Peripheral and Cerebrovascular Function during the Female Lifespan.

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Abstract

The female hormone oestrogen is thought to influence vascular physiology throughout the female lifespan particularly during life events such as menopause and pregnancy. The rapid decline in oestrogen during the menopause is associated with increased cardiovascular (CV) risk, potentially via reduced vascular function. Moreover pregnancy, where oestrogen is elevated for a prolonged period, is a time of profound physiological upheaval including large CV adaptations. Nonetheless, the impact of these important events in the female life course on cerebral and peripheral vascular risk is largely unknown and developing an understanding of such vascular responses is necessary. Importantly, exercise training has known benefits on cerebral and peripheral vascular function, however the role of exercise and physical activity (PA) in pregnancy and menopausal vascular adaptation is poorly defined. Moreover, maternal health has shown to program foetal health in-utero and is associated with immediate and long-term effects on offspring into adulthood and even in subsequent generations. Pregnancy exercise may therefore translate to the neonatal environment, although this has yet to be defined in humans. The aim of this thesis was to describe the cerebral and peripheral vascular changes that are associated with menopause and to assess the impact of exercise training during pregnancy on maternal and offspring vascular health.

In a cross-sectional design study, 50 pre-menopausal (PRE-M; 33.2±9.1years) and 50 post-menopausal (POST-M; 58.5±5.5years) women underwent assessment of brain blood flow assessed via middle cerebral artery velocity (MCAv), cerebrovascular reactivity (CBV-R) and cerebral autoregulation. Peripheral vascular function (brachial and femoral artery endothelial function via flow mediated dilation (FMD)) and structure (brachial (b), femoral (f) and carotid (c) intima-media thickness (IMT) and pulse wave velocity (PWV)) were also assessed. Cardiorespiratory fitness was determined by a maximal oxygen consumption test (VO_{2max}) and objective measures of PA and sedentary behaviour were made. All PRE-M and POST-M women were compared followed by a sub-analysis that included women close to- (Late-PRE-M, N=10) and within 5 years of menopause (Early-POST-M, N=10). POST-M women engaged in significantly less vigorous PA (5 [3, 7 min/d]) compared to PRE-M women (9 [7, 11 min/d] p=0.01) and had a lower cardiorespiratory fitness (24.8 [22.6, 26.9 ml·kg·min]) compared to PRE-M women (34.7 [32.6, 36.9 ml·kg·min] p<0.001). Early-POST-M

women engaged in significantly less light PA (354 [292, 415 min/d]) compared to Late-PRE-M women (264 [199, 329 min/d] p=0.05). There were no differences between PRE- or POST-M women for absolute CBV-R (p=0.52), relative CBV-R (p=0.18) or parameters of cerebral autoregulation including normalized gain (p=0.56) or phase in the low frequency (p=0.73). In the sub analysis, there were no differences between groups for absolute CBV-R (p=0.79), relative CBV-R (p=0.99), normalized gain (p=0.77) or phase (p=0.18) in the low frequency. POST-M women had lower brachial FMD (4.1 [2.9, 5.2 %]) compared to PRE-M women (6.4 [5.4, 7.5%] p=0.004) and a lower femoral FMD 2.8% [1.9, 3.6 %]) compared to PRE-M women (5.8 [4.9, 6.7 %] p<0.001). POST-M women also had a higher PWV (6.87 [6.5, 7.3 m/s]) compare to PRE-M women (5.45 [5.1, 5.8 m/s] p<0.001) compared to PRE-M. Only femoral FMD was lower in Early-POST-M women (2.1% [0.8, 3.5 %]) compared to Late-PRE-M women (4.1 [2.7, 5.5 %] p=0.049). Menopause was associated with higher carotid artery lumen diameter (p=0.01), as well as higher IMT at the carotid (0.70 [0.68, 0.73 mm] p<0.001), brachial (0.38 [0.36, 0.41mm] p=0.001) and femoral arteries (0.49 [0.46, 0.53 mm] p<0.001). Only cIMT (0.67 [0.63, 0.63 mm]) compared to Late-PRE-M women (0.59 [0.54, 0.65 mm] p=0.03). In summary, menopause is associated with lower MCAv, impaired central and peripheral vascular function and greater artery wall thickness compared to the PRE-M state. These decrements are not all present in early menopause suggesting that the duration of time from menopause and age may have a greater effect on these parameters than the event of menopause alone. Finally, our data provides some evidence that post-menopausal vascular decline may be partly driven by reductions in vigorous PA and/or cardiorespiratory fitness.

