
P3 v P7
P3 v P8
P4 v P7
P4 v P8
P5 v P7
P5 v P8
P6 v P8

Figure 3. 4 Time-averaged WSS pattern for planes in healthy participants at rest and exercise

Time-averaged WSS pattern for healthy participants at rest (red) and exercise (black) for each plane of the aorta. A) tWSS – plane effect, $P<0.05$; plane*mode interaction, $P<0.05$, B) aWSS – plane effect, $P<0.05$, C) cWSS – plane effect, $P<0.05$. N=10, data are presented as mean ± SE.

3.3.3.3 Impact of segment on WSS

Aortic planes were further divided into segments to show where in the aorta i.e. the inner or outer curvature had the highest levels of shear stress and whether that changed with exercise for tWSS, aWSS and cWSS (Figure 3.5). All three measures of shear stress differed across segments ($P<0.05$) and revealed significant segment*mode interactions ($P<0.05$). The segments in tWSS and aWSS followed the same pattern at rest and during exercise. At rest the highest areas of WSS was in segment 2 and the lowest was in segment 4 whereas during exercise the shear stress shifted so that segments 1 and 6 were exposed to the lowest shear stresses and segments 2, 3, 4, and 5 were exposed to the higher levels of shear stress. The pattern of cWSS was slightly different to aWSS and tWSS. At rest, cWSS was highest at segments 1 and 6 but lowest at segment 4 however, in exercise the distribution of cWSS was fairly uniform throughout the segments.

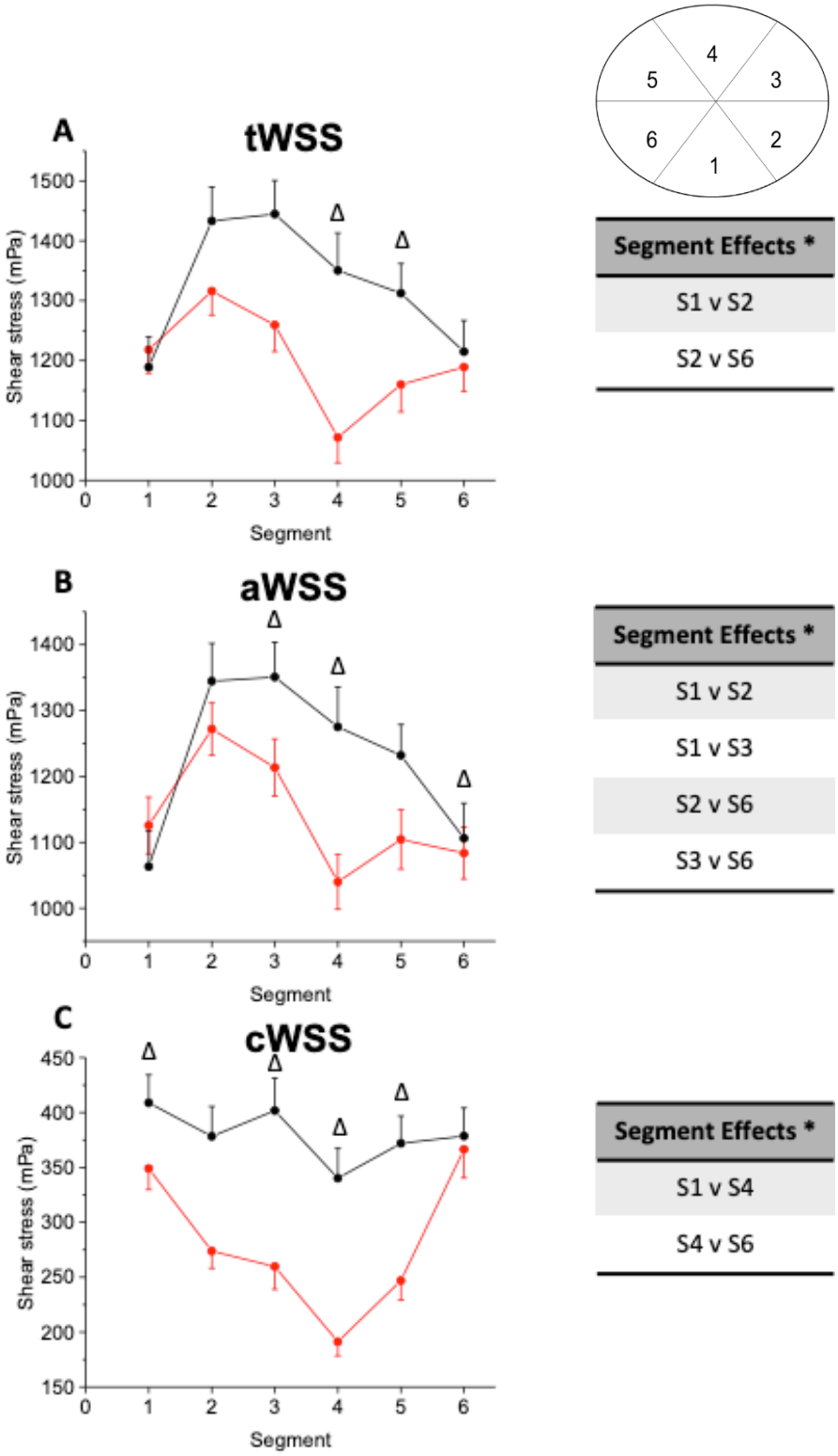


Figure 3. 5 Time-averaged WSS patterns for segments in healthy participants at rest and exercise

Time-averaged WSS pattern for healthy participants at rest (red) and exercise (black) for each segment of the aorta. A) tWSS – segment effect, $P<0.05$; segment*mode interaction, $P<0.05$, B) aWSS – segment effect, $P<0.05$; segment*mode interaction, $P<0.05$, C) cWSS – segment effect,

P<0.05; segment*mode interaction, P<0.05. N=10 data are presented as mean ± SE.

3.3.3.4 Interaction between plane and segment at rest and exercise

There was no plane*segment*mode interaction for tWSS, aWSS, or cWSS (P>0.05) however, each of tWSS, aWSS and cWSS did show a plane*segment interaction (P<0.05) showing that WSS changed across the aorta depending on the plane and segment (Figure 3.6). The tWSS from rest to exercise changed from being concentrated around segments 2 and 3 to a more even spread across segments 2-5, which is where the majority of the highest tWSS was observed in exercise. In aWSS however, there was still more of a concentration at segments 2 and 3 in each plane both at rest and during exercise. The pattern for cWSS in planes 1-4 went from a higher level of cWSS concentrated at segments 4-6 to a uniform distribution of cWSS for each segment. The cWSS in planes 6-8 changed from an almost uniform distribution to an increase in segments 5-1 for planes 6 and 7, and an increase in segments 3 and 4 for plane 8.

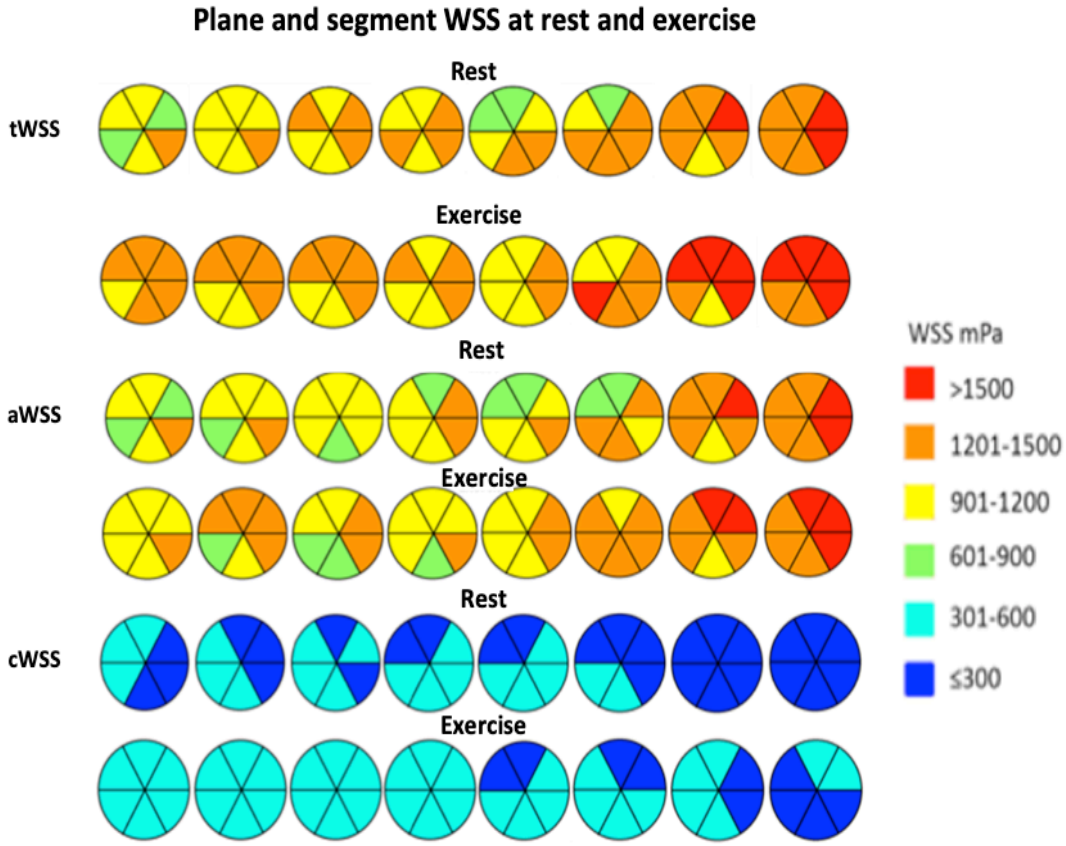


Figure 3. 6 Plane and segment WSS at rest and exercise

Plane (1 left, 8 right) and segment WSS at rest and during exercise. Each of tWSS, aWSS, and cWSS had no plane*segment*mode interaction ($P>0.05$) but did have a plane*segment interaction ($P<0.05$). N=10.

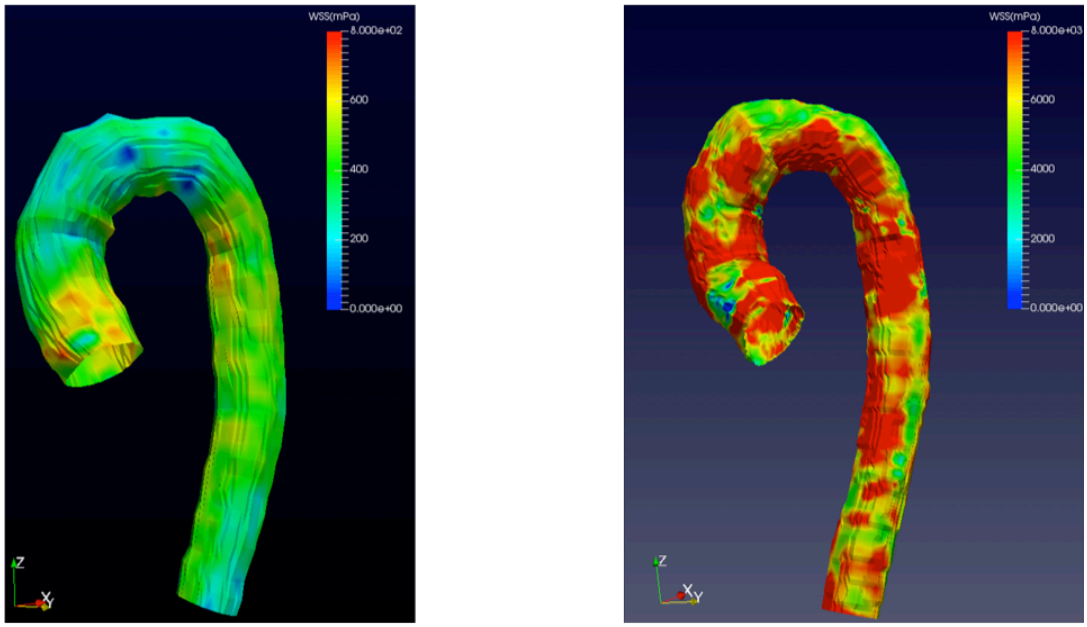


Figure 3. 7 Shear stress map of the aorta at rest and exercise

Anterior view of a representative participant WSS at rest (left) and during exercise (right).

3.3.3.5 Correlations of LV structure and function characteristics to WSS

Due to some poor spatial resolution during scans, only 9 participants had LV structure and function data that could be correlated to their corresponding WSS. There were no correlations between LV structure and function characteristics and WSS for any of the measured parameters except LVEF and cWSS ($P<0.05$) (Figures 3.8-3.10.).

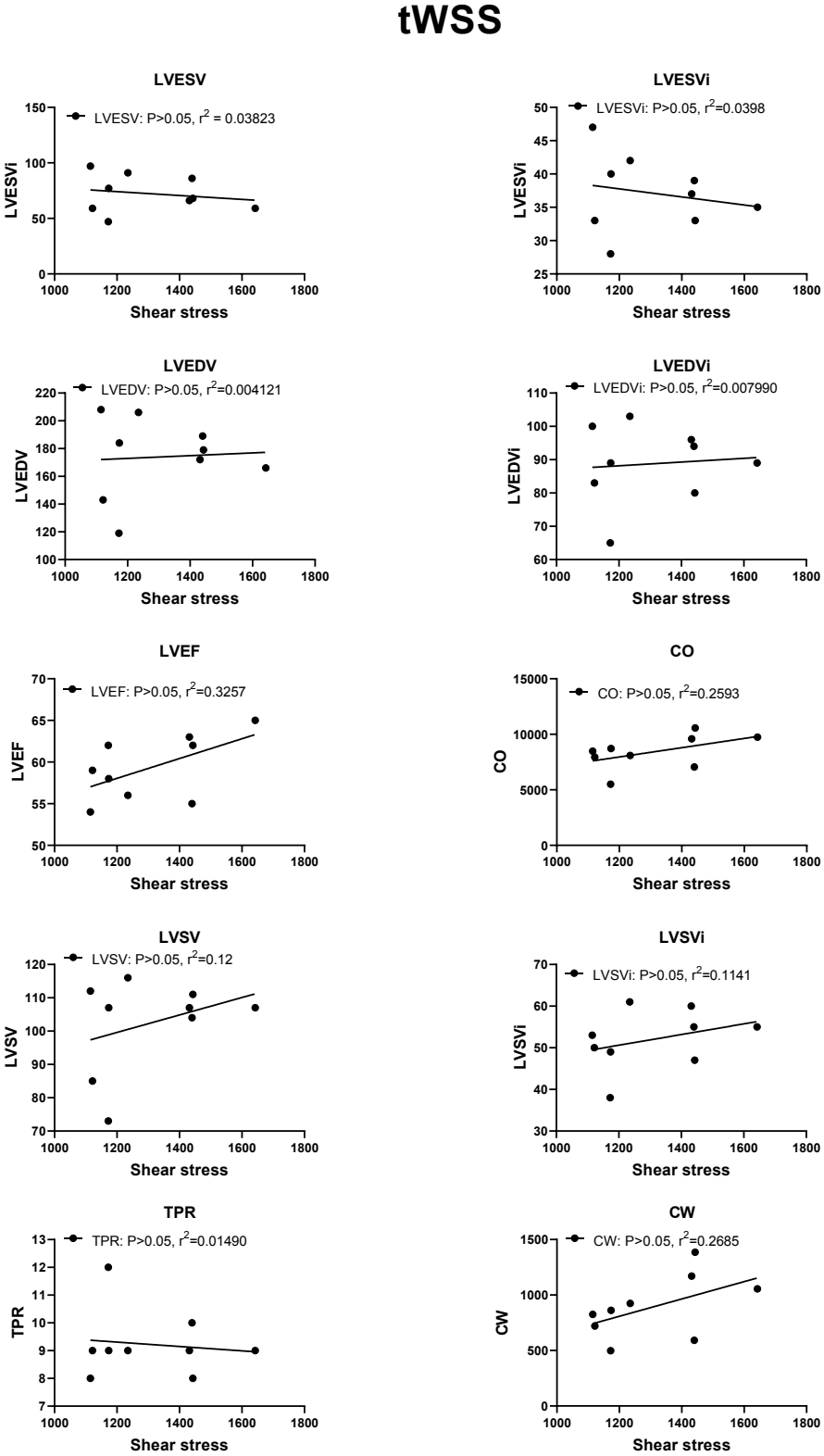


Figure 3. 8 Correlations between LV function and tWSS

Correlations between LV function and tWSS at rest and during exercise for healthy participants. N=9.

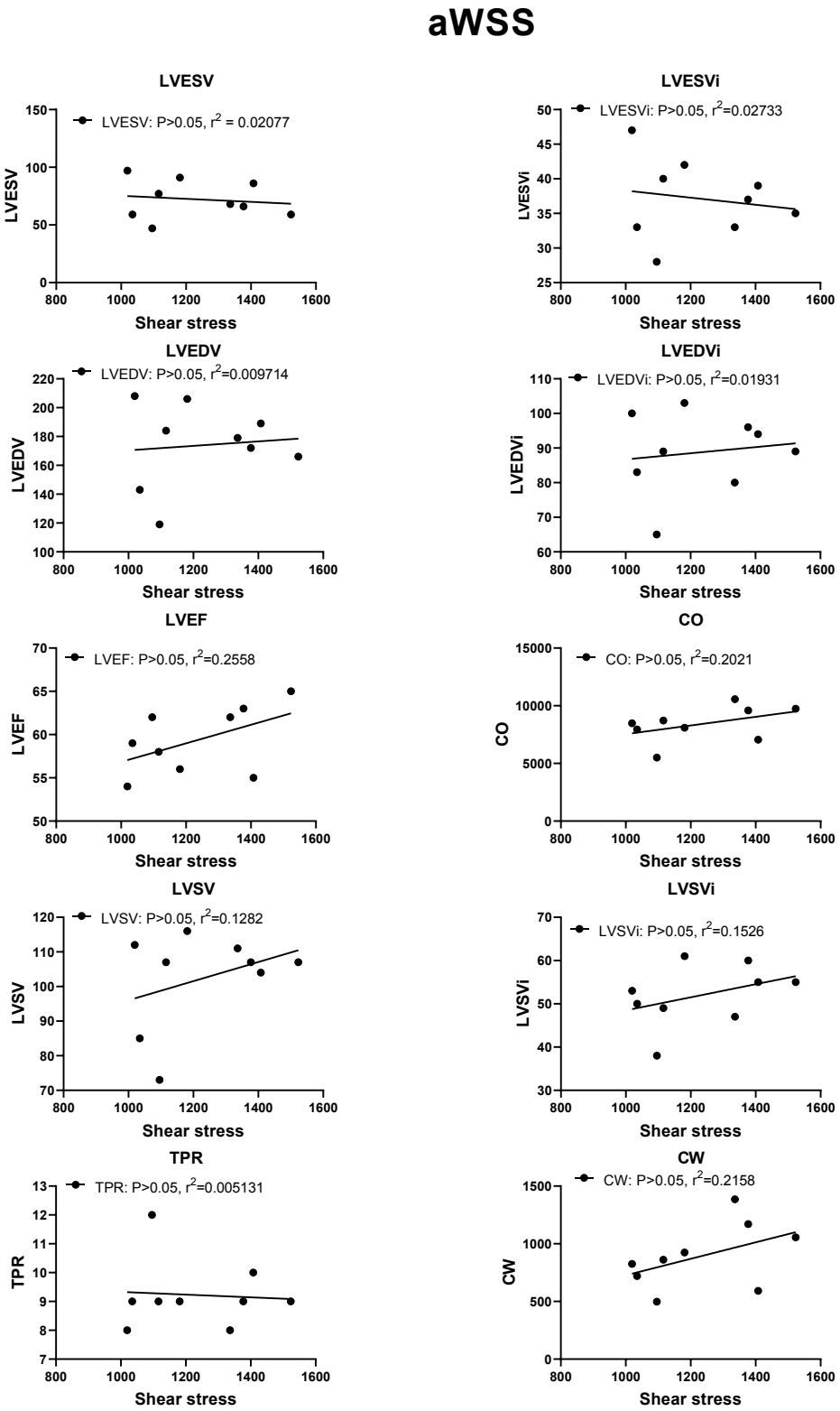


Figure 3. 9 Correlations between LV function and aWSS

Correlations between LV function and aWSS at rest and during exercise for healthy participants. N=9.

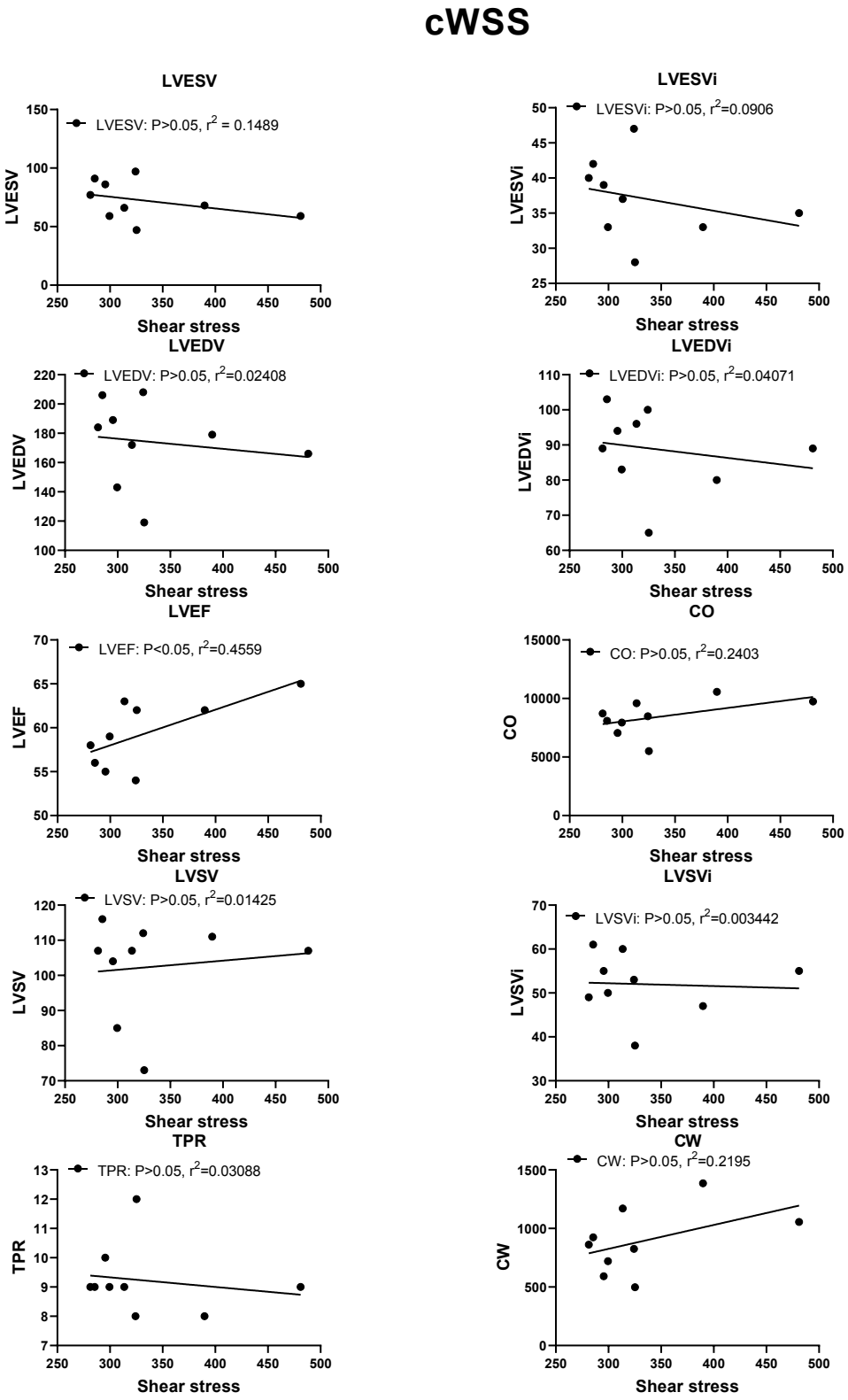


Figure 3. 10 Correlations between LV function and cWSS

Correlations between LV function and cWSS at rest and during exercise for healthy participants. N=9.