Twenty-one healthy pregnant women were recruited in trimester 1 (T1) to a partially supervised exercise (EX) or control (CONT) group. Women underwent assessment of cerebrovascular and peripheral vascular function and structural measurements at the end of T1, trimester 2 (T2), and trimester 3 (T3). Participants performed a submaximal exercise test and PA and sedentary behavior objectively monitored for 7 days at each trimester. The exercise intervention comprised of 3-4 sessions per week starting with 15-minutes progressing to 30-minutes at 60-70% maximum heart rate as per the Royal College of Obstetrics and Gynaecology (RCOG) guidelines. MCAv reduced significantly during pregnancy (-8 cm/s^{-1} [-14, -2 cm/s]; main effect of time p=0.02). There was a trend for normalised gain in the low frequency domain to increase during pregnancy (1.6% [1.5, 1.7%]; main effect of time p=0.08). There was a trend for an effect of time for increased phase in the low frequency (13.9° [29°, 39°]; main effect of time p=0.06). Femoral artery FMD decreased during pregnancy (3.5% [-6.5, -0.5%];

main effect of time p=0.03). Brachial artery diameter increased during pregnancy (0.03cm [0.01, 0.06 cm]; main effect of time p=0.03). Carotid artery diameter increased during pregnancy (0.33mm [0.07, 0.58 mm]; main effect of time p=0.04). There was a trend for a reduced PWV during pregnancy (5.01 m/s [2.8, 7.2 cm/s]; main effect of time p=0.09). However, PWV was significantly greater in the EX group (5.3 m/s [1.6, 8.9 m/s] compared to the CONT group (4.7m/s [3.0, 6.5 m/s]; main effect of intervention p=0.04). There was no time*intervention effect for PWV (p=0.68). No other intervention effects were apparent. In summary, pregnancy results in reduced MCAv, possibly due to reduced vascular resistance (carotid artery enlargement) and altered cerebral autoregulation. Pregnancy also results in increased systemic conduit artery size and a reduction in femoral FMD; changes that are seemingly unaffected by adhering to pregnancy exercise guidelines as recommended by the RCOG.

In study 3 offspring cIMT, delivery outcomes and maternal quality of life (QOL) were assessed in the women recruited for study 2. Although not significant, compared to the control group, the offspring of the EX group (N=7) had a smaller IMT (EX; 0.45±0.06mm; CONT; 0.49±0.07mm, p=0.27) and IMT/Lumen (EX; 0.15±0.02, CONT; 0.17±0.04, p=0.19) compared to the control group (N=11). The EX group also had a larger yet non-significant carotid lumen diameter (EX; 2.92±0.13, CONT; 2.88±0.40, p=0.82). Delivery outcomes and maternal QOL were unaffected by exercise. This pilot data suggests that maternal exercise participation may have some role in programming progeny vascular structure the vasculature but does not appear critical to influencing delivery outcomes or post-partum QOL.

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Declaration

I declare that the work contained within this thesis is entirely my own.

Oral Communications based on the work contained within this thesis.

Hopkins, N.D., **Brislane, Á.,** Holder, S.M., Low, D.A., Jones, H.
The effect of aerobic exercise during pregnancy on offspring vascular structure: a pilot study.
European Congress of Sport and Exercise Science. (Prague, 2019).

Brislane, Á., Jones, H., Holder, S.M., Low, D.A., Hopkins, N.D.
The effect of exercise on peripheral vascular outcomes during pregnancy: A pilot study.
European Congress of Sport and Exercise Science. (Dublin, 2018).

Brislane, Á., Jones, H., Carter, S., Holder, S.M., Low, D.A., Hopkins, N.D.
Vascular health, physical activity, sedentary behaviour and ageing in women.
Northern Vascular Biology Forum. (Liverpool, 2017).

Brislane, Á., Jones, H., Carter, S., Holder, S.M., Low, D.A., Hopkins, N.D.
The effect of sedentary behaviour on vascular outcomes in pre-, post-menopausal and pregnant women.
Sedentary Behaviour Seminar with Associate Professor Genevive Healy and Professor David Dunstan. (Liverpool, 2017).

Brislane, Á., Jones, H., Carter, S., Holder, S.M., Low, D.A., Hopkins, N.D.
Differences in Vascular Outcomes and Sedentary Behaviour in Pre- and Post-Menopausal Women.
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Poster Communications based on the work contained within this thesis.