3.3.4 MR patients wall shear stress

3.3.4.1 Total shear stress volumes at rest and during exercise

The total time-averaged wall shear stress averaged across the aortic arch for total, axial and circumferential shear stress at rest and in exercise conditions are presented in Figure 3.11. It can be seen in response to exercise that tWSS increased by 15%, aWSS increased by 11%, and cWSS increased by 41%.

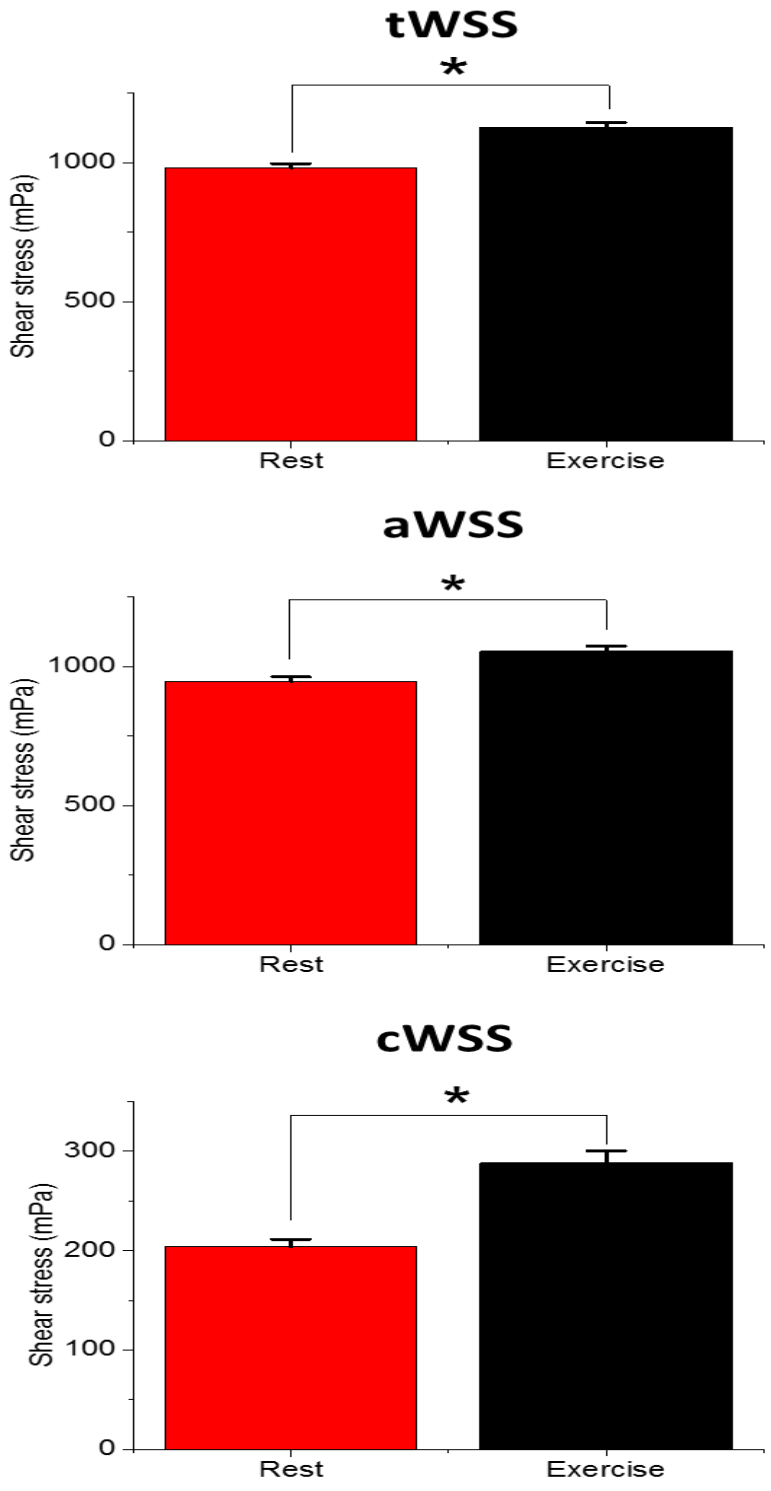
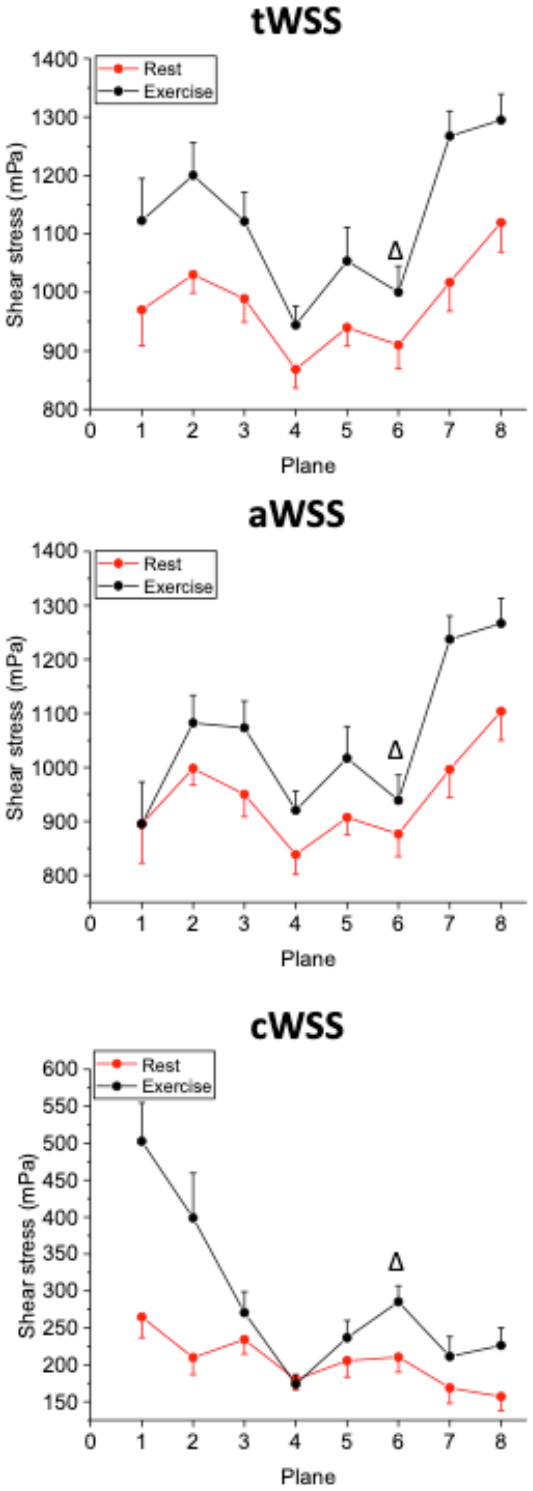


Figure 3. 11 Time-averaged WSS for MR patients at rest and exercise

Time-averaged WSS for MR patients at rest and exercise. A) tWSS is increased during exercise, B) aWSS is increased during exercise, C) cWSS is increased during exercise. N=5, * denotes significance at $P<0.05$. Data are presented as mean ± SE.

3.3.4.2 Impact of plane on wall shear stress

WSS for tWSS, aWSS and cWSS at rest and exercise for each of the 8 reported aortic planes plane are shown in Figure 3.12. tWSS differed across planes (plane effect: $P<0.05$), but the pattern of tWSS across planes did not differ from rest to exercise (plane*mode interaction $P>0.05$). Similarly, there was a change in aWSS in the planes ($P<0.05$), and there was no change in aWSS distribution in response to exercise (plane*mode interaction, $P>0.05$). However, there was a change in cWSS across the planes ($P<0.05$) as well as a change in cWSS pattern in the planes in response to exercise (plane*mode interaction, $P<0.05$). At rest for tWSS and aWSS had a very similar pattern in that planes 4, 5 and 6 had the lowest amount of shear stress and planes 7 and 8 had the highest. In exercise the pattern remained almost identical for both tWSS and aWSS except plane 1 in aWSS was lowest in exercise and did not appear to change from resting values However, cWSS at rest had a uniform distribution of shear stress throughout the aorta, but in exercise was increased at planes 1 and 2 where the other planes did not alter from resting values.



Plane Effects *
P2 v P4
P4 v P7
P4 v P8
P5 v P8
P6 v P7
P6 v P8

Plane Effects *
P1 v P8
P2 v P4
P4 v P7
P4 v P8
P5 v P8
P6 v P7
P6 v P8

Plane Effects *
P1 v P4
P1 v P5
P1 v P6
P1 v P7
P1 v P8

Figure 3. 12 Time-average WSS patterns for planes in MR patients at rest and exercise

Time-averaged WSS pattern for MR patients at rest and exercise for each plane of the aorta. A) tWSS – plane effect $P<0.05$, B) aWSS – plane effect $P<0.05$, C) cWSS – plane effect $P<0.05$; plane*mode interaction $P<0.05$. N=5, data are presented as mean ± SE.

3.3.4.3 Impact of segment on wall shear stress

As with the healthy participants, aortic planes were further divided into segments to show where in the aorta i.e. the inner or outer curvature had the highest levels of shear stress and whether that changed with exercise for tWSS, aWSS and cWSS (Figure 3.13). All three measures of shear stress differed across the segments ($P < 0.05$) and revealed that shear stress distribution across the segments differed from rest to exercise (segment*mode interaction, $P<0.05$). The segments in tWSS and aWSS followed the same pattern at rest and during exercise. At rest the highest areas of WSS were in segments 1, 2, and 6 and the lowest were in segments 4, 5, and 6. During exercise the shear stress shifted so that segment 2 was exposed to the highest shear stresses and segment 6 was exposed to the lowest levels of shear stress. The pattern of cWSS was slightly different to aWSS and tWSS. At rest, cWSS was highest at segment 1 but lowest at segments 4, 5, and 6 however, in exercise the distribution of cWSS was a fairly uniform increase throughout the segments, except in segment 6, which did not alter much from resting values.

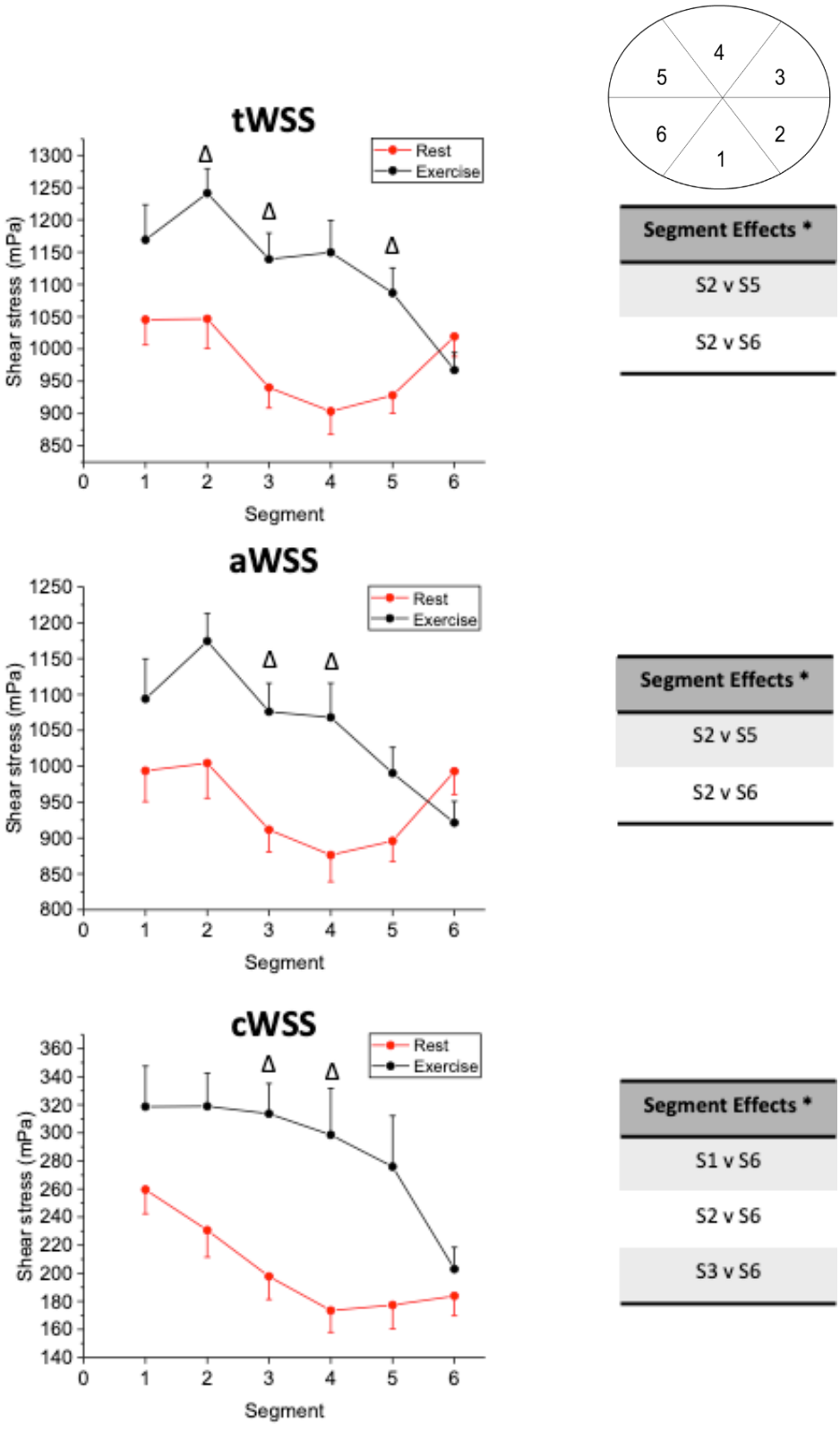


Figure 3. 13 Time-averaged WSS pattern for segment in MR patients at rest and exercise

Time-averaged WSS pattern for MR participants at rest and exercise for each segment of the aorta. A) tWSS – segment effect $P<0.05$; segment*mode interaction $P<0.05$, B) aWSS – segment effect $P<0.05$; seg-

ment*mode effect P<0.05, C) cWSS – segment effect P<0.05; segment*mode interaction P<0.05. N=5 data are presented as mean ± SE.

3.3.4.4 Interaction between plane and segment at rest and exercise

Exercise did not affect the distribution of tWSS and aWSS in the segments across the planes of the aorta (plane*segment*mode interaction, P>0.05) however; exercise did have an effect on cWSS distribution across the aorta (plane*segment*mode interaction, P<0.05). The tWSS and aWSS changed with each segment across the aorta (plane*segment interaction, P<0.05) however; cWSS did not alter in each segment across the planes of the aorta (plane*segment interaction P>0.05). This can be further illustrated in Figure 3.14 where in the almost identical patterns of tWSS and aWSS the distribution of shear stress differs in each plane, this doesn't alter with exercise. The cWSS distribution across the segments and planes could be seen to differ with exercise, most noticeably in planes 1, 2, and 6.

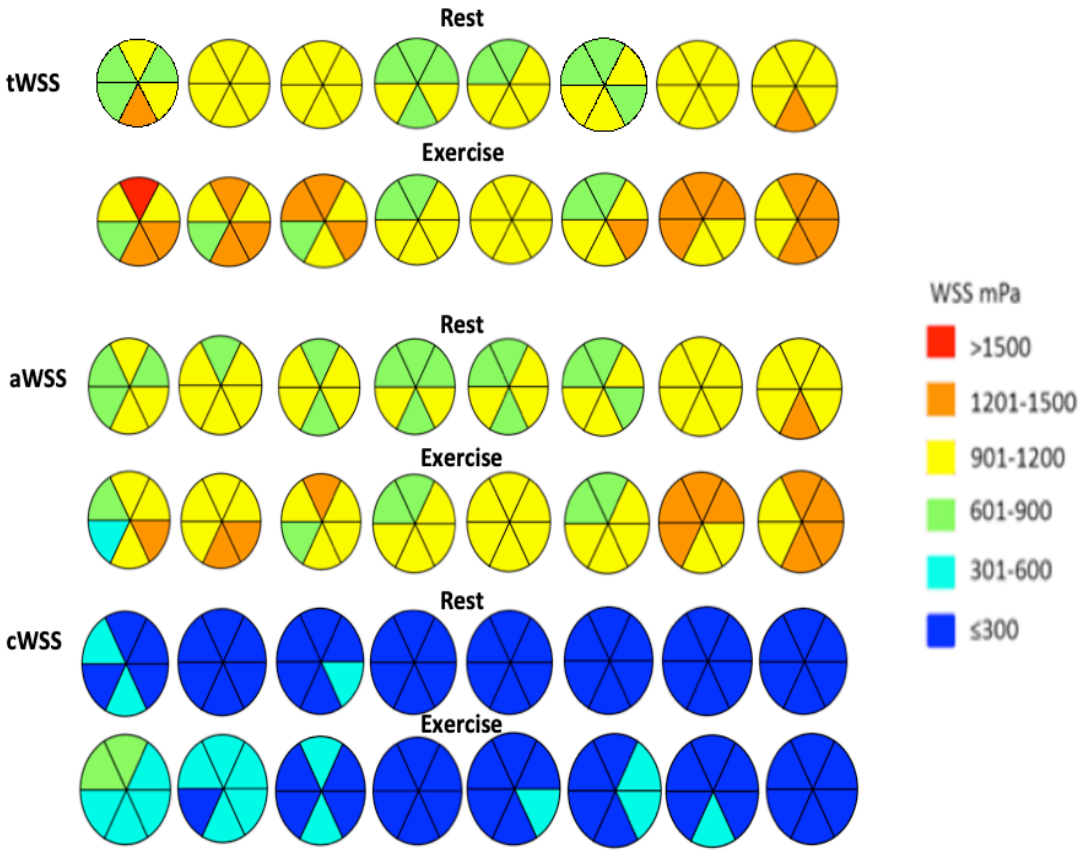


Figure 3. 14 Comparison of MR patients WSS at rest and in exercise by plane and segment

Plane (1 left, 8 right) and segment WSS at rest and during exercise. tWSS and aWSS had no plane*segment*mode interaction (P>0.05) but did have a plane*segment interaction (P<0.05). C) cWSS had a plane*segment*mode interaction (P<0.05) but no plane*segment interaction (P>0.05) N=5.

3.3.4.5 Correlations between LV structure and function characteristics and WSS

There were no correlations between LV and WSS for any of the measured parameters at rest or during exercise (Figures 3.15-3.17).

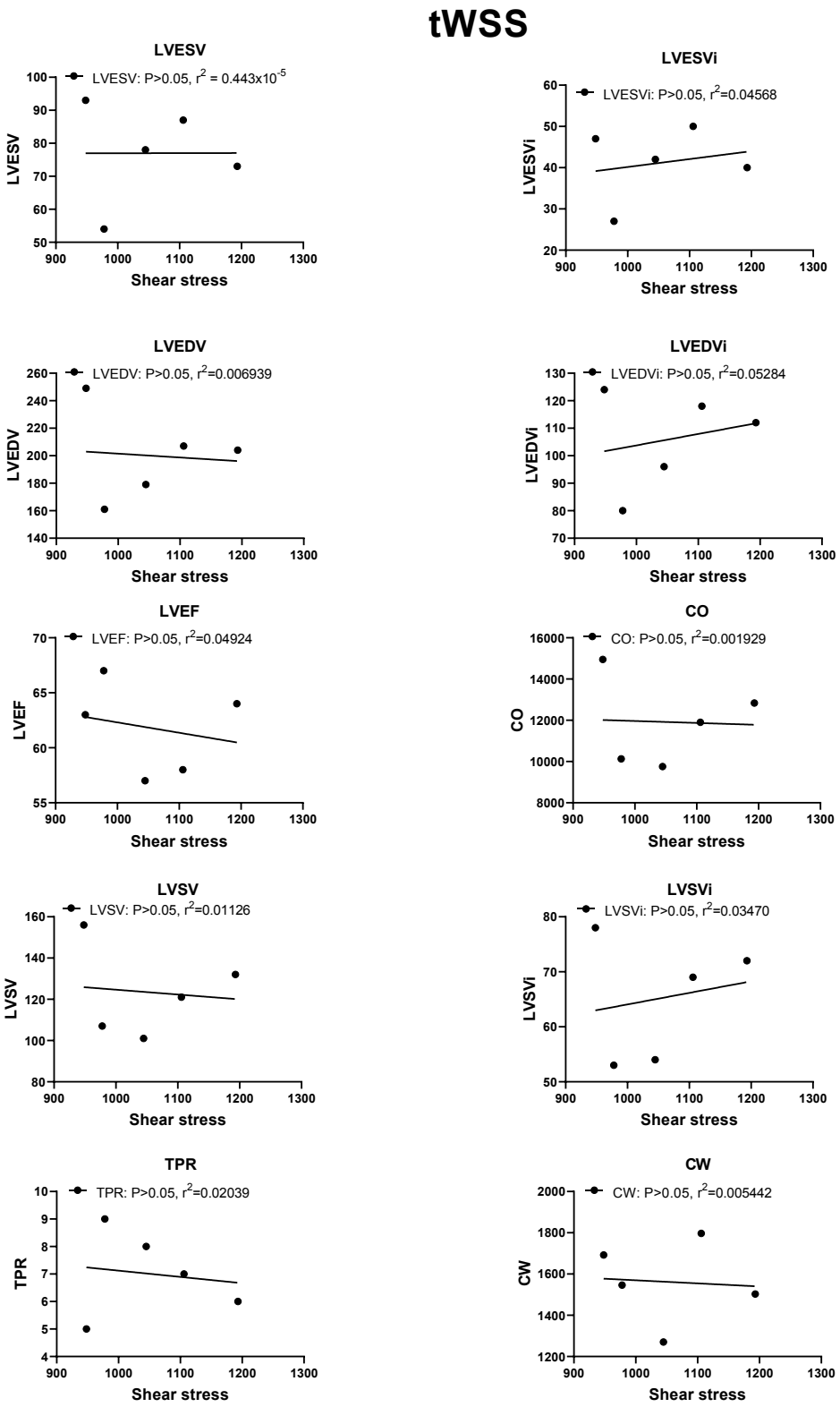


Figure 3. 15 LV function correlated to tWSS

Correlations between LV function and tWSS at rest and during exercise for healthy participants. N=5.

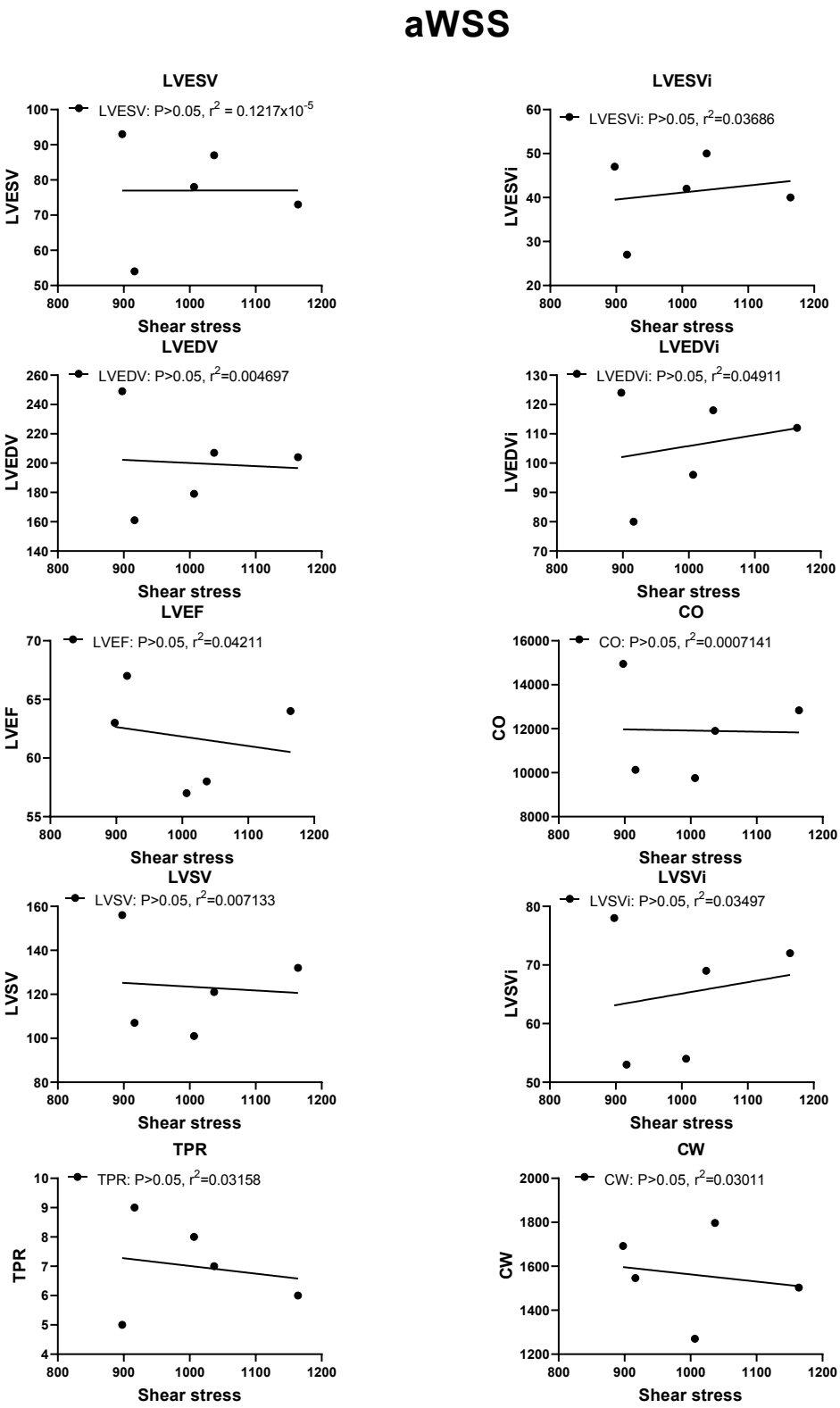


Figure 3. 16 LV function correlated to aWSS

Correlations between LV function and aWSS at rest and during exercise for healthy participants. N=5.

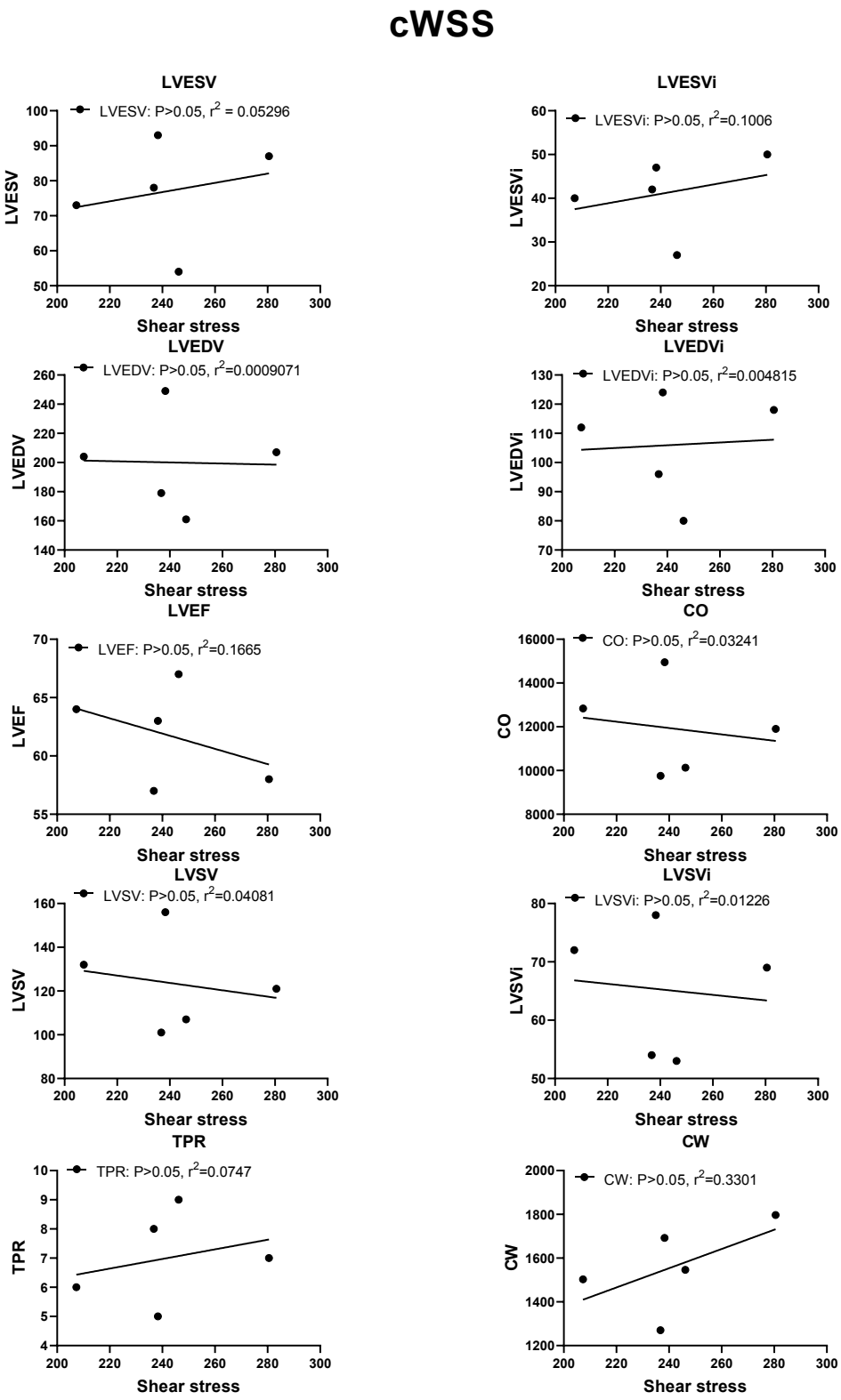


Figure 3. 17 LV function correlated to cWSS

Correlations between LV function and cWSS at rest and during exercise for healthy participants. N=5.

3.3.5 Healthy and MR shear stress comparison

Exercise for the healthy participants and MR patients was conducted at different absolute intensities thus, for this comparison data are shown for 6 healthy participants and 5 MR patients who all exercised at 30-39% HRR.

3.3.5.1 Comparison of wall shear stress volumes at rest and during exercise between healthy participants and MR patients

The results for time-averaged wall shear stress volumes for both groups at rest and exercise are displayed in Figure 3.18. Overall, exercise increased for tWSS (16%), aWSS (14%), and cWSS (33%) from resting values (mode effect, $P<0.05$) and there was a difference in tWSS, aWSS, and cWSS between the healthy and MR patients, where the healthy participants had higher tWSS (28%), aWSS (27%), and cWSS (35%) (group effect, $P<0.05$). However, the impact of exercise upon each WSS parameters did not differ between the healthy and MR patients (mode*group interaction, $P>0.05$).

Figure 3. 18 WSS comparison between healthy participants and MR patients at rest and exercise

Time-averaged WSS for healthy participants and MR patients at rest and exercise. A) tWSS – mode effect $P<0.05$; group effect $P<0.05$ B) aWSS – mode effect P0.05; group effect $P<0.05$ C) cWSS – mode effect $P<0.05$;

group effect P<0.05. No mode*group interactions were recorded (P>0.05). N=5, data are presented as mean ± SE.

3.3.5.2 Comparison of the impact of plane on wall shear stress

WSS in the eight planes of the aorta was compared between the healthy participants and MR patients at rest and in exercise (Figure 3.19). For tWSS, aWSS and cWSS WSS differed across the planes (plane effect, P<0.05). The tWSS and aWSS values did not change across the planes between the two groups (plane*group interaction, p>0.05). Furthermore, the impact of undertaking exercise did not alter the pattern of tWSS, aWSS or cWSS across the six segments (plane*mode interaction, P>0.05) in either group (group*plane*mode interaction, P>0.05).

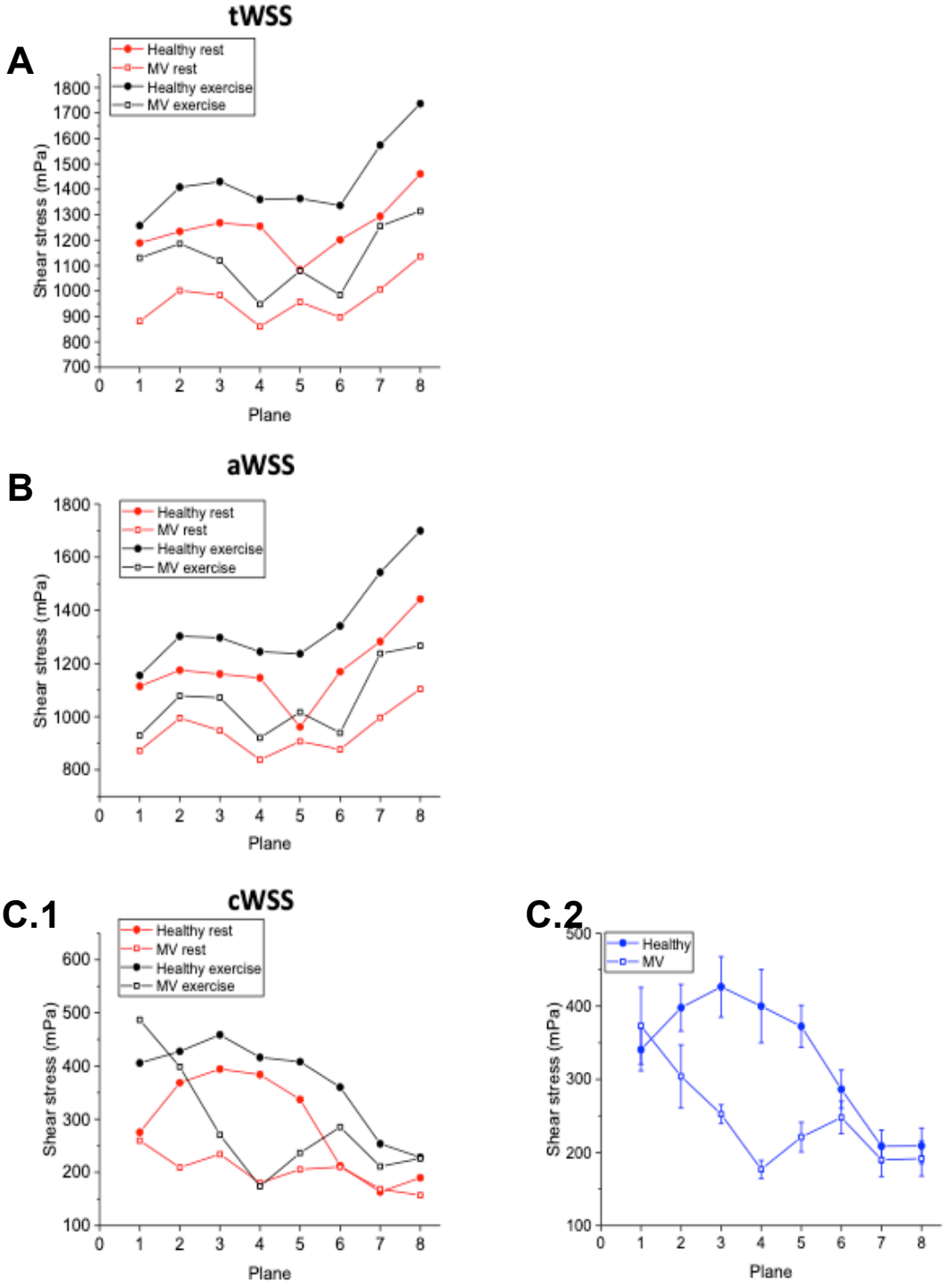


Figure 3. 19 Comparison of WSS in the planes of the aorta between healthy participants and MR patients

Time-averaged WSS pattern for healthy and MR patients at rest and exercise for each plane of the aorta. A) tWSS – plane effect $P<0.05$, B) aWSS – plane effect $P<0.05$, C.1) cWSS – plane effect $P<0.05$; C.2) cWSS – plane*group interaction $P<0.05$. N=11, data are presented as mean ± SE.

3.3.5.3 Comparison of the impact of segment on wall shear stress

WSS across the six segments of the aorta was compared between the healthy participants and MR patients at rest and in exercise (Figure 3.20.). Only tWSS changed across the segments (segment effect, $P<0.05$) whilst the distribution of aWSS and cWSS in the segments remained the same (segment effect, $P>0.05$). Neither of tWSS, aWSS or cWSS differed between the groups in the segments of the aorta (group*segment interaction, $P>0.05$). Furthermore, the impact of undertaking exercise did not alter the pattern of tWSS, aWSS or cWSS across the six segments (segment*mode interaction, $P>0.05$) in either group (group*segment*mode interaction, $P>0.05$).

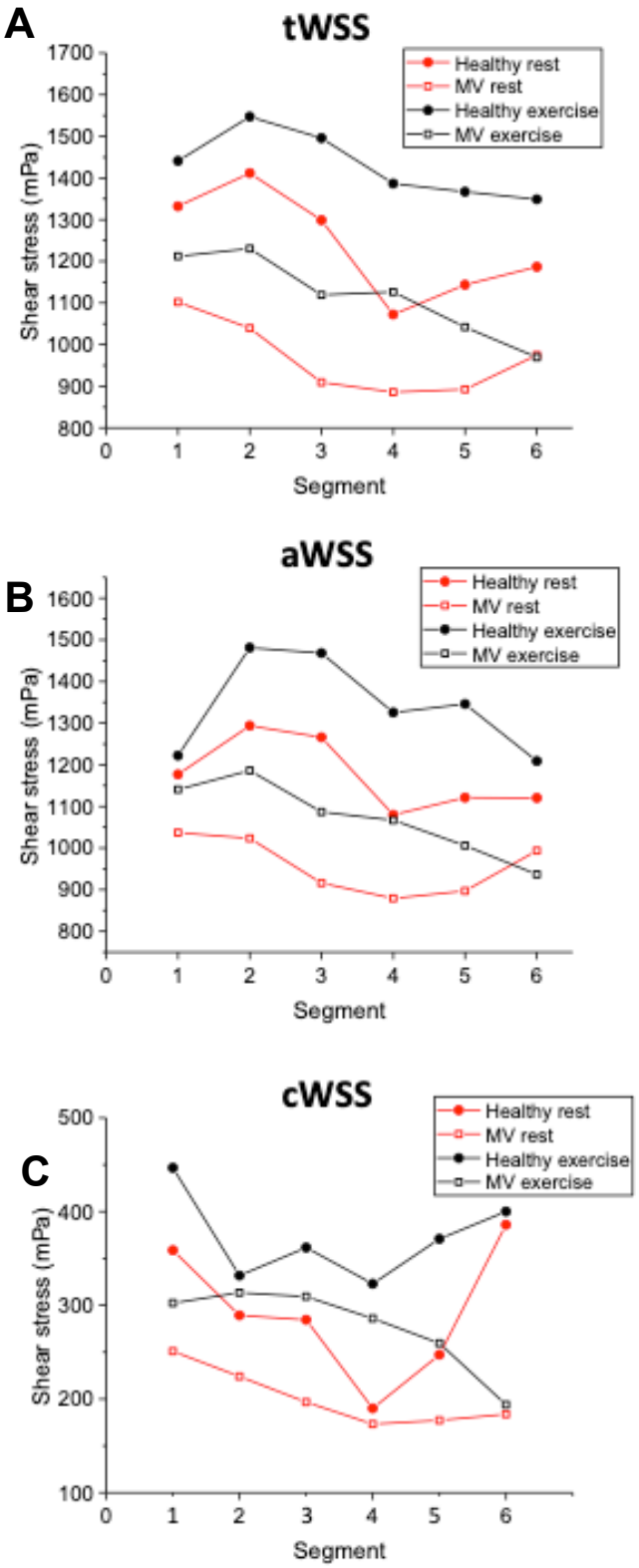


Figure 3. 20 Comparison of WSS in the segments of the aorta between healthy participants and MR patients at rest and exercise

Time-averaged WSS pattern for healthy and MR patients at rest and exercise for each segment of the aorta. A) tWSS – segment effect ($P<0.05$); B) aWSS – segment effect ($P>0.05$); C) cWSS – segment effect ($P>0.05$).

3.4 Discussion

The key findings of this study showed that LV structure and function characteristics were greater in the MR patients than the healthy participants, both the healthy participants and MR patients had an increase in WSS from rest to exercise however this was only in certain planes and segments of the aorta and not seen throughout, and finally there was no difference in the magnitude of WSS between the healthy participants and MR patients at rest and during exercise.

CO and LVSV were greater in MR patients than healthy participants in exercise. Stroke volume is increased in MR patients as a result of an enlarged left ventricle, one of the symptoms of MR (Gaasch and Meyer 2008). This is caused by the chronic volume overload as a result of an increase in end diastolic volume, which over a prolonged period of time leads to eccentric hypertrophy of the LV in order to compensate for the change in volume. The eccentric hypertrophy process occurs due to the extramyocardial matrix beginning to remodel, which leads to a dissolution of collagen causing the myocardial fibres to slip resulting in left ventricle enlargement (Ross 1985) (Perry et al. 2002). In contrast to the results of this chapter, previous studies have shown that cardiac output is generally higher in healthy participants than MR patients, indeed those with severe MR are often characterised by their low cardiac output (O'Rourke and Crawford 1984) (Gaasch and Meyer

2008) (Bakkestrom et al. 2018). In a previous study, patients with MR who could exercise at >50% predicted VO2 maximum had an increase in cardiac output and stroke volume from resting values, like the patients in this study, however, MR patients that could only exercise <50% predicted VO2 maximum did not exhibit an increase in stroke volume and only an increase in cardiac output (Lapu-Bula *et al*., 2002). Unlike the study in this chapter, Lapu-Bula *et al*. conducted their testing on an upright bike and only reported results at peak exercise, whereas this study was conducted by supine cycling, in addition they used patients that were an average NYHA class 2.6, suggesting that a wider degree of heart failure severity were used, which may account for the lack of LVSV change in the patients who could only exercise to <50% predicted VO2 maximum (Lapu-Bula et al. 2002). Previous work in healthy participants has also shown an increase in CO and LVSV with exercise (Le et al. 2017). However, these values were taken at peak exercise and during a cessation in exercise as the authors used breath-hold scans therefore, the values won't necessarily represent LV function in exercise, as HR will begin to decrease as soon as exercise ceases. This study was the first of its kind to assess LV function during continuous exercise due to the utilisation of free-breathing scanning techniques, which allowed for measurement of LV function whilst the participants exercised. As such, more work would be required to confirm these initial findings.

This study showed that WSS increased from rest to exercise in both healthy participants and in MR patients in the thoracic aorta. Other studies have

shown an increase in WSS from rest to exercise (Tang et al. 2006) (Suh et al. 2011) however, the novelty of this study comes from assessing the WSS of the thoracic aorta while exercising during MRI scanning inside the bore of the scanner. Previous studies in the abdominal aorta have not been able to be conducted inside the bore of the scanner, the closest has come from leg cycling in an open MRI scanner (Taylor et al. 2002; Suh et al. 2011) however, this limits the image quality due to the low magnet strength and the amount of movement available to the participants, which can cause artefacts on the scans and produce erroneous measurements. By using a specially adapted cycle ergometer, this has enabled this study to capture the thoracic aorta at rest and during exercise at a higher magnet strength which enabled better quality images to be captured. Whilst there were some scans that could not be used to due to excessive movement by the participants, overall this was not an issue and not a limiting factor to the study. Although there was a general increase in WSS from rest to exercise, this was in fact limited to certain parts of the aorta, namely the descending thoracic aorta for both healthy participants and MR patients. This is likely due to the descending thoracic aorta's shape being a straight section of the vessel, that are exposed to higher levels of shear stress, unlike the ascending aorta and the arch, which are curved and have a naturally lower WSS. Whilst the WSS increased in planes 5-8 for healthy participants, the MR patients only had an increase in WSS at plane 6.

The patterns of WSS in healthy participants shifted from rest to exercise having a greater increase on the outer curve of the aorta rather than the inner curve. Despite there being differences between the outer and inner curve the distribution of WSS became more uniform across the planes. Similarly, the MR patients also had a greater increase in shear stress mostly on the outer curvature as well as greater uniformity in shear stress distribution throughout each plane of the aorta. Although the patterns of shear stress have not shifted in favour of the inner curve it is still encouraging to see an increase in WSS, which may increase further if exercise was performed at a higher intensity.

Despite the greater LV structure and function characteristics in the MR patients this did not translate into a higher level of WSS than the healthy participants. This may be due to the low and turbulent shear stress displayed upstream of the aorta in the left ventricle, which, upon ejection into the aorta, is maintained hence the lower WSS than the healthy participants. It also may be because the MR patients had a higher CW and SW than the healthy participants. The CW and SW are higher in the MR patients because they require a greater amount of force from the left ventricle in order to eject the blood from the heart, which may be why, particularly in exercise, there is a high level of cWSS in the ascending aorta but nowhere else along the arch. In turn, this could contribute to the lack of change in WSS in the inner curve of the arch during exercise, so whilst WSS has increased overall with exercise, it is not increasing in the areas that are most susceptible to developing

atherosclerosis. Whether this is due to the exercise being supine or not a high enough intensity remains the subject of future investigations. This is the first study of its kind to measure WSS of the thoracic aorta at rest and during exercise in both healthy participants and MR patients. More work would need to be completed in order to confirm these results including testing a greater number of participants.

There are some limitations to this study, the main one being the capabilities of the scanner. Due to poor spatial resolution above a heart rate of ~110 bpm, this limits the intensity at which participants are able to exercise. If the spatial resolution of the scanner could be improved then a wider range of exercise intensities could be tested to assess whether the pattern of WSS changes across the thoracic aorta. However, the current intensity is the equivalent of going for a walk, which is a task that is achievable for most patients with CVD, and it showed an increase in shear stress which would be beneficial to protect against developing atherosclerosis. Higher intensities of exercise may cause blood pressure abnormalities in MR patients; indeed one patient already had a fall in BP during this exercise intensity that caused the test to be terminated prematurely.

Chapter 4 – Manipulating shear rate patterns using continuous and interval exercise: the impact on common femoral artery endothelial function

4.1 Introduction

The systemic effect of exercise upon shear stress patterns in the aorta has been established in the previous chapter, however the impact upon shear stress and endothelial function in the artery supplying to the exercising limb will be explored in this chapter. The common femoral artery (CFA) is susceptible to the development of atherosclerosis due to the bifurcation that exists when it splits into the superficial femoral artery and the profunda femoris (deep femoral artery). Additionally, it is one of the vital arteries in the body, responsible for carrying oxygenated blood to the muscles in the legs which is vital for movement and especially so in exercise, as the blood flow increases to meet the metabolic demand of the active muscles. Thus, it is an artery of significant interest when assessing endothelial function and shear stress patterns. At the bifurcations shear stress is low and flow disturbed. Enhanced blood flow associated with exercise may serve to ameliorate the impact of the factors on the progression of atherosclerosis. The relative impact of work-matched continuous (CON) and interval (INT) exercise upon femoral shear stress and acute endothelial function is unknown. A variety of methods have been utilised to measure shear stress in humans *in vivo* such as measuring shear stress of the brachial artery during hand-grip exercises (Atkinson et al. 2015) as well as during leg cycling (Padilla et al. 2011).