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Research Outputs

Holder, S.M.*, **Brislane, Á.*,** Dawson, E.A., Hopkins, N.D., Hopman, M.T.E., Cable, T.N., Jones, H., Schreuder, T.H.A., Sprung, V.S., Naylor, L., Maiorana, A., Thijssen, D.H.J., Green, D.J. (2019) Relationship between endothelial function and the eliciting shear stress stimulus in women: Changes across the lifespan differ to men. *American Heart Association,* 8(4). *****Shared first authorship.
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Holder, S.M., Dawson, E.A., **Brislane, Á.,** Jonny Hisdal, Daniel J. Green & Dick H.J. Thijssen. Fluctuation in shear rate, with unaltered mean shear rate, improves brachial artery flow-mediated dilation in healthy, young men. *Journal of Applied Physiology,* 1;126(6): p 1687-1693.
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Brislane, Á., Jones, H., Holder, S.M., Low, D.A., Hopkins, N.D. The Effect of Exercise During Pregnancy on Maternal and Offspring Vascular Outcomes: A Pilot Study. *Under Review.*

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Roberts, K., **Brislane, Á.,** Low, D., Thijssen, D. Acute epicatechin ingestion does not affect cerebrovascular function in healthy men. *In preparation.*

Contents

List of abbreviations

Abbreviation	Title
BF	Body fat
bFMD	Brachial flow mediated dilation
bIMT	Brachial intima media thickness
BMI	Body mass index
BP	Blood pressure
CCA	Common carotid artery
cIMT	Carotid intima media thickness
CO_2	Carbon dioxide
CONT	Control group
CPT	Cold pressor test
CV	Cardiovascular
CVC	Cerebrovascular conductance
CVD	Cardiovascular disease
CBV-R	Cerebrovascular reactivity
DBP	Diastolic blood pressure
ECG	Electrocardiogram
eNOS	Endothelial nitric oxide synthase
ER	Oestrogen receptor
EX	Exercise group
fFMD	Femoral flow mediated dilation
fIMT	Femoral intima media thickness
FMD	Flow mediated dilation
FSH	Follicle stimulating hormone
GDM	Gestational diabetes mellitius
GHD	Gestational hypertensive disorder
HR	Heart rate
HRT	hormone replacement therapy
ICA	Internal carotid artery
IMT	Intima media thickness
LH	Leutenising hormone
MAP	Mean arterial pressure
MCA	Middle cerebral artery
MCAv	Middle cerebral artery velocity
MHR	Maximum heart rate
NO	Nitric oxide
NVC	Neurovascular coupling
PA	Physical activity
PCA	Posterior cerebral artery
PCAv	Posterior cerebral artery velocity

$PETCO_2$	End tidal carbon dioxide
POST-M	Post-menopause
PP	Post-partum
PRE-M	Pre-menopause
PWV	Pulse wave velocity
RPE	Rate of perceived exertion
SBP	Systolic blood pressure
SD	Standard deviation
SFTM	Skin fold thickness measure
SR_{AUC}	Shear rate area under the curve
T2DM	Type 2 diabetes mellitius
TCD	Transcranial doppler ultrasound
TTP	Time to peak
VO_2	Oxygen uptake
VO_{2max}	Maximal oxygen uptake
VO_{2peak}	Peak oxygen uptake
WHO	World health organization
WHO-QOL	World health organization quality of life

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Chapter 1: Introduction

The female phenotype undergoes significant physiological change throughout the lifespan, underpinned by fluctuating levels of reproductive hormones. The primary ovarian hormone oestrogen exerts a profound influence on the integrative mechanisms that regulate the cardiovascular (CV) and cerebrovascular system. Endogenous oestrogen is secreted cyclically from the ovaries during the female menstrual cycle and is thought to enhance endothelial function and cerebral blood flow via the vascular endothelium. Oestrogen does this by contributing to the release and bio-availability of nitric oxide (NO); a potent vasodilator (Holm and Nilsson, 2013), which mediates arterial vasodilation, vascular tone, scavenges free radicals, inhibits vasoconstrictors and as a result, prevents vascular dysfunction (Chakrabarti et al., 2014, Forstermann and Sessa, 2012). Contrarily, following menopause, oestrogen levels decline increasing the vulnerability of the CV and cerebrovasculature and an elevated risk of endothelial dysfunction. It is therefore unsurprising that menopause is recognised as a critical event in the progression of women's cardiovascular disease (CVD) risk.