However, it is not yet known if there are any differences in the pattern and magnitude of shear stress in the common femoral artery during different types of exercise for example continuous vs interval exercise. Interval exercise has been shown to provide a greater improvement in VO_2 peak, a prognostic marker in CVD populations, compared to continuous exercise (Wisloff et al. 2007), and is thus worthy of further investigation into whether its benefits extend directly to the vasculature. A previous study by Tinken *et al.* (2009) has shown that increases in anterograde shear stresses elicit a beneficial change in endothelial function as measured by flow-mediated dilation (FMD) in the brachial artery following exercise training (Tinken et al. 2009). Many of these studies have not measured shear stress and FMD in the artery supplying blood to the working muscle during exercise and, even in those that have, only one type of exercise has been measured. It could be that, as seen in VO_2 peak, an interval approach is more beneficial than continuous exercise, or it may be that the oscillations in work rate, leading to oscillations in shear stress, are more damaging to the endothelium than a constant shear stress that would be seen from continuous exercise.

4.1.1 Principles of flow-mediated dilation

Cardiovascular health, specifically endothelial function can be assessed non-invasively by FMD. FMD is an NO-dependent measure that assesses the blood vessel's response to blood flow after a period of ischemia. A healthy individual will see an increase in artery diameter in response to occlusion as the surge in blood flow, and consequently shear stress, following cuff release will trigger an increase in NO production in the endothelium,

which will signal the VSMC layer to vasodilate. In an individual with endothelial dysfunction the artery will not dilate due to an absence in NO production, usually as a result of poor NO bioavailability.

The aims therefore of this study were to i) establish and compare the impact of work-matched continuous and interval exercise upon femoral shear stress during exercise, and ii) assess and compare the acute response of endothelial function, as measured by FMD, in continuous and interval exercise.

4.2 Methods

4.2.1 Participants

10 healthy participants (25 ± 3 years, n=5 female) free of any history of cardiovascular disease were recruited to take part in this study. Recruitment was via posters advertisements displayed around the university as well as by word of mouth. Each was given a participant information sheet to read before commencement of the study and gave their written informed consent. Participants were told that they were free to withdraw from the study at any time without reason. Ethics was granted from the University of Leeds Faculty of Biological Sciences ethics committee in accordance with the Declaration of Helsinki (BIOSCI 15-008).

Participants were screened in accordance to standard laboratory procedure. Exclusion criteria for the study included: current or a history of cardiovascular disease; pregnancy; pulmonary disease; metabolic disease; smoking;

and any musculoskeletal injury or impairment that could prevent them from completing the exercise.

4.2.2 Experimental protocol

Participants were invited to the University of Leeds exercise physiology laboratory on three separate days. The first visit involved completing a modified PAR-Q health questionnaire and giving informed consent, followed by a ramp incremental test (RIT) on a supine cycle ergometer for determination of further work rates. The second and third visits were conducted no more than one week post-RIT and separated by a minimum of 24 hours, participants' endothelial function was assessed by FMD of the CFA followed by an exercise session of either continuous or interval supine cycling at 125% lactate threshold (LT), during which ultrasound scans of the CFA were taken at regular intervals. FMD was repeated ten minutes post-exercise.

Participants were required to be overnight fasted, (at least 8 hours) and have not consumed any caffeinated or alcoholic beverages in the 12 hours preceding the visit. Additionally, participants were instructed to not partake in any strenuous exercise in the 24 hours before each assessment. For female participants testing took place in the same phase of the menstrual cycle in order to reduce variability caused by hormonal changes, participants on the oral contraceptive pill were included in this study.

4.2.3 Ramp incremental test

A supine RIT was performed for each participant in order to determine LT and thus calculate the work rate at 125% LT. The test was performed supine as this was the mode in which the exercise sessions were conducted. On entry to the laboratory participants received an explanation of the protocol and invited to ask questions before beginning. A 12-lead ECG was fitted to each participant to monitor heart rate (HR) and observe any abnormalities that may occur during maximal exercise and cause the test to be terminated prematurely. Once the ECG was positioned, participants lay supine on the couch and their feet were strapped in the pedals of the cycle ergometer. The position of the ergometer was adjusted so that participants could comfortably cycle without over-extending their knees. When this position was established, it was recorded and used for future sessions. Breath-by-breath gas exchange was measured at rest, during, and after exercise using Med-Graphics Ultima (MGC Diagnostics, USA). Participants breathed through a mouthpiece and wore a nose clip to prevent any nasal breathing. Before the test started the gas analysers were calibrated against known concentrations of oxygen and carbon dioxide in a pre-mixed gas cylinder, and airflow was calibrated against a known volume (3L) at different speeds of inspiration and expiration.

The test began with ~2 minutes of rest followed by a warm up period of 2-3 minutes at 10 W, with a cadence of 50–60 rpm. Once oxygen uptake (VO2) and rest expiratory ratio (RER) (RER = 0.75-0.9) had stabilised, the ramp section of the test at a rate of 20 W/min began. Participants were instructed

to cycle at a cadence of >50 rpm and continued to cycle until they reached volitional fatigue. Volitional fatigue was described as being unable to cycle above a cadence of 50 rpm despite strong verbal encouragement. Once this point was reached, participants entered an active recovery period where they cycled at a load of 10 W at their own pace until HR and VO2 returned back towards baseline levels.

Post-RIT in data analysis, expired gases were graphed and a 95% confidence limit was fitted to the curve; any breaths outside of these limits were considered outliers and excluded from any further analysis of LT and $VO2_{peak}$.

4.2.4 Estimation of lactate threshold

The lactate threshold was determined using the pulmonary gas exchange data generated using the V-slope method (Beaver, Wasserman, and Whipp 1986). This method uses the inflection point of the VO2 against volume of exhaled carbon dioxide (VCO2) curve to non-invasively estimate LT. An estimated value of LT was produced by at least two researchers and the average was reported. This value was then used to calculate 125% LT and subsequently the work load at which the participants would be exercising for both the CON and INT protocols.

4.2.5 Assessment of common femoral artery endothelial function

FMD was measured to assess the endothelial function of the CFA. Participants lay in the supine position completely at rest in a quiet, dark, temperature-controlled room for ten minutes before a resting baseline measurement Doppler blood velocity was recorded in Duplex mode on a Vivid E9 ultrasound machine for 20 seconds at a rate of 15 frames per second. Blood flow measurements were assessed by ultrasound using a 10 MHz 9L linear array probe was used in B-mode to obtain high-resolution images and the probe was placed ~2-3 cm proximal to the CFA bifurcation. The angle of insonation used was 60° in order to obtain the clearest image and most accurate measurement of blood velocity. A blood pressure cuff was placed ~10 cm below the inguinal ligament and inflated to 200 mmHg for 5 minutes. Images were recorded 4 minutes 30 seconds into occlusion and lasted for a total of 180 seconds. Images were live streamed from the ultrasound machine to a PC and recorded with Vascular Imager. In order to replicate the scans between visits, the previous scan was used to act as a guide and only when the images matched were any measurements taken. Edge-detection software was used to analyse CFA diameter to reduce experimenter bias, and any images with a confidence value <70 % were discarded.

In order to ensure minimal error by poor scanning technique, training was undertaken over a period of nine months, including assessing the reliability of ultrasound scans in the common femoral artery, before any participants were enrolled in the study. Reliability assessments involved recruiting 10 healthy participants to undergo scans of the common femoral artery at rest and a measurement of FMD on two separate occasions. The technique is

the same as described above for the exercise sessions. This process yielded a coefficient of variation score of 10.6% was deemed a suitable error to be able to conduct ultrasound scans with minimal risk of incorrect data due to poor scanning technique.

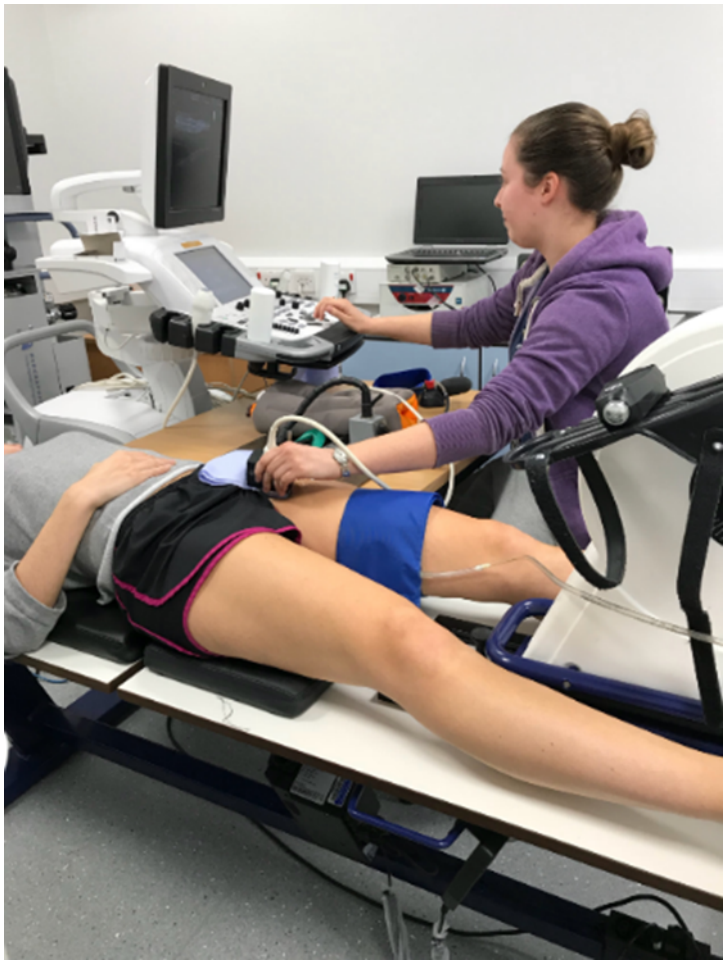


Figure 4. 1 Common femoral artery FMD scan set-up

FMD procedure in the common femoral artery. Participants lie supine with legs extended while the probe is placed on their common femoral artery. A blood pressure cuff was located ~10 cm below the inguinal ligament and inflated to >200 mmHg for 5 minutes to occlude blood flow.

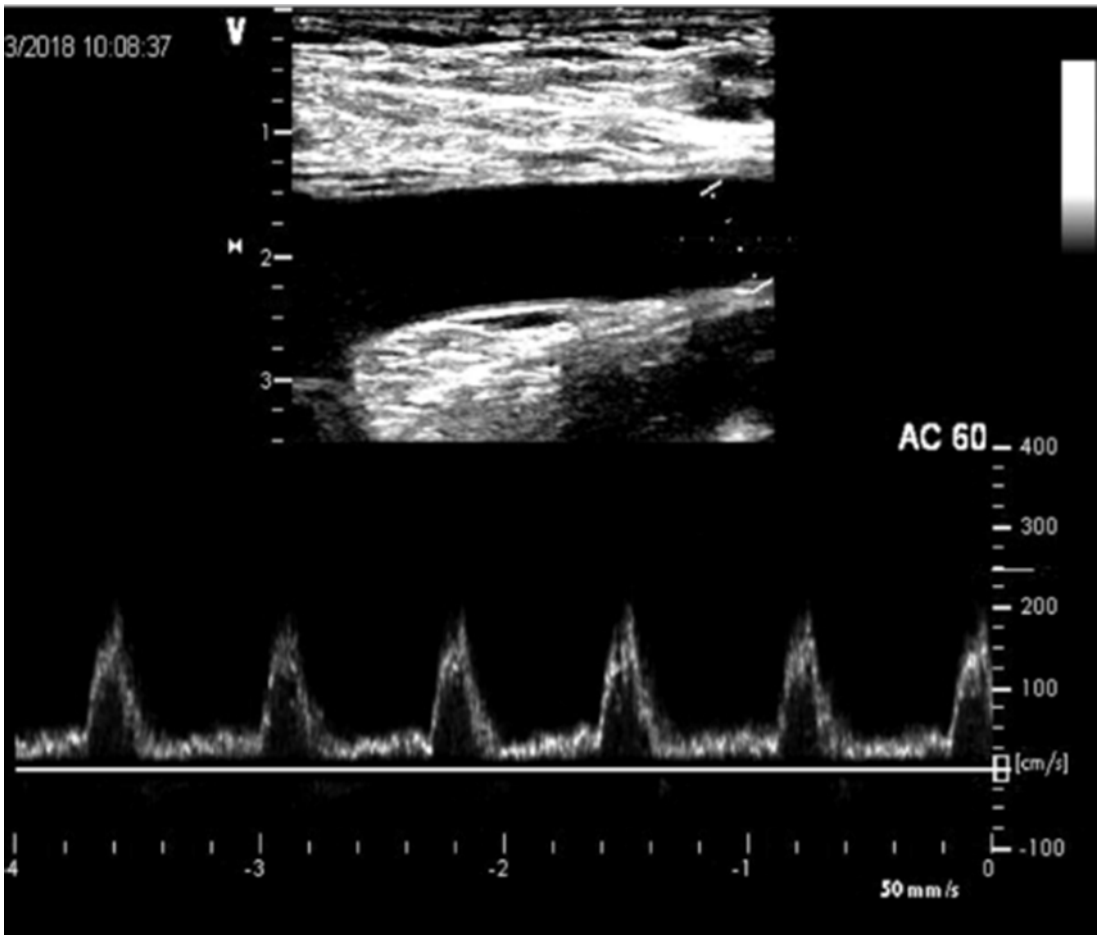


Figure 4. 2 Representative ultrasound image of the common femoral artery

Ultrasound image of the common femoral artery (top) and blood flow velocity trace (bottom) in the off-transient of exercise.

4.2.6 Exercise protocols and in-exercise measures

In order to assess shear stress patterns throughout both continuous (CON) and interval (INT) exercise two intensity-matched protocols were used. Both protocols were conducted at a work rate equivalent to 125% LT as determined by the RIT. The CON exercise bout consisted of 13.5 minutes of supine cycling, whilst the INT bout consisted of 12 repetitions of 60 seconds work interspersed with repeated rest periods of 60 seconds. Pulmonary gas exchange from breathing through the mouthpiece (as described 4.2.3) and

heart rate were recorded throughout exercise via Medgraphics and 12-lead ECG respectively. For the purposes of assessing shear the 10 MHz linear transducer was placed upon the common femoral artery, as described above (4.2.4.2), during brief cessations in exercise. Due to the location of the common femoral artery it was not possible to scan continuously throughout cycling exercise due to probe displacement by leg movement. Therefore, participants stopped cycling with a fully extended right leg and recordings were taken immediately for 30 seconds of the off-transient of exercise. During the CON protocol, participants paused for 30 seconds on four occasions (Figure 4.3). During INT exercise ultrasound scans for 30 seconds were obtained during the rest periods (Figure 4.3).

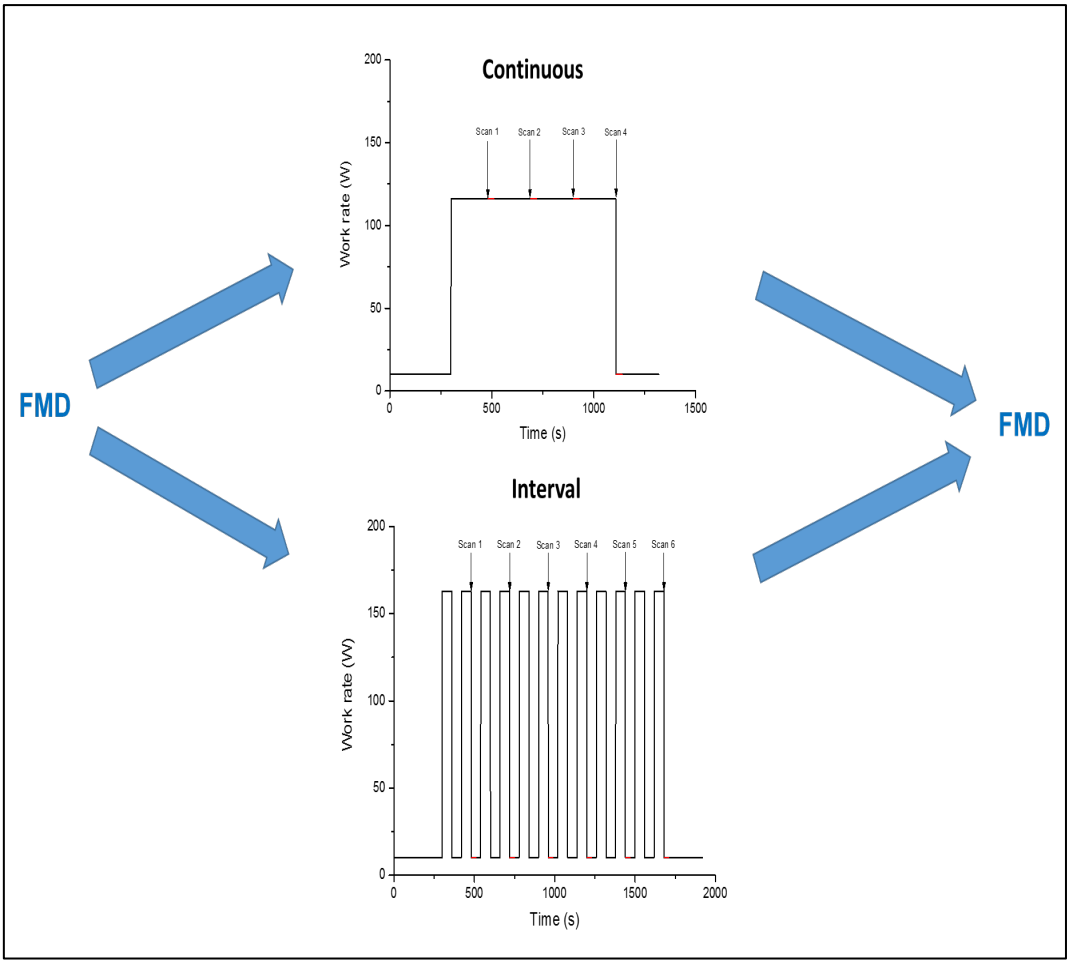


Figure 4. 3 Exercise session protocol schematic

Exercise session protocols using mean work rate from all participants. Red lines indicate 30 s scanning periods. FMD was assessed pre- and 10 minutes post-exercise on both visits. Exercise protocols were matched for total work done at 125% LT, and duration (12 minutes).

For the CON protocol, participants were instructed to cycle immediately after the scan at the prescribed work rate. However during the INT protocol, in order to retain the work-matched element of the study, participants were not instructed to cycle until 10 seconds before the work interval started in order to prepare for the higher work rate and reduce the inertia of the fly wheel. These 10 seconds were unloaded pedalling.

4.2.7 Analysis of data

All pulmonary gas exchange and HR data were analysed in OriginPro. Ultrasound scans were analysed in Brachial Analyser to determine arterial diameter and blood flow velocities. These values were then exported to an Excel spreadsheet where volumetric flow, shear rate and OSI were calculated.

$$Volumetric\ flow\ (mL\ min^{-1}) = (cross\ sectional\ area \times mean\ flow) \times 60$$

$$Shear\ rate\ (s^{-1}) = \left(\frac{mean\ flow}{artery\ diameter}\right) \times 8$$

$$OSI = \frac{retrograde\ shear}{(retrograde\ shear + anterograde\ shear)}$$

Cross sectional area (cm^2), mean flow ($mL\ min^{-1}$), artery diameter (cm).

The shear stress patterns recorded during cycling were calculated using an assumption of blood viscosity at high shear rates. Blood viscosity here is an assumption based upon previous research at high shear stress. The relationship between blood viscosity and shear rate is linear up until ~100 s^{-1} where it plateaus between 3-4 mPa.s at 37°C (Chien 1970). A more recent study has corroborated these findings with a blood viscosity value of 3.26 ± 0.43 mPa.s at a shear rate of 100 s^{-1} at a temperature of 37°C (Rosenson, McCormick, and Uretz 1996). Shear rate is not the only factor which affects blood viscosity, it is also sensitive to: temperature; haematocrit and other components of blood; viscosity of plasma; age of red blood cells; disease state; and gender (Roberston, Sequeira, and Kameneva 2008). In regards to gender, females have a lower blood viscosity than males at a shear rate of

100 s^{-1}, and temperature of 37°C (females: 2.93 ± 0.43 mPa.s; males 3.39 ± 0.34 mPa.s; P < 0.0001) (Rosenson, McCormick, and Uretz 1996). When temperature increases blood viscosity decreases, for example from 22°C to 37°C, viscosity decreases from 6.3 mPa.s to 3.8 mPa.s (Rand et al. 1964). As participants underwent exercise protocols in the heavy intensity domain that would have elicited an increase in body temperature, it is fair to assume that this will cause a decrease in blood viscosity. With these factors (gender and temperature) in mind, we have chosen to use 3 mPa.s as the viscosity constant, at the lower end of the 3-4 mPa.s plateau as shown previously.

Statistical analyses were completed in SPSS. Statistics used were one-way ANOVA or T test and have been stated in the corresponding figure legends. Data are presented as mean ± SD unless otherwise stated. A P value of $P<0.05$ was accepted as statistically significant.

4.3 Results

4.3.1 Participant characteristics

Participant characteristics from this study have been summarised and can be seen in table 4.1.

Table 4. 1 Participant characteristics.

Data are reported as mean ± SD where appropriate.

Subject Characteristics	
Participants (% female)	10 (50%)
Age (yr)	26 ± 2
Height (m)	1.71 ± 0.08
Body mass (kg)	70.4 ± 12.7
RIT max work (W)	212 ± 35
RIT VO_2 peak (L/min)	2.66 ± 0.41
CON work rate (W)	116 ± 24
INT work rate (W)	163 ± 37

4.3.2 Heart rate and blood flow

Participants reached a steady state, confirmed by the VO2 output, during the CON protocol and HR remained consistent throughout (113±7 bpm; P>0.05, one-way ANOVA). In contrast heart rate in the INT protocol fluctuated in response to the bouts of exercise and rest, but end bout HR appeared to plateau and remained consistent throughout the protocol (133±9 bpm; P>0.05, one-way ANOVA).

Blood flow measurements mirrored the HR response, as peak blood flow remained constant throughout the CON protocol (5575±1517 mL/min; P>0.05, one-way ANOVA) (Figure 4.4a). In the INT protocol, the blood flow also mirrored the HR pattern, and peak blood flow appeared to plateau and remained consistent throughout the protocol (6308±1442 mL/min; P>0.05, one-way ANOVA) (Figure 4.4b). In comparing the two protocols, there was no difference in peak (CON: 5575±1517 mL/min; INT: 6308±1442 mL/min;

P>0.05, Ttest) or mean blood flow (CON: 5151±1250 mL/min; INT: 5642±1284 mL/min; P>0.05, Ttest).

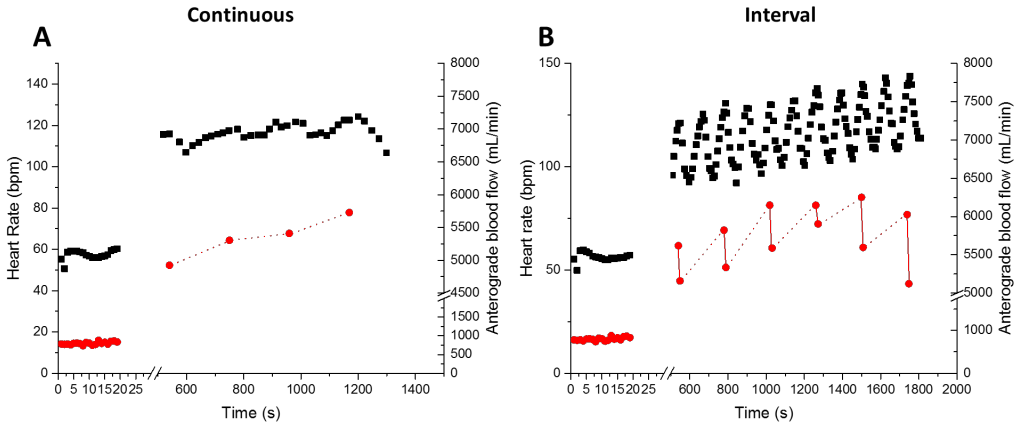


Figure 4. 4 Heart rate and blood flow in continuous and interval exercise

Heart rate (black squares) and anterograde blood flow (red circles) at rest and during exercise for continuous (panel A) and interval (panel B) protocols. Dotted lines show an inferred relationship between data points and solid lines show a change in blood flow over time. No alteration in mean heart rate and blood flow were observed in either protocol during exercise (P>0.05). Mean heart rate and blood flow increased from rest to exercise in both protocols (P<0.05). N=10.

4.3.4 Shear stress

4.3.4.1 Anterograde and retrograde

Anterograde shear stress increased significantly from resting values (P<0.05) but reached a plateau during exercise in both protocols, and there was no difference in mean (CON: 1169±370 mPa; INT 1362±478 mPa; P>0.05) and peak (1292±412 mPa; INT: 1521±525 mPa; P>0.05) shear stress between CON and INT exercise. There was no difference between CON and INT mean (CON: 54±68 mPa; INT: 28±19 mPa; P>0.05) and peak

(CON: 93±96 mPa; INT: 77±38 mPa; P>0.05) retrograde shear stress, which does not significantly change throughout each protocol.

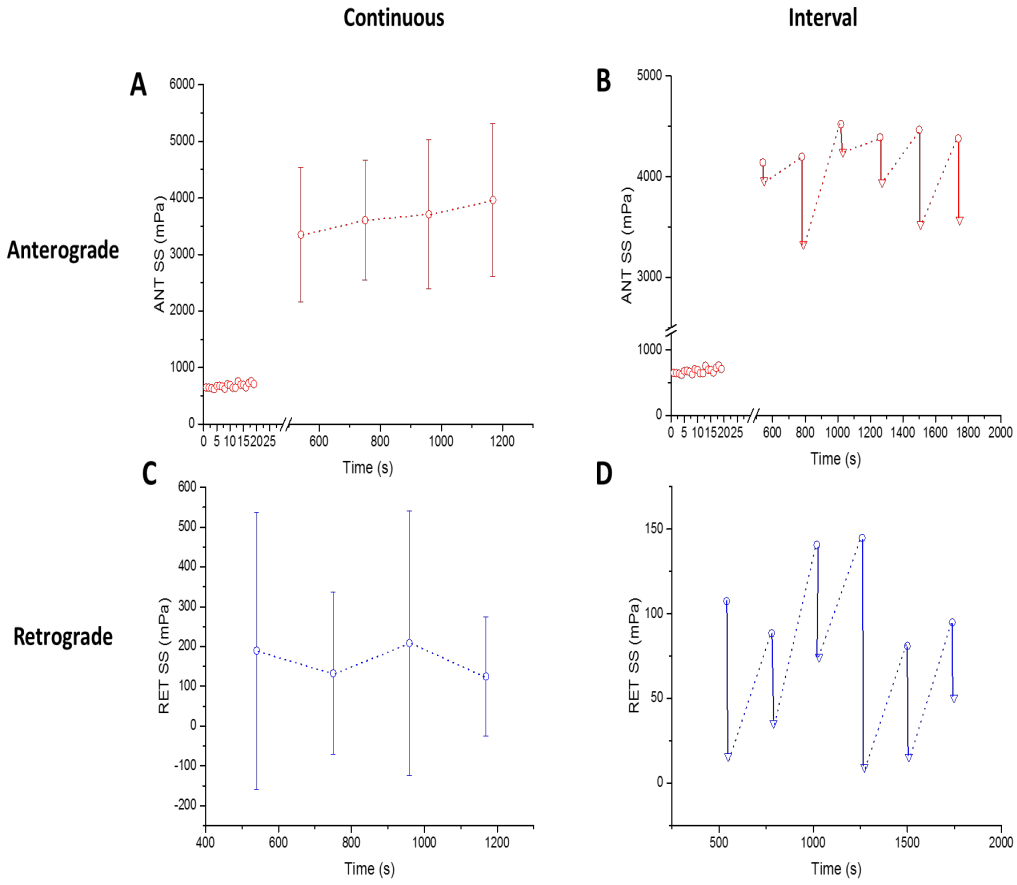


Figure 4. 5 Anterograde and retrograde shear stress in continuous and interval exercise

Anterograde (panels A and B) and retrograde (panels C and D) shear stress in the off-transient of CON (panels A and C) and INT (panels B and D) exercise protocols. There was no difference in peak anterograde or retrograde shear stress between protocols (P>0.05) or during protocols (P>0.05). Dotted lines indicate an inferred relationship between data points and solid lines show a change in shear stress over time. N=10.

4.3.4.2 Total shear stress

The results show that there was a greater total anterograde shear stress volume in interval than continuous exercise (P<0.05) however, there was no

difference in total retrograde shear stress volumes between protocols (P>0.05).

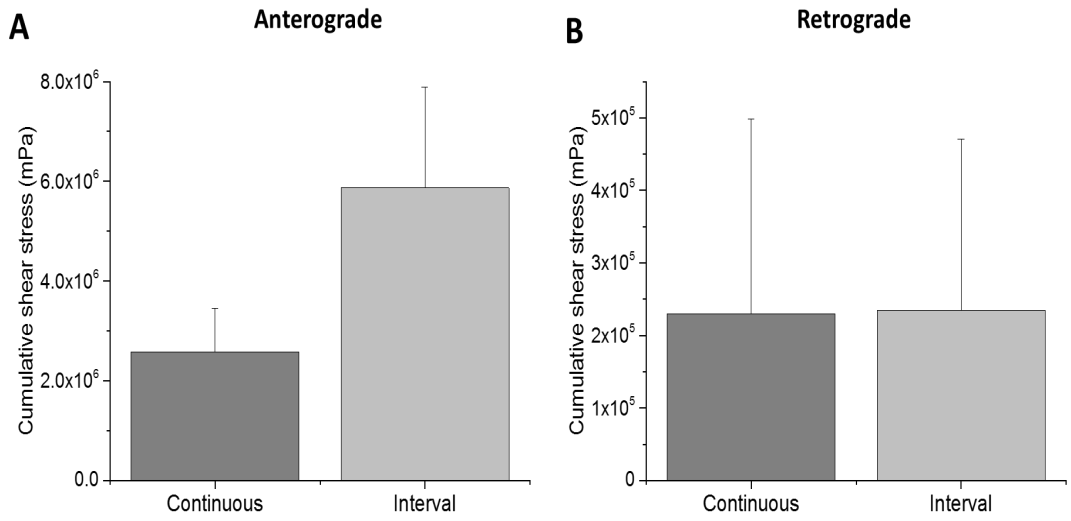


Figure 4. 6 Total anterograde and retrograde shear stress in continuous and interval exercise

Total anterograde and retrograde shear stress for continuous and interval exercise. Total anterograde shear stress (panel A) was greater in the interval than continuous protocol (P<0.05). There was no difference in retrograde shear stress between the two protocols (panel B) (P>0.05). N=10.

4.3.4.3 Oscillatory Shear Index

Oscillatory shear index (OSI) is a ratio of anterograde to retrograde shear stress and indicates the level of oscillation between anterograde and retrograde shear stress. OSI mean (CON: 0.05±0.06 AU; INT: 0.04±0.04 AU; P>0.05) and peak (CON: 0.08±0.08 AU; INT: 0.09±0.06; P>0.05) did not alter between protocols, and no time was spent in purely oscillatory shear (>0.5 AU) in either CON or INT protocol (Figure 7).

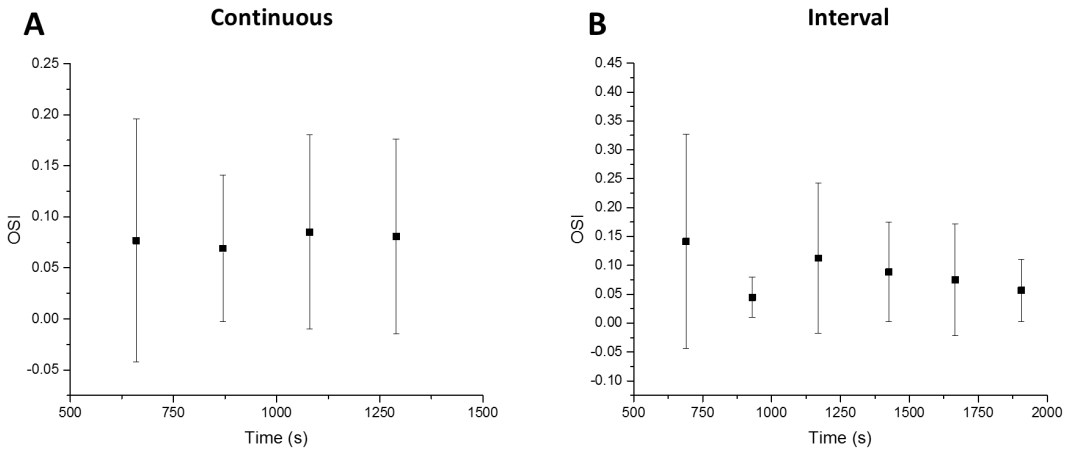


Figure 4. 7 Oscillatory shear index in continuous and interval exercise

Peak oscillatory shear index assessment during the off-transient of exercise for CON (panel A) and INT (panel B) exercise. OSI remained constant throughout both protocols (P>0.05) and was not different between protocols (P>0.05). N=10.

4.3.4 FMD

FMD was assessed pre- and acutely post-exercise in the common femoral artery, as described above. There were no significant changes in FMD% in response to an acute bout of either CON (pre: 9.9±8.1%, post: 8.4±6.2%, P>0.05) or INT (pre: 9.1±5.9%, post: 6.6±3.4%, P>0.05) exercise when measured acutely 10 minutes post-exercise (Figure 4.8).

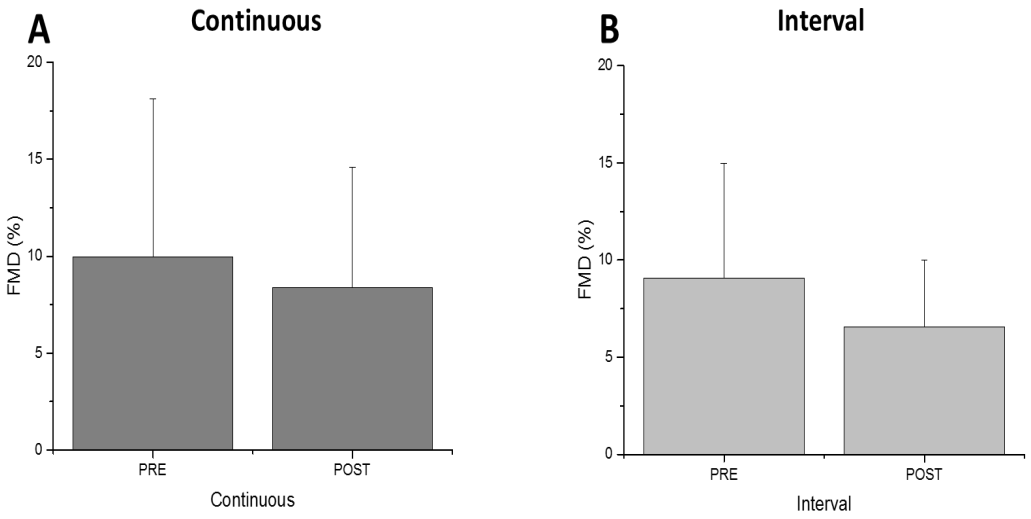


Figure 4. 8 FMD of the common femoral artery pre- and post- exercise for continuous and interval sessions

Common femoral artery FMD % does not change pre- to post-exercise in either continuous (panel A) or interval (panel B) protocol (P>0.05). Data are group mean ± SD, n = 10.

In order to determine if there were any alterations in the drivers of FMD pre- to post-exercise or between protocols, area under the curve (AUC) 60 and 90, and peak hyperaemia were assessed. There was no difference in AUC60 pre- to post-exercise in CON (pre: 4324±1611; post: 3988±1611; P>0.05) or INT (pre: 4347±1657; post: 4556±2025; P>0.05) however; the INT protocol had a significantly higher AUC60 than the CON protocol post-exercise (CON: 3988±1611; INT: 4556±2025; P<0.05). Similarly, there was no difference in AUC90 pre- to post-exercise in either CON (pre: 8642±3167; post: 7958±3361; P>0.05) or INT (pre: 8895±3455; post: 9323±4659; P>0.05) protocol, and AUC90 did not alter post-exercise between protocols (CON: 7958±3361; INT: 9323±4659; P>0.05). In addition, peak hyperaemia did not differ pre- to post-exercise for CON (pre: 16.8±5.8 cm/s; post:

22.4±17.1 cm/s; P>0.05) or INT (pre: 24.9±11.8 cm/s; post: 33±23.1 cm/s; P>0.05) exercise, and peak hyperaemia was not significantly different post-exercise in either CON or INT protocol (CON: 22.4±17.1 cm/s; INT: 33±23.1 cm/s; P>0.05).

4.4 Discussion

The aim of this study was to determine if there were any differences in shear stress patterns in the artery supplying blood to the working limbs with different exercise protocols and whether this subsequently had an effect upon endothelial function acutely post-exercise. The above-presented results have shown that whilst there is no difference in peak and mean shear stress, the total anterograde shear stress experienced by the CFA in each protocol is markedly different, as INT exercise has a significantly higher total volume of anterograde shear stress than the CON protocol. Interestingly, OSI did not reach above 0.5 AU, the threshold for pure oscillatory shear. This is encouraging as oscillating and disturbed patterns of shear stress are attributed to endothelial inflammation resulting in decreased eNOS (Gambillara et al. 2006), and increased VCAM-1 (Chappell et al. 1998), ICAM-1 (Hsiai et al. 2003), and MCP-1 (Hwang et al. 2003).

As the FMD% didn’t change following each protocol, it cannot be determined immediately as to whether this is beneficial or detrimental to the vasculature. The drivers of FMD (AUC60, AUC90, and peak hyperaemia) showed no dif-

ference pre- to post-exercise thus indicating why FMD% did not change immediately post-exercise. As there was no increase or decrease in ischemia-induced shear stress or peak hyperaemia, there cannot be a change in FMD% pre- to post-exercise. The greater exposure to high shear stress could be beneficial to the endothelium and promote an increase in NO bioavailability. However, this does not line up with the results of the FMD data, which shows that there's no difference in endothelial function pre- to post-exercise suggesting that the greater total shear stress is not translating to benefits to the endothelium on a cellular level, at least, not acutely. This is backed up by a previous study by Dawson et al. (2008) who showed that acutely post exercise brachial FMD showed no change and the femoral FMD was decreased (Dawson et al. 2008). Furthermore, in a study that examined the brachial artery vasodilatory response to bouts of fast and slow contractions in hand-grip exercise showed that acutely (30 minutes) post-exercise there was no change in brachial FMD after the fast contractions and a significant reduction in FMD post-exercise for the slow contractions, compared to resting measures (Gonzales et al. 2011). Finally in a study of brachial FMD response post leg-exercise at different intensities in a laboratory-based setting also showed that FMD either did not change (50% HRmax) or decreased (70 and 85% HRmax) when compared to a resting baseline (Birk et al. 2013). No matter the mode in which exercise was conducted, these studies (and many others) have all shown the same results: brachial artery FMD does not change or decreases acutely post-exercise. It is interesting to see that the CFA behaves in the same manner as the brachial artery in this respect indicating that whether exercise is local or systemic to the artery of in-

terest, the immediate effect of exercise is that it does not confer a positive effect upon endothelial function.

There have been other studies that have demonstrated an increase in FMD post-exercise. (Zhu et al. 2010; Tinken et al. 2009; Harvey et al. 2005; Harris et al. 2008). All of the above mentioned studies that saw an improvement in brachial FMD were conducted 1 hour post-exercise, except Tinken et al. (2009) where the measurements were taken 5 minutes post-exercise (Tinken et al. 2009). This, alongside the studies that showed a decrease in FMD post-exercise, indicates that in many cases improvements in endothelial function are delayed until at least an hour post-exercise. However, the time period for which beneficial effects, if any, are to be seen in the CFA remains as yet unknown and would be the subject of future investigations.

The mechanism of action for this apparent nadir in FMD followed by an increase ~1 hour post-exercise is currently the subject of speculation as no one is sure as to why this happens. Perhaps however, this should not be a completely surprising result. Experiments conducted *in vitro* have indicated that endothelial cells exhibit a biphasic response to an increase in shear stress with an initial surge in inflammatory markers such as MCP-1 (Shyy et al. 1994), VCAM-1 and ICAM-1 and a down-regulation of eNOS, followed by an increase in anti-inflammatory markers.

In order to determine the effect of shear stress generated by CON and INT exercise on the endothelium a more in-depth analysis needs to be undertaken to understand the mechanism of shear stress in these two protocols and how that affects the endothelium over time at a molecular level.

The main limitation to this study is the small sample size of participants. Whilst they were of a similar age and self-reported as recreationally active, there was likely some heterogeneity among the participants response to exercise and outliers. Having a larger sample size would enable easier identification of outliers and generate a more robust statistical output. If this study were to be repeated tor expanded into a training study then the sample size should be larger. As an assessment of reliability was made prior to enrolling participants into the study, it is unlikely that any variation between scans was down to operator error.

Chapter 5 – replicating *in vivo* shear stress patterns on endothelial cells *in vitro*

5.1 Introduction

The two previous chapters have explored the effects of exercise on shear stress systemically and locally to the exercising muscle, and by utilising FMD as a measure of endothelial function, the effects of endothelium to exercise have been explored. In this chapter, this was taken one step further by analysing the gene expression of anti-inflammatory and inflammatory markers implicated in endothelial dysfunction and the development of atherosclerosis, to understand the mechanistic effect of exercise upon endothelial cells.

To develop a deeper understanding of how exercise effects endothelial function, there needs to be a mechanistic approach. In the healthy endothelium under chronic high laminar shear stress there is an abundance of NO, KLF2 and KLF4 conferring an anti-inflammatory effect (Niu et al. 2019) however there are areas of the vasculature that have an inflammatory phenotype at areas of curvature and bifurcations due to the presence of low and oscillatory shear stress, characterised by increased levels of MCP-1, VCAM-1 and ICAM-1 (Shyy et al. 1994). What is not known is whether heavy intensity exercise is going to exacerbate the inflammatory response or enhance the anti-inflammatory response and if INT or CON exercise would be a better method of delivery to enhance any anti-inflammatory effects of the increased shear

stress. If high-intensity exercise by either CON or INT exercise is found to enhance the anti-inflammatory response in an area traditionally associated with plaque development, it could be used as potential preventative measure or therapy to those at risk or already have atherosclerosis to regress existing plaques and reduce the blood flow restriction and subsequently the associated cardiac events that come from developing atherosclerosis. Based upon the results of the previous chapter and that of the existing literature it was hypothesised that INT exercise would exhibit a greater amount of anti-inflammatory gene expression than the CON and rest protocols.

The aims of this study were to i) replicate the shear stress measured in the femoral artery during cycling in chapter 4 across human coronary artery endothelial cell in vitro; ii) assess the anti-inflammatory and inflammatory response of these endothelial cells; and iii) examine the time course of related gene expression changes.

5.2 Methods

5.2.1 Cell culture

Human coronary artery endothelial cells (HCAEC) from donors were purchased from PromoCell (PromoCell GmbH, Heidelberg, Germany). Cells were cultured in endothelial cell growth medium (PromoCell GmbH, Heidelberg, Germany) in a T75 flask (Sarstedt AG & Co. KG, Nümbrecht, Germany) in a 37 °C and 5% CO_2 incubator until passage 3-5.

5.2.2 Ibidi pump parallel plate system

HCAEC at passage 3-5 were seeded onto a 1% gelatin coated Ibidi µ 0.4 Luer slides at a density of ~250,000 cells/slide in 120 µL endothelial cell growth medium. After a period of 24h cells were assessed for a confluent monolayer. The slide was then attached to the Ibidi pump as per the manufacturer's instructions. Flow parameters were inputted to the Ibidi pump controller software (v 1.5.3) and the cells were exposed to flowing media by the pressure from the pumps feeding into the reservoirs of the fluidic units. The parameters studied to replicate the exercise sessions detailed in chapter 4, followed by a period of rest were: i) 43 dynes/cm^2 for one minute then 33 dynes/cm^2 for one minute, repeated 15 times followed by 24 hours at 6 dynes/cm^2 (interval protocol); ii) 36 dynes/cm^2 for 30 minutes followed by 24 hours at 6 dynes/cm^2 (continuous protocol); or iii) 6 dynes/cm^2 for 24.5 hours (rest protocol). Slides and pump apparatus were enclosed in a cell culture incubator at 37 °C and 5% CO_2.

5.2.3 RNA extraction from endothelial cells

After the HCAECs had been exposed to the prescribed shear stress, the slide was removed from the Ibidi flow unit and the media was removed from the slide and replaced with 150 µL PBS to wash the cells by pipetting the PBS up and down the slide 3-4 times. The lysis buffer was prepared by adding 0.01% of β-mercaptoethanol into the RLT lysis buffer. The PBS was removed and replaced with 150 µL lysis buffer and incubated at room tem-

perature for 3-5 minutes. After checking cells had been lysed, the lysis buffer was removed from the slide, placed into an Eppendorf tube, and topped up with a further 200 µL lysis buffer. Total RNA was extracted using the RNeasy mini kit following the manufacturer's instructions. RNA concentration and purity were assessed by spectrophotometer using a Nano Drop ND-1000 (Life Technologies). Analysis of RNA quality involved measurement of optical density to determine 260-280 nm and 260-230 nm ratios using the ND-1000 v 3.7.0 software.

5.2.4 cDNA synthesis

Gene expression was assessed by quantitative real-time PCR (qRT-PCR). Total RNA at a concentration of 200-250 ng/µL was used for reverse transcription and cDNA synthesis using the iScript cDNA synthesis kit. Constituents for the reaction were: 4 µL of 5x iScript reaction mix; 1 µL iScript reverse transcriptase; x µL RNA template; and x µL nuclease-free water to give a total reaction volume of 20 µL. A thermal cycler was used to synthesis the cDNA. The profile for the reaction was: step 1 at 25 °C for 5 mins; step 2 at 42 °C for 30 mins, step 3 at 85 °C for 5 mins; and step 4 at 4 °C for 10 mins.

5.2.5 qRT-PCR

A 384 well plate was used to set up these experiments. A total of 10 µL reaction volume was used in each well consisting of 4.4 µL cDNA and 5.6 µL of qRT-PCR SsoAdvanced universal SYBR Green mastermix (0.3 µL forward

primer, 0.3 µL reverse primer, and 5 µL SsoAdvanced SYBR Green mastermix was added per well). The reaction was carried out in triplicate for each gene of interest. The 384 well plate was sealed with a clear adhesive film and centrifuged at 1000 rpm for 2 mins. All qRT-PCR experiments were conducted using a CFX384 Real-time instrument (Bio-Rad, CA, USA). The profile used was: step 1 at 95 °C for 3 mins; step 2 at 95 °C for 5 secs; step3 at 60 °C for 30 seconds x 40 repeats.

Table 5. 1 Table of primer sequences used for qRT-PCR

Gene Name	Forward sequence	Reverse sequence
HPRT	TTGGTCAGGCAGTATAATCC	GGGCATATCCTACAACAAAC
eNOS	TGAAGCACCTGGAGAATGAG	TTGACCATCTCCTGATGGAA
MCP-1	GCAGAAGTGGGTTCAGGATT	TGGGTTGTGGAGTGAGTGTT
KLF4	GAACCCACACAGGTGAGAAA	CCCGTGTGTTTACGGTAGTG
VCAM-1	CATTGACTTGCAGCACCACA	AGATGTGGTCCCCTCATTCG
ICAM-1	CACAAGCCACGCCTCCCTGAACCTA	TGTGGGCCTTTGTGTTTTGATGCTA

5.2.4 Analysis of data

Analysis of qRT-PCR data were conducted via the Bio-Rad CFX manager 3.0 software, which determined the Ct value for each gene under investigation. These data were subsequently analysed using the $2^{-\Delta CT}$ method. Statistical analyses were conducted in SPSS using linear mixed model to examine differences in parameters between time and protocol (time and condition) as within subject effects and have been stated where appropriate. Data are presented as mean ± SD unless otherwise stated, and data are accepted as statistically significant at $P<0.05$.