Emerging evidence illustrates that more women than men encounter cognitive decline, Alzheimer's disease, dementia (Beam et al, 2018) and stroke (Reeves et al, 2008) proposedly due to a longer life expectancy (Townsend et al, 2015; Mazure and Swendsen, 2016). Despite this, very little is known regarding cerebrovascular senescence system across the female life span. It has been speculated that menopause contributes to cerebrovascular decline (Li and Singh, 2014; Wong, Evans and Howe, 2016) however, such speculation requires further research to clarify. This is in contrast to the large evidence base linking examining the impact of oestrogen reduction on functional and structural measures of the peripheral vasculature.

The cerebral and peripheral vasculature are structurally similar; however, they have distinct differences in terms of mechanistic control. The brain controls its own blood flow via cerebral

autoregulation (CA) and responds to changes in carbon dioxide (CO_2), neural activity and blood pressure (BP) (Payne, 2016). Differentially, the peripheral vessels are tolerant to changes in flow and pressure, responding with vaso- dilation or –constriction, dependent on the endothelium response to a given stimulus (Vlachopoulos, O'Rourke and Nichols, 2011). Exercise training increases cardiorespiratory fitness , improves peripheral vascular function (Black et al., 2009, Swift et al., 2011), increases MCAv (Bailey et al., 2016) and improves cerebrovascular reactivity (CBV-R) in post-menopausal women (Leslie and Briggs, 2016). Along with aging, PA and cardiorespiratory fitness, may therefore be critical confounders in any study which aims to assess vascular outcomes in post-menopausal women. Yet, no study has attempted to objectively quantify the difference in PA and cardiorespiratory fitness in conjunction with cerebral and peripheral vascular function between pre and post-menopausal women. Therefore, it is incorrect to assume that the changes in the peripheral vessels with the menopause and aging also occur in the cerebral vessels. Therefore, research studies describing the impact of the menopause on the cerebrovascular system are required. With that, ***aim 1*** of this thesis is to is to investigate the effect of menopause on cerebrovascular function, as well as peripheral vascular function and structure, with simultaneous measurement of PA and cardiorespiratory fitness.

Another significant life event for females is pregnancy, which is also characterised by profound changes to reproductive hormones and advanced CV adaptation. The CV changes that arise in brief, include increased cardiac output, decreased mean arterial pressure (MAP), increased blood volume and systemic vasodilation (Gongora and Wenger, 2015); all of which are necessary for adequate delivery of nutrients and oxygen to the foetus. During a healthy uncomplicated pregnancy, peripheral vascular function is enhanced (Torrado et al, 2015). Nevertheless, some women who are seemingly healthy prior to pregnancy develop complications during the gestational period potentially due to maladaptation of the vascular endothelium. This can result in systemic vascular related gestational issues including the onset of gestational diabetes and pre-eclampsia which

increased the risk of CVD.

In the non-pregnant state, exercise has shown to have positive effect on cerebral (Ainslie et al, 2008; Brown et al, 2010; Tyndall et al, 2013) and peripheral vascular funciton (Currie, McKelvie and Macdonald, 2012; Birk et al, 2013; Dawson et al, 2013). Furthermore, exercise has been shown to improve endothelial function during pregnancy (Ramirez-Velez et al, 2011) and is associated with reduced risk of pregnancy-related complications (Davies et al., 2003). Despite this, pregnancy has been shown to have a marked effect on women's propensity to engage in physical exercise (Clarke and Gross, 2004), and often results in the reduction of overall levels of physical activity (PA) (Evenson and Wen, 2011; Hegaard et al, 2011; Amezcua-Prieto et al, 2013). Moreover, it has been demonstrated that during pregnancy women spend a large proportion of time engaging in sedentary behaviour (Downs et al, 2012; Di Fabio et al, 2015). Given the benefit on the vascular system, exercise is a viable means to reduce pregnancy related complications (Mosca et al, 2011). There remains a gap in the literature to identify the normal cerebral and peripheral vascular adaptation to exercise throughout pregnancy. Therefore, ***aim 2*** of this thesis is to investigate the cerebral and peripheral vascular responses to a 6-month pregnancy exercise intervention compared to a non-exercise control, in previously inactive women.