5.3 Results

5.3.1 Anti-inflammatory gene expression

Gene expression relative to a housekeeping gene (HPRT) was measured for anti-inflammatory genes KLF4 and eNOS. Whilst no significant changes were observed for KLF4, there was a greater level of eNOS at the 24 hour time point compared to at 2 hours (time effect, $P<0.05$). There was a 1800%, 2182%, and 2% increase in eNOS expression from 2 to 24 h for rest, interval and continuous protocols, respectively (figure 5.1). There were no condition*time interactions for either gene.

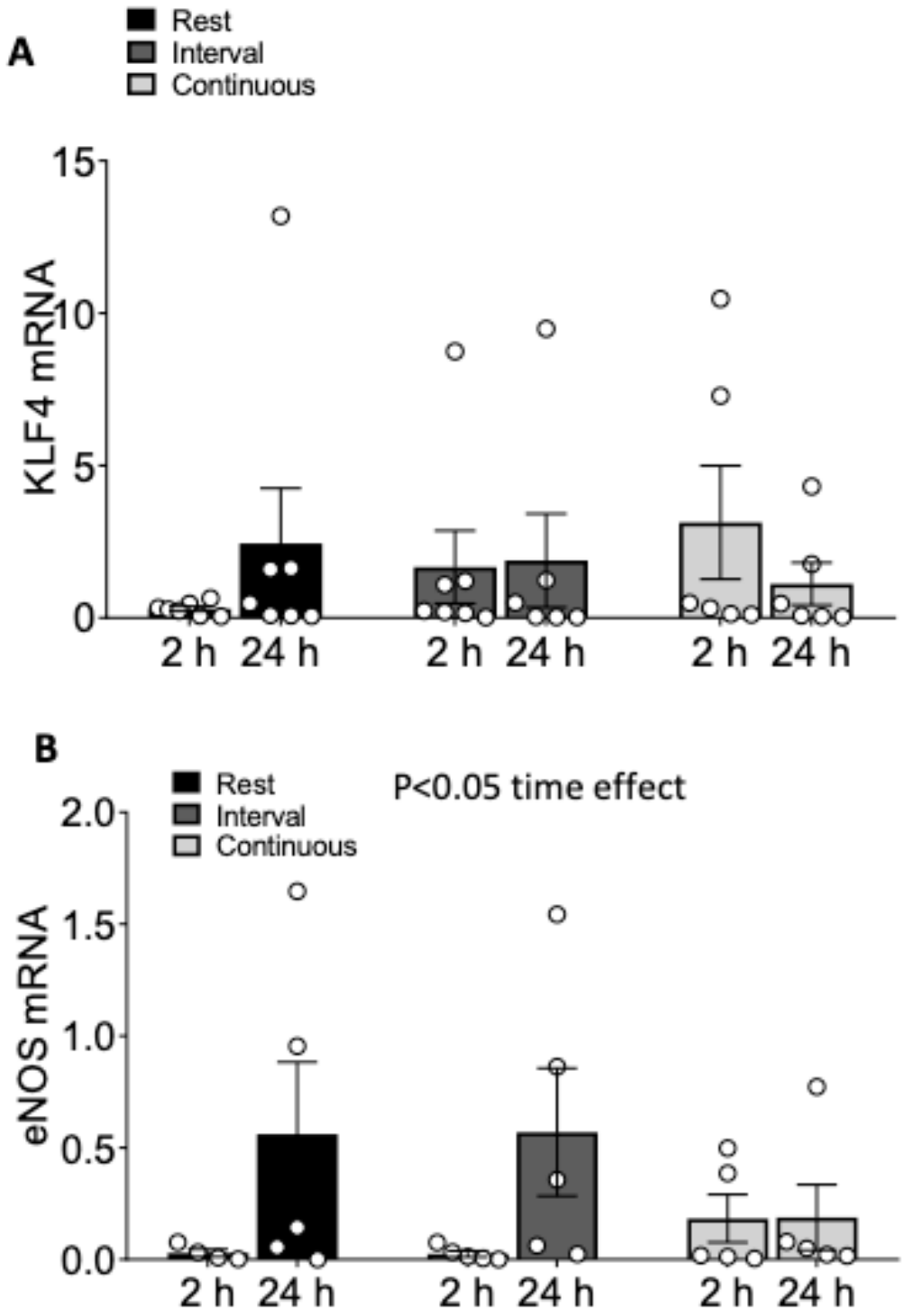


Figure 5. 1 Anti-inflammatory gene expression at 2 and 24 hours post-intervention

Anti-inflammatory gene expression relative to HPRT in HCAECs 2 and 24 h post-intervention. A) KLF4, B) eNOS (time effect, P<0.05).

5.3.2 Inflammatory gene expression

Inflammatory gene expression was measured in MCP-1, VCAM-1 and ICAM-1. Whilst VCAM-1 showed no changes with condition or time, both MCP-1 and ICAM-1 showed that gene expression decreased from 2 hours to 24 hours (time effect, $P<0.05$). MCP-1 had a decrease of 69%, 88%, and 85% from 2 to 24 h for rest, interval, and continuous protocols, respectively. There was a decrease in ICAM-1 from 2 to 24 hours of 98%, 97%, and 96% for rest, interval, and continuous protocols, respectively (figure 5.2). There were no condition*time interactions for either genes.

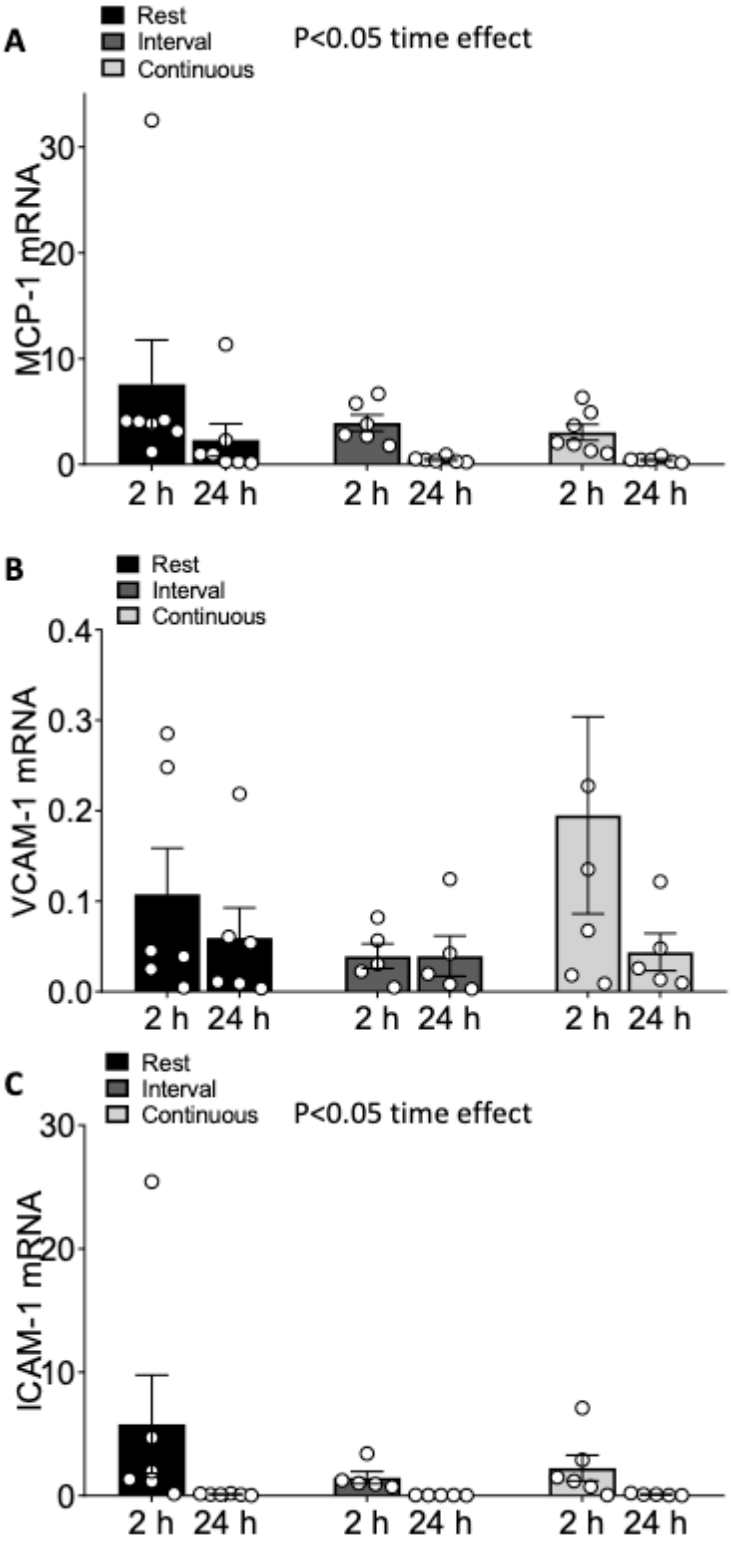


Figure 5. 2 Inflammatory gene expression 2 and 24 hours post-intervention

Inflammatory gene expression relative to HPRT in HCAECs 2 and 24 h post-intervention. A) MCP-1 (time effect, P<0.05), B) VCAM-1, C) ICAM-1 (time effect, P<0.05).

5.3.3 Correlation of gene expression

Gene expression of anti-inflammatory and pro-inflammatory markers in HCAECs were correlated in total and at the 2 h and 24 h time points. Overall there was a positive correlation of KLF4 and eNOS (P<0.05) as well as a positive correlation of MCP-1 and ICAM-1 (P<0.05). At the 2 h time point there were positive correlations between; KLF4 and eNOS (P<0.05), eNOS and VCAM-1 (P<0.05), and MCP-1 and ICAM-1 (P<0.05). At the 24 h time point there were positive correlations between; KLF4 and eNOS (P<0.05), KLF4 and MCP-1 (P<0.05), eNOS and MCP-1 (P<0.05), and VCAM-1 and ICAM-1 (P<0.05).

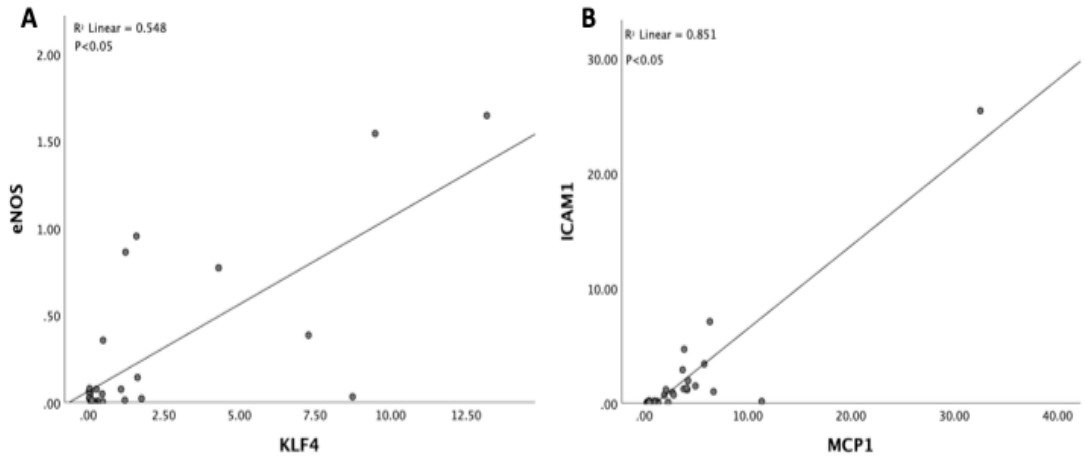


Figure 5. 3 Correlations between gene expression at all time points

Correlations between genes expressed post-intervention for all groups and time points. A) KLF4 and eNOS (P<0.05), B) MCP-1 and ICAM-1 (P<0.05).

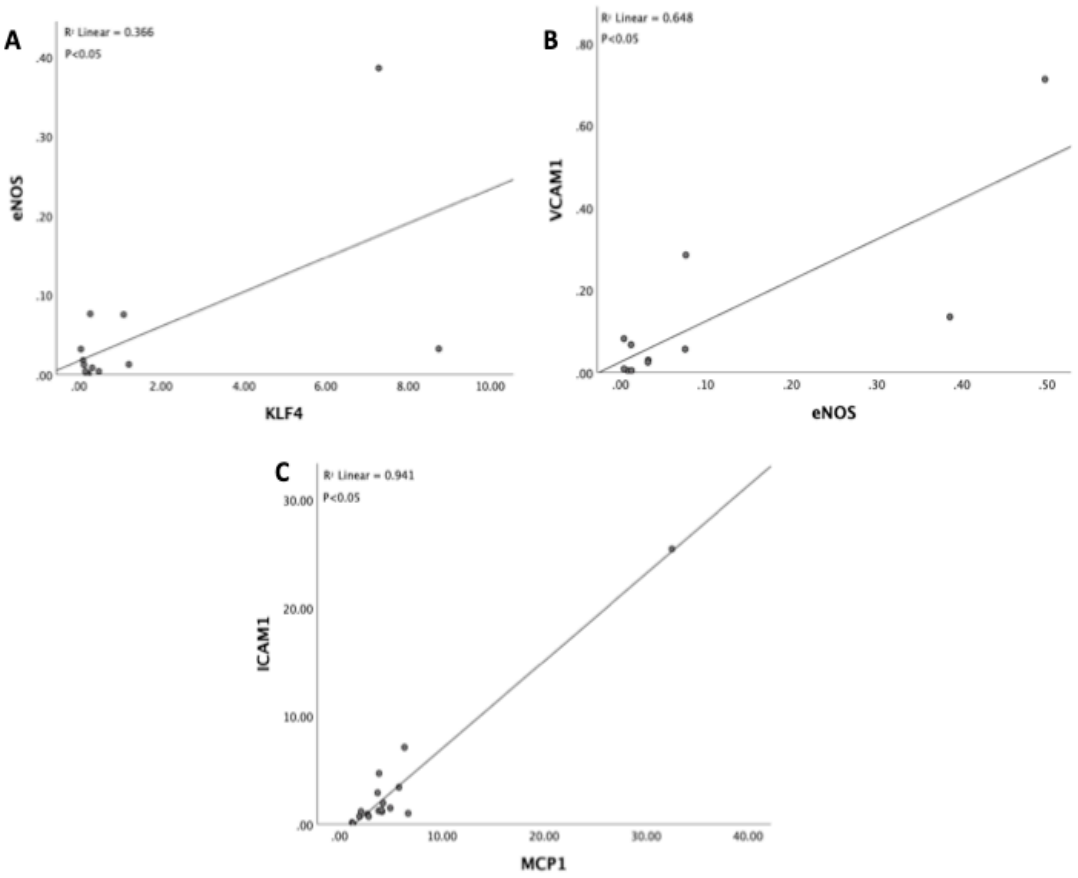


Figure 5. 4 Correlations between genes expressed at 2 hours post-intervention

Correlations between genes expressed at 2 h post-intervention. A) KLF4 and eNOS (P<0.05), B) eNOS and VCAM-1 (P<0.05), C) MCP-1 and ICAM-1 (P<0.05).

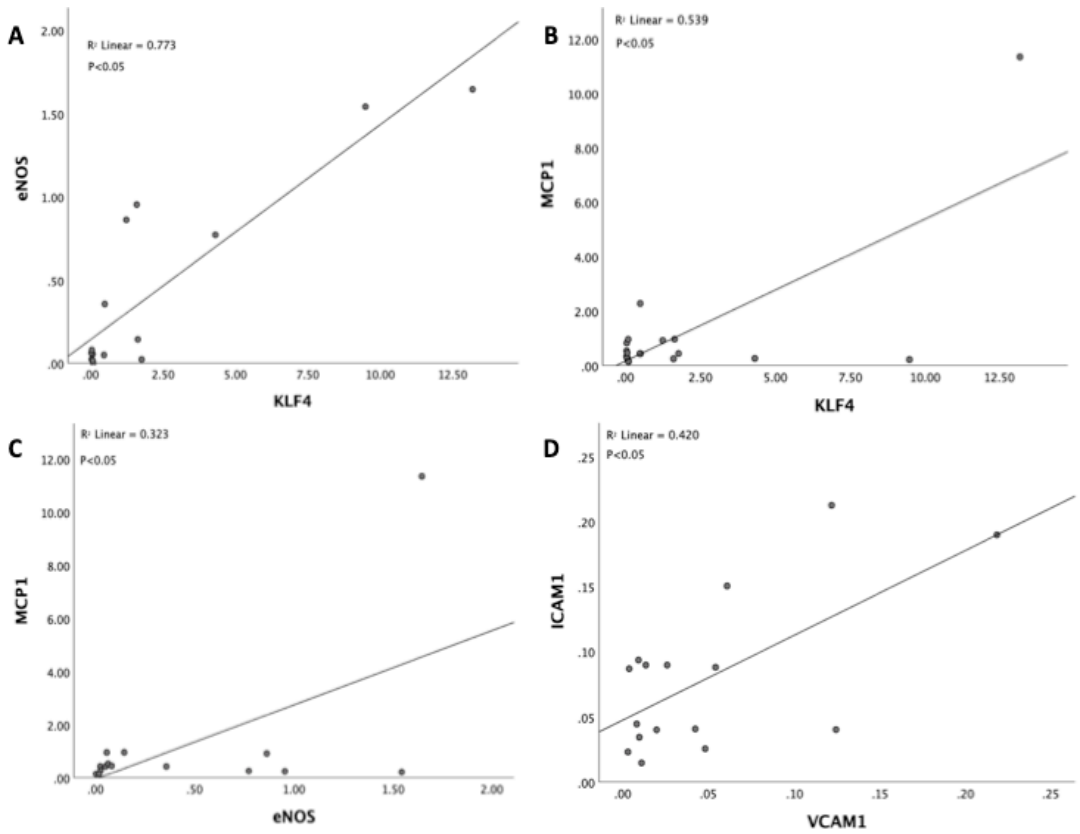


Figure 5. 5 Correlations between genes expressed 24 hours post-intervention

Correlations between genes expressed at 24 h post-intervention. A) KLF4 and eNOS (P<0.05), B) KLF4 and MCP-1 (P<0.05), C) eNOS and MCP-1 (P<0.05), D) VCAM-1 and ICAM-1 (P<0.05).

5.4 Discussion

The key findings of this chapter are that expression of the anti-inflammatory gene eNOS increases from 2 to 24 hours, and expression of inflammatory genes MCP-1 and ICAM-1 decrease from 2 to 24 hours. Furthermore there were positive correlations between anti-inflammatory and pro-inflammatory genes.

The change in gene expression over time for both anti-inflammatory and inflammatory genes is further evidence of endothelial cells biphasic response to shear stress. Previous research by Shyy *et al.* (1994) showed that HUVECs subjected to a shear stress of 16 dynes/cm^2 exhibited a 2-3 fold increase in MCP-1 expression after 1.5 hours of shear stress but after 4 hours of the same level of shear stress, this returned to basal levels (Shyy et al. 1994). Also supporting the results of this chapter, Nagel *et al.* (1994) showed that ICAM-1 showed an increase in expression in HUVECs at 2 hours, which was sustained up to 8 hours before decreasing at 24 hours (Nagel et al. 1994). Furthermore, this study also showed a similar result to this chapter in that VCAM-1 was not upregulated at any time point or in response to different levels of shear stress. The difference between ICAM-1 and VCAM-1 is thought to be due to the lack of the shear stress response element (SSRE) in the VCAM-1 promoter region. VCAM-1 is expressed in response to inflammation however; this is not due to shear stress but rather due to the presence of cytokines such as IL-1. Nagel *et al.* (1994) subjected HUVECs to a dose of IL-1β and observed an upregulation in VCAM-1 cell surface expression in comparison to static cultures (Nagel et al. 1994). In regards to the anti-inflammatory markers increasing from 2 to 24 hours as seen in this chapter, this has been demonstrated to be a usual effect of eNOS. Davis *et al.* in 2004 showed that eNOS promoter activity increased significantly from static conditions after 12 and 24 hours of shear stress at 15 dynes/cm^2 but no statistically significant increases were seen before that point (Davis et al. 2004). As with the results of this chapter, there appears to be a delay in re-

sponse of eNOS expression. This could be due to the initial reaction of the endothelial cells going from a static to shear stress condition, the cells' initial response is inflammatory but over time, as the shear stress stimulus is sustained, this returns to an anti-inflammatory state. Furthermore, combining the evidence from this chapter and previous studies it would appear that the delayed response is positively correlated with the level of inflammatory gene expression in the endothelial cells. A greater level of inflammatory gene expression is correlated with a higher level of anti-inflammatory gene expression indicating that a greater anti-inflammatory response is required in order to neutralise the effects of the initial inflammatory response.

There was no difference in gene expression between conditions at any time point. Although there seems to be a trend for a higher expression of eNOS in the INT at 24h compared to the CON, this did not reach statistical significance, and the same applies for CON seemingly having a greater expression of eNOS at 2h compared to the INT. It is however interesting to see that CON eNOS expression doesn't appear to change between 2 and 24 h, which indicates either a sustained expression of eNOS or the peak of gene expression happens earlier and was missed. This is perhaps unsurprising that there were no differences between continuous and interval exercise as the dose of shear stress was the same however the similarities to the rest protocol were unexpected. This may be due to the endothelial cells not being pre-exposed to shear stress before beginning the exercise protocols. In this study, the cells were not pre-exposed to shear stress primarily to replicate

the acute nature of the exercise that it's based on from the previous chapter. Previous studies have indicated that inflammatory genes are upregulated acutely but suppressed after chronic exposure to shear stress. Indeed multiple studies (Brooks, Lelkes, and Rubanyi 2002; Chen et al. 2001; McCormick et al. 2001; Garcia-Cardena et al. 2001) have used DNA microarray to analyse endothelial response to shear stress and whilst their methods and data analysis techniques have varied slightly, the overall message remains the same: i) chronic shear stress (at least 24 hours) conditions suppresses more genes than are activated; and ii) application of acute shear stress induces gene expression related to endothelial activation. The change from a static to shear stress state is thought to be why inflammatory genes such as MCP-1 and ICAM-1 rise acutely and are then suppressed after chronic exposure when anti-oxidant genes such as cytochrome 1B1 and 1A1 as well as eNOS. This is a likely explanation for the results of this chapter, despite the differences in magnitudes of shear stress, the fact that the cells were all static before the application of shear stress appears to be more important than the protocol applied.

In this study by measuring shear stress *in vivo* and applying that to an *in vitro* model there was a direct physiological translation from the human into the cell model. This was the first study of its kind to mimic the shear stress seen in exercise and apply it to endothelial cells to measure gene expression of endothelial health and atherogenesis. Previous work that has imitated exercise shear stress magnitudes on endothelial cells has been to test

different flow systems (Estrada 2011, Wang 2016) however only Wang et al. used a physiological measurement of shear stress from a single exercising participant. One study however not only tested a new lab-on-a-chip flow system but also analysed the reactive oxygen species of simulated exercise shear stress levels in a diabetic patient (Chin 2011). By putting HUVECs under an “exhaustive exercise” protocol of 30 dynes/cm^2 and a pulsatile rate of 140 bpm in a high glucose media, the authors found an increase in ROS after 60 minutes of shear stress but this was not using an observed physiological result from exercising participants but instead was based upon existing exercise literature. Like this chapter’s results, there was an increase in inflammatory markers (ROS) but as they did not test any further time points the chronic effects remain unknown.

In terms of atherosclerosis, this chapter’s results are novel in understanding the effect of exercise-induced shear stress upon the endothelium. The results have shown that even with the application of very high shear stresses, cells are able to recover and return to similar levels of anti-inflammatory genes expressed as seen at resting shear stress. The hypothesis was that INT exercise would generate a greater amount of anti-inflammatory gene expression than the rest and CON protocols however, there was no difference between the gene expression for any gene between the protocols. Future work should pre-shear the cells using the resting shear stress values for 24 hours before exposing them to the exercise shear stress levels then analyse them with a time course up to 24 hours post exercise to get a more de-

tailed picture of the effect of exercise-induced shear stress on endothelial cells.

The main limitation to this study was the small sample size. Only seven donors were used and there were clear outliers. A greater sample size would enable a more statistically robust data set and outliers could be excluded from the final analysis. Furthermore, there was not much known about the cells aside from age and sex of the donor, other contraindications such as diabetes and coronary artery disease could affect the cells response to a shear stress stimulus. Additionally age is known to be a factor in endothelial function with younger people tending to have a greater bioavailability of NO than older people. As the ages varied greatly between the donors, this could partly explain the outliers in the data and adds to the need of a greater sample size so that differences in response by age can be identified should they occur.

Chapter 6 – General discussion

6.1 Changes in wall shear stress are not uniform throughout the vasculature

One of the main aims of this thesis was to determine where exercise-induced changes in WSS occurred both local to the exercising muscle and systemically. Whilst increases were seen from rest to exercise in both chapter 3 and chapter 4, chapter 3 highlighted that not all areas of the vasculature receive these increases equally or in the same way, indeed not even in the same blood vessel. In the aorta only planes in the descending region of the thoracic aorta experienced any statistically significant rise in WSS in both the healthy participants and MR patients in response to exercise. This is likely due to the descending thoracic aorta being a straight artery with usually high levels of laminar shear stress, as there are no curves WSS can increase without further increasing turbulent flow or retrograde shear stress. The ascending aorta and the arch did not have an increase in WSS, which may be in part because the exercise stimulus was not great enough for the anterograde shear stress to increase beyond a certain level. If the exercise stimulus was increased then that might translate to an increase in shear stress in areas that are more susceptible to atherosclerosis i.e. the inner curvature of the aorta.

In terms of how the patterns changed across the aorta, in healthy participants the only noticeable change from rest to exercise in tWSS and aWSS

was in plane 5 where shear stress was of a similar level to that of planes 4 and 6. As such there was more of a sigmoidal curve pattern across the aorta in terms of shear stress magnitudes. Furthermore, the distribution of tWSS and aWSS became more uniform for each plane with exercise, but in planes where there were lower shear stresses, they were still concentrated along the inner curvature of the aorta. For cWSS plane 1 had a much lower shear stress magnitude than planes 2-5 at rest however, during exercise planes 1-5 were exposed to a similar level of shear stress, which then descended gradually through planes 6-8. The distribution of cWSS in each plane was highly variable between each plane at rest however during exercise cWSS was distributed evenly throughout planes 1-4 but became more variable in planes 5-8 as the shear stress decreased in the outer curvature (planes 5 and 6) then shifted towards the inner curvature further along the aorta (planes 7 and 8). Interestingly, in the MR patients the overall pattern of shear stress across the planes did not change in tWSS or aWSS from rest to exercise however the distribution of shear stress in each plane became more uniform throughout the aorta, particularly in planes 4-8 with exercise. The overall pattern of cWSS magnitudes at rest in MR patients is relatively uniform across the aorta however with exercise cWSS in plane 1 is much higher than the other planes, which decreases down to plane 3 and remains relatively similar throughout the other planes. The distribution of cWSS in each plane at rest is similar throughout the aorta however in exercise in plane 1 and 2 there is an increase in cWSS on the outer curvature whereas the rest of the planes did not change as much. Whilst there are some shifts in WSS pattern for both healthy and MR patients it would appear that it is not always

in favour of the inner curvature where an increase in WSS would be most beneficial in preventing atherosclerosis.

Although WSS could not be measured over time, the stable heart rates of the participants suggest that shear stress is likely to remain relatively consistent throughout exercise in the thoracic aorta. In the femoral artery however, WSS was only able to be assessed in one region of the common femoral artery but could be measured throughout the exercise, and showed that after the initial increase from rest to exercise WSS stabilised throughout the duration of the intervention, with each time point having a similar level of shear stress at the cessation of exercise. This is in contrast to existing literature in the brachial artery that showed an increase in WSS over time during leg cycling exercise (Lyall 2019, Padilla 2011, Tinken, 2009). This may be due to the different anatomical structure of the vessels as the CFA is a curved vessel with a bifurcation whereas the brachial artery is a straight vessel thus; both arteries are exposed to different kinds of shear stresses, which may contribute to the different shear stress patterns. More likely however, is the systemic vs local effect of shear stress. During leg cycling the arm muscles are doing very little and the blood flow demand so shear stress doesn't increase drastically. Instead there is a gradual increase likely in response to vasodilation and a rise in heart rate so whilst the majority of the blood flow is directed towards the working muscles in the legs due to a greater metabolic demand (Thijssen, Steendijk, and Hopman 2009) (Green et al. 2005). The reason why shear stress appears to plateau in the CFA is likely because the

stimulus is unchanging. This is particularly evident in the continuous protocol where participants cycle at the same workload for 12 minutes and once the metabolic demand has been met and heart rate has stabilised there are no further changes in shear stress until exercise ceases. Interestingly, this was also the case with the interval exercise for whilst there were oscillations in heart rate, blood flow and shear stress, the peak shear stress did not change over the course of exercise.

The nature of the shear stress response to exercise from these two chapters suggests that i) a targeted approach to exercise may need to be taken to provide increases in shear stress to areas particularly affected by atherosclerosis, ii) exercise needs to be above 110 bpm in order to confer positive shear stress benefits in vessels with curvatures and bifurcations, and iii) shear stress patterns do appear to slightly change to give a more uniform distribution around the aorta.

6.2 Endothelial function does not differ between continuous and interval exercise in the common femoral artery

Chapters 4 and 5 explored the endothelial response to exercise-induced shear stress via FMD and gene expression, respectively. Both chapters showed that acutely (0-2 hrs) post-exercise there is no improvement in endothelial function with chapter 4 showing there was no change in FMD compared to pre-exercise for either CON or INT exercise, and chapter 5 showing that there was a spike in inflammatory genes 2 hrs post-exercise and anti-

inflammatory gene expression did not increase until 24 hrs post-exercise. Despite the greater anterograde shear stress in the INT exercise this did not translate into a beneficial effect to the endothelium immediately post-exercise. By coupling this data with that of the gene expression it can be seen that the initial acute rise in shear stress actually generates an inflammatory response to the endothelium, which is likely due to it being different from the normal levels of shear stress the cells experience. However, over time, as seen in chapter 5, the inflammatory genes initially triggered diminish to almost nothing whereas anti-inflammatory genes increase, especially eNOS. This has been seen in various *in vivo* (Dawson 2008, Gonzales 2011, Birk 2013) and *in vitro* (Garcia-Cardena 2001, McCormick 2001, Chen 2001, Brooks 2002) studies separately but is the first time the data have been linked together using physiological data derived directly from exercise and applied to an *in vitro* model.

What these chapters have shown is that INT exercise has a greater amount of anterograde shear stress than CON exercise in the CFA and that in vivo measures of shear stress can successfully be translated to an in vitro model in order to determine the gene expression resulting from heavy intensity exercise. Whilst there were no differences in gene expression between protocols, it is encouraging that these initial experiments have followed similar patterns in gene expression over time that other studies have produced and with a slight change to the protocol and time course, more details may emerge about INT v CON effects of exercise. However, what this means in

terms of patients with atherosclerosis is unclear at present. Previous research (Burke 1999) has suggested that sudden increases in shear stress may cause ruptures to the most unstable plaques so a gradual increase in exercise intensity might be the best way forward in those patient groups (Leon 2005) however, these answers can only be gained by recruiting patients to an exercise training study and assessing plaque stability and plaque burden over time. In terms of the general population, high intensity interval and continuous exercise does not have any negative effects acutely and therefore may be beneficial in preventing atherosclerosis in susceptible arteries such as the CFA in the long term. Continual exposure of the endothelium to low shear stress with turbulent flow can lead to the development of atherosclerotic plaques therefore, by exercising and exposing the endothelium to higher shear stress could prevent development of atherosclerosis.

6.3 Future perspectives

The results from this thesis have come a long way in helping the wider scientific community understand the implication of exercise-induced increases in shear stress and the effect that has upon the endothelium however, there is further research to be done. This further research should focus on understanding the effects of exercise-induced shear stress in patients with CVD and those at risk of developing CVD.

The exploration of exercise-induced shear stress in the thoracic aorta of healthy participants and MR patients needs to have a greater number of both healthy and MR participants. Furthermore, an age-matched healthy population to the MR patients would be beneficial to allow for greater comparison. Additionally this would serve as an extra comparison to the young healthy participants so that the implication of age upon exercise-induced WSS can be further explored and understood. Until such time as the MRI technology is able to conduct 4D flow scans at higher heart rates, the 110 bpm limit will still have to be adhered to however all future studies should conduct the exercise section at 30-39% HRR to allow for all participants to be compared. However what is encouraging is that the slight increase in HR in the MR patients was enough to increase shear stress in the thoracic aorta, which from a clinical perspective could be prescribed as an exercise regime to patients. This could improve their vascular health not just in the aorta but systemically. Additionally, as 110 bpm is an achievable HR for these patients to reach with low effort (i.e. a brisk walk) and could easily be incorporated into their daily routines thus making it a more sustainable mode of exercise in the long term whilst also reducing the risk of a major adverse cardiac event posed by an intense exercise regime.

The femoral study should be expanded to include a larger number of healthy participants in the first instance and two extra FMD measures should also be included at 1 hour and 24 hours post-exercise. As there were no changes to FMD immediately pre- to post-exercise the time course could help in under-

standing when the beneficial effects of exercise start to emerge. This could help tailor exercise programmes to the healthy population as well as people with cardiovascular disease by identify when to exercise to get the greatest benefit to the vasculature, which could prevent decline in endothelial function. Furthermore if the time-course showed continuous or interval exercise to be more beneficial, this could also be incorporated into prescriptions for exercise where applicable.

Next a training study should be conducted to assess changes in endothelial function and shear stress in the CFA. Once these changes, if any, have been established in a healthy population then a disease group (atherosclerosis) should also be enrolled onto the study and carry out the same intervention as the healthy participants in order to generate a comparison and also to see the effects of an increase in shear stress on their plaque burden. If these patients are older than the current healthy participants then a healthy age-matched population should also be recruited for a direct comparison and to provide an age comparison to the young healthy group. This would also aid in the clinical use of exercise as a therapy, particularly if plaques regressed or stabilised, preventing a decline into more severe cardiovascular disease.

To expand upon the results of the *in vitro* study firstly the same protocols should be repeated but with a 24 hour period of expose to resting levels of shear stress before the exercise intervention is applied, followed by a time course to plot when exactly the anti-inflammatory genes begin to increase

and overtake the inflammatory genes. Further to this a training study in ApoE mice should be conducted to allow for a deeper understanding of how exercise affects development of atherosclerosis in a model of atherosusceptible mice. By having this approach with animal and cellular studies, it could help to identify specific mechanisms involved in atherosclerosis and identify how and why exercise generates improvements in endothelial function. Furthermore, by identifying specific genes or proteins involved in this mechanism could help to identify potential drug targets to develop pharmaceutical agents that could be given to patients to control disease progression.

By combining the results from the human studies alongside a mouse model and cell models we can increase our understanding of atherosclerosis development and when best to apply an exercise intervention. Not only applying an intervention to prevent the onset of disease but even to potentially reverse it. Research from Costopoulos et al. (2019) has shown that high shear stress reduces plaque burden and can reduce plaque size (Costopoulos et al. 2019) so a targeted approach to exercise in areas prone to plaque formation could prove to be a highly effective treatment. Further to this there should also be studies to understand whether there is a limit to plaque regression and if there are some atherosclerotic plaque formations that cannot be reversed at all. Over time plaques develop weaknesses and become vulnerable to sudden increases in shear stress, and if it’s the case that high-intensity exercise puts patients at risk of plaque rupture then studies would need to be conducted to find which types of exercise can increase shear

stress to be effective in promoting endothelial health without risking plaque rupture.

This thesis set out to explore the hypothesis “exercise-induced changes in shear stress lead to an increase in shear stress in atherosusceptible regions of arteries, and improve endothelial function at a cellular level” (section 2.5.1). From the data presented in these chapters it can be stated with some confidence that exercise-induced changes in shear stress can increase shear stress in atherosusceptible regions. This is evidenced in chapters 3 and 4 where increases in shear stress were seen in both the thorachic aorta and the common femoral artery. However, the improvement in endothelial function on a cellular level requires further investigation for the reasons outlined in the above chapter. Furthermore, there was no improvement in endothelial function immediately post-exercise in the common femoral artery, which requires further investigation to see if there is a delayed response to exercise, as seen in previous studies and in the cell model from chapter 5.

Each of the aims presented were also addressed in this thesis (section 2.5.2). The shear stress patterns were successfully assessed in both healthy participants and patients with severe mitral valve regurgitation. It was determined that high-intensity interval exercise elicited the greatest response in terms of anterograde shear stress and the oscillations in exercise pattern did not cause greater oscillations in shear stress, and there was no negative effect on the endothelium as a consequence of these increased shear stress-

es. Finally, gene expression was measured over time in response to shear stress acquired from the common femoral artery showing that anti-inflammatory genes increased over time, and inflammatory genes decreased over time in response to exercise.

References

Aboyans, V., R. L. McClelland, M. A. Allison, M. M. McDermott, R. S. Blumenthal, K. Macura, and M. H. Criqui. 2011. 'Lower extremity peripheral artery disease in the absence of traditional risk factors. The Multi-Ethnic Study of Atherosclerosis', *Atherosclerosis*, 214: 169-73.

Adams, V., B. Reich, M. Uhlemann, and J. Niebauer. 2017. 'Molecular effects of exercise training in patients with cardiovascular disease: Focus on skeletal muscle, endothelium and myocardium', *Am J Physiol Heart Circ Physiol*: ajpheart 00470 2016.

Al-Wakeel, N., J. F. Fernandes, A. Amiri, H. Siniawski, L. Goubergrits, F. Berger, and T. Kuehne. 2015. 'Hemodynamic and energetic aspects of the left ventricle in patients with mitral regurgitation before and after mitral valve surgery', *J Magn Reson Imaging*, 42: 1705-12.

Amarenco, P., A. Cohen, C. Tzourio, B. Bertrand, M. Hommel, G. Besson, C. Chauvel, P. J. Touboul, and M. G. Bousser. 1994. 'Atherosclerotic disease of the aortic arch and the risk of ischemic stroke', *N Engl J Med*, 331: 1474-9.

Atkinson, C. L., H. H. Carter, L. H. Naylor, E. A. Dawson, P. Marusic, D. Hering, M. P. Schlaich, D. H. Thijssen, and D. J. Green. 2015. 'Opposing effects of shear-mediated dilation and myogenic constriction on artery diameter in response to handgrip exercise in humans', *J Appl Physiol (1985)*, 119: 858-64.

Bakkestrom, R., A. Banke, N. L. Christensen, R. Pecini, A. Irmukhamedov, M. Andersen, B. A. Borlaug, and J. E. Moller. 2018. 'Hemodynamic Characteristics in Significant Symptomatic and Asymptomatic Primary Mitral Valve Regurgitation at Rest and During Exercise', *Circ Cardiovasc Imaging*, 11: e007171.

Baldwin, A. L., G. Thurston, and H. al Naemi. 1998. 'Inhibition of nitric oxide synthesis increases venular permeability and alters endothelial actin cytoskeleton', *Am J Physiol*, 274: H1776-84.

Beaver, W. L., K. Wasserman, and B. J. Whipp. 1986. 'A new method for detecting anaerobic threshold by gas exchange', *J Appl Physiol (1985)*, 60: 2020-7.

BHF. 2017. 'Cardiovascular Disease Statistics 2017', BHF, Accessed 20/09/17. https://www.bhf.org.uk/research/heart-statistics/heart-statistics-publications/cardiovascular-disease-statistics-2017.

Birk, G. K., E. A. Dawson, C. Atkinson, A. Haynes, N. T. Cable, D. H. Thijssen, and D. J. Green. 2012. 'Brachial artery adaptation to lower limb exercise training: role of shear stress', *J Appl Physiol (1985)*, 112: 1653-8.

Birk, G. K., E. A. Dawson, A. M. Batterham, G. Atkinson, T. Cable, D. H. Thijssen, and D. J. Green. 2013. 'Effects of exercise intensity on flow mediated dilation in healthy humans', *Int J Sports Med*, 34: 409-14.

Black, M. A., N. T. Cable, D. H. Thijssen, and D. J. Green. 2009. 'Impact of age, sex, and exercise on brachial artery flow-mediated dilatation', *Am J Physiol Heart Circ Physiol*, 297: H1109-16.

Brooks, A. R., P. I. Lelkes, and G. M. Rubanyi. 2002. 'Gene expression profiling of human aortic endothelial cells exposed to disturbed flow and steady laminar flow', *Physiol Genomics*, 9: 27-41.

Brown, A. J., Z. Teng, P. C. Evans, J. H. Gillard, H. Samady, and M. R. Bennett. 2016. 'Role of biomechanical forces in the natural history of coronary atherosclerosis', *Nat Rev Cardiol*, 13: 210-20.

Burgomaster, K. A., S. C. Hughes, G. J. Heigenhauser, S. N. Bradwell, and M. J. Gibala. 2005. 'Six sessions of sprint interval training increases muscle oxidative potential and cycle endurance capacity in humans', *J Appl Physiol (1985)*, 98: 1985-90.

Burke, A. P., A. Farb, G. T. Malcom, Y. Liang, J. E. Smialek, and R. Virmani. 1999. 'Plaque rupture and sudden death related to exertion in men with coronary artery disease', *JAMA*, 281: 921-6.

Caro, C. G., J. M. Fitz-Gerald, and R. C. Schroter. 1969. 'Arterial wall shear and distribution of early atheroma in man', *Nature*, 223: 1159-60.

Caro, C. G., and R. M. Nerem. 1973. 'Transport of 14 C-4-cholesterol between serum and wall in the perfused dog common carotid artery', *Circ Res*, 32: 187-205.

Celermajer, D. S., K. E. Sorensen, D. J. Spiegelhalter, D. Georgakopoulos, J. Robinson, and J. E. Deanfield. 1994. 'Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women', *J Am Coll Cardiol*, 24: 471-6.

Chappell, D. C., S. E. Varner, R. M. Nerem, R. M. Medford, and R. W. Alexander. 1998. 'Oscillatory shear stress stimulates adhesion molecule expression in cultured human endothelium', *Circ Res*, 82: 532-9.

Chen, B. P., Y. S. Li, Y. Zhao, K. D. Chen, S. Li, J. Lao, S. Yuan, J. Y. Shyy, and S. Chien. 2001. 'DNA microarray analysis of gene expression in endothelial cells in response to 24-h shear stress', *Physiol Genomics*, 7: 55-63.

Chien, S. 1970. 'Shear dependence of effective cell volume as a determinant of blood viscosity', *Science*, 168: 977-9.

Coon, B. G., N. Baeyens, J. Han, M. Budatha, T. D. Ross, J. S. Fang, S. Yun, J. L. Thomas, and M. A. Schwartz. 2015. 'Intramembrane binding of VE-cadherin to VEGFR2 and VEGFR3 assembles the endothelial mechanosensory complex', *J Cell Biol*, 208: 975-86.

Costopoulos, C., L. H. Timmins, Y. Huang, O. Y. Hung, D. S. Molony, A. J. Brown, E. L. Davis, Z. Teng, J. H. Gillard, H. Samady, and M. R. Bennett. 2019. 'Impact of combined plaque structural stress and wall shear stress on coronary plaque progression, regression, and changes in composition', *Eur Heart J*, 40: 1411-22.

Davis, M. E., I. M. Grumbach, T. Fukai, A. Cutchins, and D. G. Harrison. 2004. 'Shear stress regulates endothelial nitric-oxide synthase promoter activity through nuclear factor kappaB binding', *J Biol Chem*, 279: 163-8.

Dawson, E. A., D. J. Green, N. T. Cable, and D. H. Thijssen. 2013. 'Effects of acute exercise on flow-mediated dilatation in healthy humans', *J Appl Physiol (1985)*, 115: 1589-98.

Dawson, E. A., G. P. Whyte, M. A. Black, H. Jones, N. Hopkins, D. Oxborough, D. Gaze, R. E. Shave, M. Wilson, K. P. George, and D. J. Green. 2008. 'Changes in vascular and cardiac function after prolonged strenuous exercise in humans', *J Appl Physiol (1985)*, 105: 1562-8.

DeSouza, C. A., L. F. Shapiro, C. M. Clevenger, F. A. Dinenno, K. D. Monahan, H. Tanaka, and D. R. Seals. 2000. 'Regular aerobic exercise prevents and restores age-related declines in endothelium-dependent vasodilation in healthy men', *Circulation*, 102: 1351-7.

Dewey, C. F., Jr., S. R. Bussolari, M. A. Gimbrone, Jr., and P. F. Davies. 1981. 'The dynamic response of vascular endothelial cells to fluid shear stress', *J Biomech Eng*, 103: 177-85.

Ekelund, L. G., W. L. Haskell, J. L. Johnson, F. S. Whaley, M. H. Criqui, and D. S. Sheps. 1988. 'Physical fitness as a predictor of cardiovascular mortality in asymptomatic North American men. The Lipid Research Clinics Mortality Follow-up Study', *N Engl J Med*, 319: 1379-84.

Faxon, D. P., V. Fuster, P. Libby, J. A. Beckman, W. R. Hiatt, R. W. Thompson, J. N. Topper, B. H. Annex, J. H. Rundback, R. P. Fabunmi, R. M. Robertson, J. Loscalzo, and Association American Heart. 2004. 'Atherosclerotic Vascular Disease Conference: Writing Group III: pathophysiology', *Circulation*, 109: 2617-25.

Fernstrom, M., U. Fernberg, G. Eliason, and A. Hurtig-Wennlof. 2017. 'Aerobic fitness is associated with low cardiovascular disease risk: the impact of lifestyle on early risk factors for atherosclerosis in young healthy Swedish individuals - the Lifestyle, Biomarker, and Atherosclerosis study', *Vasc Health Risk Manag*, 13: 91-99.

Francois, M. E., C. Durrer, K. J. Pistawka, F. A. Halperin, and J. P. Little. 2016. 'Resistance-based interval exercise acutely improves endothelial function in type 2 diabetes', *Am J Physiol Heart Circ Physiol*, 311: H1258-H67.

Frangos, J. A., S. G. Eskin, L. V. McIntire, and C. L. Ives. 1985. 'Flow effects on prostacyclin production by cultured human endothelial cells', *Science*, 227: 1477-9.

Franzoni, F., L. Ghiadoni, F. Galetta, Y. Plantinga, V. Lubrano, Y. Huang, G. Salvetti, F. Regoli, S. Taddei, G. Santoro, and A. Salvetti. 2005. 'Physical activity, plasma antioxidant capacity, and endothelium-dependent vasodilation in young and older men', *Am J Hypertens*, 18: 510-6.

Fukumoto, Y., T. Hiro, T. Fujii, G. Hashimoto, T. Fujimura, J. Yamada, T. Okamura, and M. Matsuzaki. 2008. 'Localized elevation of shear stress is related to coronary plaque rupture: a 3-dimensional intravascular ultrasound study with in-vivo color mapping of shear stress distribution', *J Am Coll Cardiol*, 51: 645-50.

Gaasch, W. H., and T. E. Meyer. 2008. 'Left ventricular response to mitral regurgitation: implications for management', *Circulation*, 118: 2298-303.

———. 2018. 'Secondary mitral regurgitation (part 1): volumetric quantification and analysis', *Heart*, 104: 634-38.

Gambillara, V., C. Chambaz, G. Montorzi, S. Roy, N. Stergiopulos, and P. Silacci. 2006. 'Plaque-prone hemodynamics impair endothelial function in pig carotid arteries', *Am J Physiol Heart Circ Physiol*, 290: H2320-8.

Garcia-Cardena, G., J. Comander, K. R. Anderson, B. R. Blackman, and M. A. Gimbrone, Jr. 2001. 'Biomechanical activation of vascular endothelium as a determinant of its functional phenotype', *Proc Natl Acad Sci U S A*, 98: 4478-85.

Gijsen, F. J., J. J. Wentzel, A. Thury, F. Mastik, J. A. Schaar, J. C. Schuurbiers, C. J. Slager, W. J. van der Giessen, P. J. de Feyter, A. F. van der Steen, and P. W. Serruys. 2008. 'Strain distribution over plaques in human coronary arteries relates to shear stress', *Am J Physiol Heart Circ Physiol*, 295: H1608-14.

Gonzales, J. U., B. C. Thompson, J. R. Thistlethwaite, and B. W. Scheuermann. 2011. 'Association between exercise hemodynamics and changes in local vascular function following acute exercise', *Appl Physiol Nutr Metab*, 36: 137-44.

Grabowski, E. F., E. A. Jaffe, and B. B. Weksler. 1985. 'Prostacyclin production by cultured endothelial cell monolayers exposed to step increases in shear stress', *J Lab Clin Med*, 105: 36-43.

Green, D. J., W. Bilsborough, L. H. Naylor, C. Reed, J. Wright, G. O'Driscoll, and J. H. Walsh. 2005. 'Comparison of forearm blood flow responses to incremental handgrip and cycle ergometer exercise: relative contribution of nitric oxide', *J Physiol*, 562: 617-28.

Green, D. J., H. H. Carter, M. G. Fitzsimons, N. T. Cable, D. H. Thijssen, and L. H. Naylor. 2010. 'Obligatory role of hyperaemia and shear stress in microvascular adaptation to repeated heating in humans', *J Physiol*, 588: 1571-7.

Green, D. J., M. T. Hopman, J. Padilla, M. H. Laughlin, and D. H. Thijssen. 2017. 'Vascular Adaptation to Exercise in Humans: Role of Hemodynamic Stimuli', *Physiol Rev*, 97: 495-528.

Hagmar, M., M. J. Eriksson, C. Lindholm, K. Schenck-Gustafsson, and A. L. Hirschberg. 2006. 'Endothelial function in post-menopausal former elite athletes', *Clin J Sport Med*, 16: 247-52.

Hahn, C., and M. A. Schwartz. 2009. 'Mechanotransduction in vascular physiology and atherogenesis', *Nat Rev Mol Cell Biol*, 10: 53-62.

Harris, R. A., J. Padilla, K. P. Hanlon, L. D. Rink, and J. P. Wallace. 2008. 'The flow-mediated dilation response to acute exercise in overweight active and inactive men', *Obesity (Silver Spring)*, 16: 578-84.

Harvey, P. J., B. L. Morris, T. Kubo, P. E. Picton, W. S. Su, C. F. Notarius, and J. S. Floras. 2005. 'Hemodynamic after-effects of acute dynamic exercise in sedentary normotensive postmenopausal women', *J Hypertens*, 23: 285-92.

Haskell, W. L., I. M. Lee, R. R. Pate, K. E. Powell, S. N. Blair, B. A. Franklin, C. A. Macera, G. W. Heath, P. D. Thompson, A. Bauman, Medicine American College of Sports, and Association American Heart. 2007. 'Physical activity and public health: updated recommendation for

adults from the American College of Sports Medicine and the American Heart Association', *Circulation*, 116: 1081-93.

Higashi, Y., S. Sasaki, N. Sasaki, K. Nakagawa, T. Ueda, A. Yoshimizu, S. Kurisu, H. Matsuura, G. Kajiyama, and T. Oshima. 1999. 'Daily aerobic exercise improves reactive hyperemia in patients with essential hypertension', *Hypertension*, 33: 591-7.

Hillsley, M. V., and J. M. Tarbell. 2002. 'Oscillatory shear alters endothelial hydraulic conductivity and nitric oxide levels', *Biochem Biophys Res Commun*, 293: 1466-71.

Hopkins, P. N. 2013. 'Molecular biology of atherosclerosis', *Physiol Rev*, 93: 1317-542.

Hsiai, T. K., S. K. Cho, P. K. Wong, M. Ing, A. Salazar, A. Sevanian, M. Navab, L. L. Demer, and C. M. Ho. 2003. 'Monocyte recruitment to endothelial cells in response to oscillatory shear stress', *FASEB J*, 17: 1648-57.

Huynh, J., N. Nishimura, K. Rana, J. M. Peloquin, J. P. Califano, C. R. Montague, M. R. King, C. B. Schaffer, and C. A. Reinhart-King. 2011. 'Age-related intimal stiffening enhances endothelial permeability and leukocyte transmigration', *Sci Transl Med*, 3: 112ra22.

Hwang, J., M. H. Ing, A. Salazar, B. Lassegue, K. Griendling, M. Navab, A. Sevanian, and T. K. Hsiai. 2003. 'Pulsatile versus oscillatory shear stress regulates NADPH oxidase subunit expression: implication for native LDL oxidation', *Circ Res*, 93: 1225-32.

Johnson, B. D., J. Padilla, and J. P. Wallace. 2012. 'The exercise dose affects oxidative stress and brachial artery flow-mediated dilation in trained men', *Eur J Appl Physiol*, 112: 33-42.

Jones, E. F., and G. A. Donnan. 1995. 'The proximal aorta: a source of stroke', *Baillieres Clin Neurol*, 4: 207-20.

Korenaga, R., J. Ando, H. Tsuboi, W. Yang, I. Sakuma, T. Toyo-oka, and A. Kamiya. 1994. 'Laminar flow stimulates ATP- and shear stress-dependent nitric oxide production in cultured bovine endothelial cells', *Biochem Biophys Res Commun*, 198: 213-9.

Kroger, K., A. Kucharczik, H. Hirche, and G. Rudofsky. 1999. 'Atherosclerotic lesions are more frequent in femoral arteries than in carotid arteries independent of increasing number of risk factors', *Angiology*, 50: 649-54.

Ku, D. N., D. P. Giddens, C. K. Zarins, and S. Glagov. 1985. 'Pulsatile flow and atherosclerosis in the human carotid bifurcation. Positive correlation between plaque location and low oscillating shear stress', *Arteriosclerosis*, 5: 293-302.

Kuchan, M. J., and J. A. Frangos. 1994. 'Role of calcium and calmodulin in flow-induced nitric oxide production in endothelial cells', *Am J Physiol*, 266: C628-36.

Kuchan, M. J., H. Jo, and J. A. Frangos. 1994. 'Role of G proteins in shear stress-mediated nitric oxide production by endothelial cells', *Am J Physiol*, 267: C753-8.

Kurose, I., P. Kubes, R. Wolf, D. C. Anderson, J. Paulson, M. Miyasaka, and D. N. Granger. 1993. 'Inhibition of nitric oxide production. Mechanisms of vascular albumin leakage', *Circ Res*, 73: 164-71.

Lapu-Bula, R., A. Robert, D. Van Craeynest, A. M. D'Hondt, B. L. Gerber, A. Pasquet, J. A. Melin, M. De Kock, and J. L. Vanoverschelde. 2002. 'Contribution of exercise-induced mitral regurgitation to exercise stroke volume and exercise capacity in patients with left ventricular systolic dysfunction', *Circulation*, 106: 1342-8.

Le, T. T., J. A. Bryant, A. E. Ting, P. Y. Ho, B. Su, R. C. Teo, J. S. Gan, Y. C. Chung, D. P. O'Regan, S. A. Cook, and C. W. Chin. 2017. 'Assessing exercise cardiac reserve using real-time cardiovascular magnetic resonance', *J Cardiovasc Magn Reson*, 19: 7.

Leon, A. S. 1987. 'Age and other predictors of coronary heart disease', *Med Sci Sports Exerc*, 19: 159-67.

———. 2005. 'Does long-term aerobic exercise slow progression of atherosclerosis?', *Clin J Sport Med*, 15: 285-6.

Li, M. F., Y. Ren, C. C. Zhao, R. Zhang, L. X. Li, F. Liu, J. X. Lu, Y. F. Tu, W. J. Zhao, Y. Q. Bao, and W. P. Jia. 2014. 'Prevalence and clinical characteristics of lower limb atherosclerotic lesions in newly diagnosed patients with ketosis-onset diabetes: a cross-sectional study', *Diabetol Metab Syndr*, 6: 71.

Lyall, G. K., M. J. Davies, C. Ferguson, K. E. Porter, and K. M. Birch. 2019. 'In-exercise vascular shear rate during acute continuous and interval exercise: impact on endothelial function and miR-21', *J Appl Physiol (1985)*, 127: 1754-62.

McCormick, S. M., S. G. Eskin, L. V. McIntire, C. L. Teng, C. M. Lu, C. G. Russell, and K. K. Chittur. 2001. 'DNA microarray reveals changes in gene expression of shear stressed human umbilical vein endothelial cells', *Proc Natl Acad Sci U S A*, 98: 8955-60.

Merghani, A., V. Maestrini, S. Rosmini, A. T. Cox, H. Dhutia, R. Bastiaenen, S. David, T. J. Yeo, R. Narain, A. Malhotra, M. Papadakis, M. G. Wilson, M. Tome, K. AlFakih, J. C. Moon, and S. Sharma. 2017. 'Prevalence of Subclinical Coronary Artery Disease in Masters Endurance Athletes with a Low Atherosclerotic Risk Profile', *Circulation*.

Mitranun, W., C. Deerochanawong, H. Tanaka, and D. Suksom. 2014. 'Continuous vs interval training on glycemic control and macro- and microvascular reactivity in type 2 diabetic patients', *Scand J Med Sci Sports*, 24: e69-76.

Mo, M., S. G. Eskin, and W. P. Schilling. 1991. 'Flow-induced changes in Ca2+ signaling of vascular endothelial cells: effect of shear stress and ATP', *Am J Physiol*, 260: H1698-707.

Mohamied, Y., E. M. Rowland, E. L. Bailey, S. J. Sherwin, M. A. Schwartz, and P. D. Weinberg. 2015. 'Change of direction in the biomechanics of atherosclerosis', *Ann Biomed Eng*, 43: 16-25.

Molmen-Hansen, H. E., T. Stolen, A. E. Tjonna, I. L. Aamot, I. S. Ekeberg, G. A. Tyldum, U. Wisloff, C. B. Ingul, and A. Stoylen. 2012. 'Aerobic interval training reduces blood pressure and improves myocardial function in hypertensive patients', *Eur J Prev Cardiol*, 19: 151-60.

Mora, S., N. Cook, J. E. Buring, P. M. Ridker, and I. M. Lee. 2007. 'Physical activity and reduced risk of cardiovascular events: potential mediating mechanisms', *Circulation*, 116: 2110-8.

Morishima, T., R. M. Restaino, L. K. Walsh, J. A. Kanaley, and J. Padilla. 2017. 'Prior exercise and standing as strategies to circumvent sitting-induced leg endothelial dysfunction', *Clin Sci (Lond)*, 131: 1045-53.

Myers, J., M. Prakash, V. Froelicher, D. Do, S. Partington, and J. E. Atwood. 2002. 'Exercise capacity and mortality among men referred for exercise testing', *N Engl J Med*, 346: 793-801.

Nagel, T., N. Resnick, W. J. Atkinson, C. F. Dewey, Jr., and M. A. Gimbrone, Jr. 1994. 'Shear stress selectively upregulates intercellular adhesion molecule-1 expression in cultured human vascular endothelial cells', *J Clin Invest*, 94: 885-91.

Naylor, L. H., H. Carter, M. G. FitzSimons, N. T. Cable, D. H. Thijssen, and D. J. Green. 2011. 'Repeated increases in blood flow, independent of exercise, enhance conduit artery vasodilator function in humans', *Am J Physiol Heart Circ Physiol*, 300: H664-9.

Nerem, R. M., M. J. Levesque, and J. F. Cornhill. 1981. 'Vascular endothelial morphology as an indicator of the pattern of blood flow', *J Biomech Eng*, 103: 172-6.

Niu, N., S. Xu, Y. Xu, P. J. Little, and Z. G. Jin. 2019. 'Targeting Mechanosensitive Transcription Factors in Atherosclerosis', *Trends Pharmacol Sci*, 40: 253-66.

O'Rourke, R. A., and M. H. Crawford. 1984. 'Mitral valve regurgitation', *Curr Probl Cardiol*, 9: 1-52.

Padilla, J., G. H. Simmons, L. C. Vianna, M. J. Davis, M. H. Laughlin, and P. J. Fadel. 2011. 'Brachial artery vasodilatation during prolonged lower limb exercise: role of shear rate', *Exp Physiol*, 96: 1019-27.

Parikh, J., A. Kapela, and N. M. Tsoukias. 2017. 'Can endothelial hemoglobin-alpha regulate nitric oxide vasodilatory signaling?', *Am J Physiol Heart Circ Physiol*, 312: H854-H66.

Perk, J., G. De Backer, H. Gohlke, I. Graham, Z. Reiner, M. Verschuren, C. Albus, P. Benlian, G. Boysen, R. Cifkova, C. Deaton, S. Ebrahim, M. Fisher, G. Germano, R. Hobbs, A. Hoes, S. Karadeniz, A. Mezzani, E. Prescott, L. Ryden, M. Scherer, M. Syvanne, W. J. Scholte op Reimer, C. Vrints, D. Wood, J. L. Zamorano, F. Zannad, Prevention European Association for Cardiovascular, Rehabilitation, and E. S. C. Committee for Practice Guidelines. 2012. 'European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts)', *Eur Heart J*, 33: 1635-701.

Perry, G. J., C. C. Wei, G. H. Hankes, S. R. Dillon, P. Rynders, R. Mukherjee, F. G. Spinale, and L. J. Dell'Italia. 2002. 'Angiotensin II receptor blockade does not improve left ventricular function and remodeling in subacute mitral regurgitation in the dog', *J Am Coll Cardiol*, 39: 1374-9.

Pierce, G. L., I. Eskurza, A. E. Walker, T. N. Fay, and D. R. Seals. 2011. 'Sex-specific effects of habitual aerobic exercise on brachial artery flow-mediated dilation in middle-aged and older adults', *Clin Sci (Lond)*, 120: 13-23.

Rand, P. W., E. Lacombe, H. E. Hunt, and W. H. Austin. 1964. 'Viscosity of Normal Human Blood under Normothermic and Hypothermic Conditions', *J Appl Physiol*, 19: 117-22.

Restaino, R. M., S. W. Holwerda, D. P. Credeur, P. J. Fadel, and J. Padilla. 2015. 'Impact of prolonged sitting on lower and upper limb micro- and macrovascular dilator function', *Exp Physiol*, 100: 829-38.

Richardson, P. D., M. J. Davies, and G. V. Born. 1989. 'Influence of plaque configuration and stress distribution on fissuring of coronary atherosclerotic plaques', *Lancet*, 2: 941-4.

Roberston, A.M., A. Sequeira, and MV. Kameneva. 2008. 'Hemorheology.' in, *Hemodynamical flows* (Springer).

Roger, V. L., A. S. Go, D. M. Lloyd-Jones, R. J. Adams, J. D. Berry, T. M. Brown, M. R. Carnethon, S. Dai, G. de Simone, E. S. Ford, C. S. Fox, H. J. Fullerton, C. Gillespie, K. J. Greenlund, S. M. Hailpern, J. A. Heit, P. M. Ho, V. J. Howard, B. M. Kissela, S. J. Kittner, D. T. Lackland, J. H. Lichtman, L. D. Lisabeth, D. M. Makuc, G. M. Marcus, A. Marelli, D. B. Matchar, M. M. McDermott, J. B. Meigs, C. S. Moy, D. Mozaffarian, M. E. Mussolino, G. Nichol, N. P. Paynter, W. D. Rosamond, P. D. Sorlie, R. S. Stafford, T. N. Turan, M. B. Turner, N. D. Wong, J. Wylie-Rosett, Committee American Heart Association Statistics, and Subcommittee Stroke Statistics. 2011. 'Heart disease and stroke statistics--2011 update: a report from the American Heart Association', *Circulation*, 123: e18-e209.

Rosenson, R. S., A. McCormick, and E. F. Uretz. 1996. 'Distribution of blood viscosity values and biochemical correlates in healthy adults', *Clin Chem*, 42: 1189-95.

Ross, J., Jr. 1985. 'Afterload mismatch in aortic and mitral valve disease: implications for surgical therapy', *J Am Coll Cardiol*, 5: 811-26.

Ross, R., T. N. Wight, E. Strandness, and B. Thiele. 1984. 'Human atherosclerosis. I. Cell constitution and characteristics of advanced lesions of the superficial femoral artery', *Am J Pathol*, 114: 79-93.

Rywik, T. M., M. R. Blackman, A. R. Yataco, P. V. Vaitkevicius, R. C. Zink, E. H. Cottrell, J. G. Wright, L. I. Katzel, and J. L. Fleg. 1999. 'Enhanced endothelial vasoreactivity in endurance-trained older men', *J Appl Physiol (1985)*, 87: 2136-42.

Shen, J., F. W. Luscinskas, A. Connolly, C. F. Dewey, Jr., and M. A. Gimbrone, Jr. 1992. 'Fluid shear stress modulates cytosolic free calcium in vascular endothelial cells', *Am J Physiol*, 262: C384-90.

Shyy, Y. J., H. J. Hsieh, S. Usami, and S. Chien. 1994. 'Fluid shear stress induces a biphasic response of human monocyte chemotactic protein 1 gene expression in vascular endothelium', *Proc Natl Acad Sci U S A*, 91: 4678-82.

Slager, C. J., J. J. Wentzel, F. J. Gijsen, A. Thury, A. C. van der Wal, J. A. Schaar, and P. W. Serruys. 2005. 'The role of shear stress in the destabilization of vulnerable plaques and related therapeutic implications', *Nat Clin Pract Cardiovasc Med*, 2: 456-64.

Son, D. J., S. Kumar, W. Takabe, C. W. Kim, C. W. Ni, N. Alberts-Grill, I. H. Jang, S. Kim, W. Kim, S. Won Kang, A. H. Baker, J. Woong Seo, K. W. Ferrara, and H. Jo. 2013. 'The atypical mechanosensitive

microRNA-712 derived from pre-ribosomal RNA induces endothelial inflammation and atherosclerosis', *Nat Commun*, 4: 3000.

Spain, D. M. 1966. 'Atherosclerosis', *Sci Am*, 215: 48-56.

Stary, H. C., A. B. Chandler, R. E. Dinsmore, V. Fuster, S. Glagov, W. Insull, Jr., M. E. Rosenfeld, C. J. Schwartz, W. D. Wagner, and R. W. Wissler. 1995. 'A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association', *Circulation*, 92: 1355-74.

Straub, A. C., A. W. Lohman, M. Billaud, S. R. Johnstone, S. T. Dwyer, M. Y. Lee, P. S. Bortz, A. K. Best, L. Columbus, B. Gaston, and B. E. Isakson. 2012. 'Endothelial cell expression of haemoglobin alpha regulates nitric oxide signalling', *Nature*, 491: 473-7.

Suh, G. Y., A. S. Les, A. S. Tenforde, S. C. Shadden, R. L. Spilker, J. J. Yeung, C. P. Cheng, R. J. Herfkens, R. L. Dalman, and C. A. Taylor. 2011. 'Hemodynamic changes quantified in abdominal aortic aneurysms with increasing exercise intensity using mr exercise imaging and image-based computational fluid dynamics', *Ann Biomed Eng*, 39: 2186-202.

Tanaka, H., S. Shimizu, F. Ohmori, Y. Muraoka, M. Kumagai, M. Yoshizawa, and A. Kagaya. 2006. 'Increases in blood flow and shear stress to nonworking limbs during incremental exercise', *Med Sci Sports Exerc*, 38: 81-5.

Tang, B. T., C. P. Cheng, M. T. Draney, N. M. Wilson, P. S. Tsao, R. J. Herfkens, and C. A. Taylor. 2006. 'Abdominal aortic hemodynamics in young healthy adults at rest and during lower limb exercise: quantification using image-based computer modeling', *Am J Physiol Heart Circ Physiol*, 291: H668-76.

Tate, J. R. 2008. 'Troponin revisited 2008: assay performance', *Clin Chem Lab Med*, 46: 1489-500.

Taylor, C. A., C. P. Cheng, L. A. Espinosa, B. T. Tang, D. Parker, and R. J. Herfkens. 2002. 'In vivo quantification of blood flow and wall shear stress in the human abdominal aorta during lower limb exercise', *Ann Biomed Eng*, 30: 402-8.

Thijssen, D. H., S. Steendijk, and M. T. Hopman. 2009. 'Blood redistribution during exercise in subjects with spinal cord injury and controls', *Med Sci Sports Exerc*, 41: 1249-54.

Tinken, T. M., D. H. Thijssen, M. A. Black, N. T. Cable, and D. J. Green. 2008. 'Time course of change in vasodilator function and capacity in response to exercise training in humans', *J Physiol*, 586: 5003-12.

Tinken, T. M., D. H. Thijssen, N. Hopkins, M. A. Black, E. A. Dawson, C. T. Minson, S. C. Newcomer, M. H. Laughlin, N. T. Cable, and D. J. Green. 2009. 'Impact of shear rate modulation on vascular function in humans', *Hypertension*, 54: 278-85.

Tinken, T. M., D. H. Thijssen, N. Hopkins, E. A. Dawson, N. T. Cable, and D. J. Green. 2010. 'Shear stress mediates endothelial adaptations to exercise training in humans', *Hypertension*, 55: 312-8.

Tjonna, A. E., S. J. Lee, O. Rognmo, T. O. Stolen, A. Bye, P. M. Haram, J. P. Loennechen, Q. Y. Al-Share, E. Skogvoll, S. A. Slordahl, O. J. Kemi, S. M. Najjar, and U. Wisloff. 2008. 'Aerobic interval training versus continuous moderate exercise as a treatment for the metabolic syndrome: a pilot study', *Circulation*, 118: 346-54.

Trinity, J. D., H. J. Groot, G. Layec, M. J. Rossman, S. J. Ives, and R. S. Richardson. 2014. 'Impact of age and body position on the contribution of nitric oxide to femoral artery shear rate: implications for atherosclerosis', *Hypertension*, 63: 1019-25.

Tunick, P. A., J. L. Perez, and I. Kronzon. 1991. 'Protruding atheromas in the thoracic aorta and systemic embolization', *Ann Intern Med*, 115: 423-7.

Tzima, E., M. Irani-Tehrani, W. B. Kiosses, E. Dejana, D. A. Schultz, B. Engelhardt, G. Cao, H. DeLisser, and M. A. Schwartz. 2005. 'A mechanosensory complex that mediates the endothelial cell response to fluid shear stress', *Nature*, 437: 426-31.

Wang, C., B. M. Baker, C. S. Chen, and M. A. Schwartz. 2013. 'Endothelial cell sensing of flow direction', *Arterioscler Thromb Vasc Biol*, 33: 2130-6.

Wentzel, J. J., Y. S. Chatzizisis, F. J. Gijsen, G. D. Giannoglou, C. L. Feldman, and P. H. Stone. 2012. 'Endothelial shear stress in the evolution of coronary atherosclerotic plaque and vascular remodelling: current understanding and remaining questions', *Cardiovasc Res*, 96: 234-43.

Wisloff, U., A. Stoylen, J. P. Loennechen, M. Bruvold, O. Rognmo, P. M. Haram, A. E. Tjonna, J. Helgerud, S. A. Slordahl, S. J. Lee, V. Videm, A. Bye, G. L. Smith, S. M. Najjar, O. Ellingsen, and T. Skjaerpe. 2007. 'Superior cardiovascular effect of aerobic interval training versus moderate continuous training in heart failure patients: a randomized study', *Circulation*, 115: 3086-94.

WorldHealthOrganization. 2009. *Global health risks: mortality and burden of disease attributable to selected major risks* (World Health Organization).

Zhu, W., J. Zeng, J. Yin, F. Zhang, H. Wu, S. Yan, and S. Wang. 2010. 'Both flow-mediated vasodilation procedures and acute exercise improve endothelial function in obese young men', *Eur J Appl Physiol*, 108: 727-32.