Optimising maternal health is known to influence the foetal environment and relates to offspring risk of metabolic and CVD (Gluckman et al, 2008). The earliest site of atherosclerosis begins in the abdominal aorta and has previously been measured non-invasively using ultrasound to quantify intima-media thickness (IMT) and has shown to be greater among offspring with intrauterine growth restriction compared with normal birthweight offspring (Skilton et al, 2005). Intrauterine vascular adaptation is critical as preclinical changes to vascular structure such as arterial wall thickening, are apparent in high risk individuals before plaque formation and presentation of clinical events affirming its prognostic value (Celermajer et al, 1992; O'Leary et al, 1999). As the intrauterine

environment is paramount to offspring long term health in accordance with the Barker hypothesis (Barker, 1997), optimising maternal health is pivotal to offspring disease prevention in adult life. Recent animal studies have shown maternal exercise to program the intrauterine environment and affect offspring vascular function providing rationale to explore vascular outcomes within a human cohort following delivery. Therefore, ***aim 3*** of this thesis is to investigate the impact of maternal aerobic exercise in healthy pregnant women on birth outcomes and offspring carotid IMT.

Aims

The aims of this thesis are to:

1. Describe the cerebral and peripheral vascular differences between pre- and post-menopausal women.
2. Investigate the effect of pregnancy and exercise on the cerebral and peripheral vascular system throughout pregnancy in previously inactive women.
3. Determine the effect of maternal exercise on offspring carotid artery intima media thickness.

Objectives

The aims outlined above will be achieved through the following objectives listed here.

In line with Aim 1:

1. Directly measure a battery of cerebral and peripheral vascular components in a cohort of healthy ageing women.
2. Compare results of cerebral and peripheral measures between i) pre- and post-menopausal women and ii) between women prior to and beyond the menopause (<5years).

In line with Aim 2:

1. Recruit previously inactive women in early pregnancy (~12 weeks gestation) and engage in a 6-month partially supervised moderate intensity aerobic exercise intervention in line with current guidelines.
2. Directly measure a battery of cerebral and peripheral vascular outcomes at the end of the first, second and third trimester.
3. Compare the effects of exercise training on cerebral and peripheral vascular outcomes with a non-exercise control group.

In line with Aim 3:

1. Directly measure carotid artery intima media thickness in offspring born to all pregnant women in the exercise training study.
2. Compare results of carotid artery intima media thickness between offspring born to exercise and non-exercise trained women.

Chapter 2: Literature Review

2.0 Overview

The changes in reproductive hormones, specifically oestrogen, that occur during a woman's life include cyclical variation during the menstrual cycle that occurs between the age of menarche and the menopause, chronic reduction in the post-menopausal period and, in some women, temporary elevation during pregnancy. The onset of menopause and pregnancy are two important events where substantial changes to oestrogen levels occur and these changes coincide with changes in cardiovascular function which may ultimately impact risk of CVD and cerebrovascular events such as stroke (Maturana et al., 2007, Hunter, 1990, Regitz-Zagrosek et al., 2011). This literature review will focus on the known cerebral and peripheral adaptations that arise during the female lifespan and how these changes may contribute to CV risk.

2.1 Healthy Ageing

The normal menstrual cycle. The normal menstrual cycle involves the synergistic interaction between the hypothalamus, pituitary gland, ovaries and uterus to initiate cyclical hormonal changes which are essential for human reproduction. This cyclical variation in reproductive hormones, lasting approximately 26-35 days, begins at the age of menarche and ceases following the transition to menopause. The menstrual cycle comprises of three phases; follicular, ovulatory and luteal, orchestrated by the stimulation and inhibition of follicle stimulating hormone (FSH), leuteinising hormone (LH), oestrogen and progesterone. The hypothalamic releasing factor gonadotropin stimulates the secretion of FSH and LH from the anterior pituitary which results in the release and growth of a dominant ovarian follicle. The ovary secretes progesterone and oestrogens in response to elevated levels of FSH and LH. During the follicular phase, oestrogens secreted by ovarian follicles suppress the production of LH until a threshold is reached and a subsequent surge in LH is released to prepare the body for ovulation. During the luteal phase, FSH and LH cause the dominant follicle to transform into the corpus luteum and secrete progesterone, further activating the

production of oestrogens in the luteal phase. Increased levels of oestrogen suppress the release of FSH and LH causing the corpus luteum to regress due to declining progesterone levels initiating menstruation (Dawson and Reilly, 2009) (Figure 2.1). Of the hormones involved in controlling menstrual function, oestrogen is recognised for its impact on the CV system (Mendelsohn and Karas, 1999). The mechanism surrounding oestrogen enhancement of NO production is not completely understood however, it is thought that oestrogen receptors ER-α and ER-β located at the endothelium, mediate endothelium derived NO activation which help to maintain vascular function, reduce oxidative stress, inhibit vasoconstrictors and prevent endothelial dysfunction (Chakrabarti et al., 2014, Forstermann and Sessa, 2012).

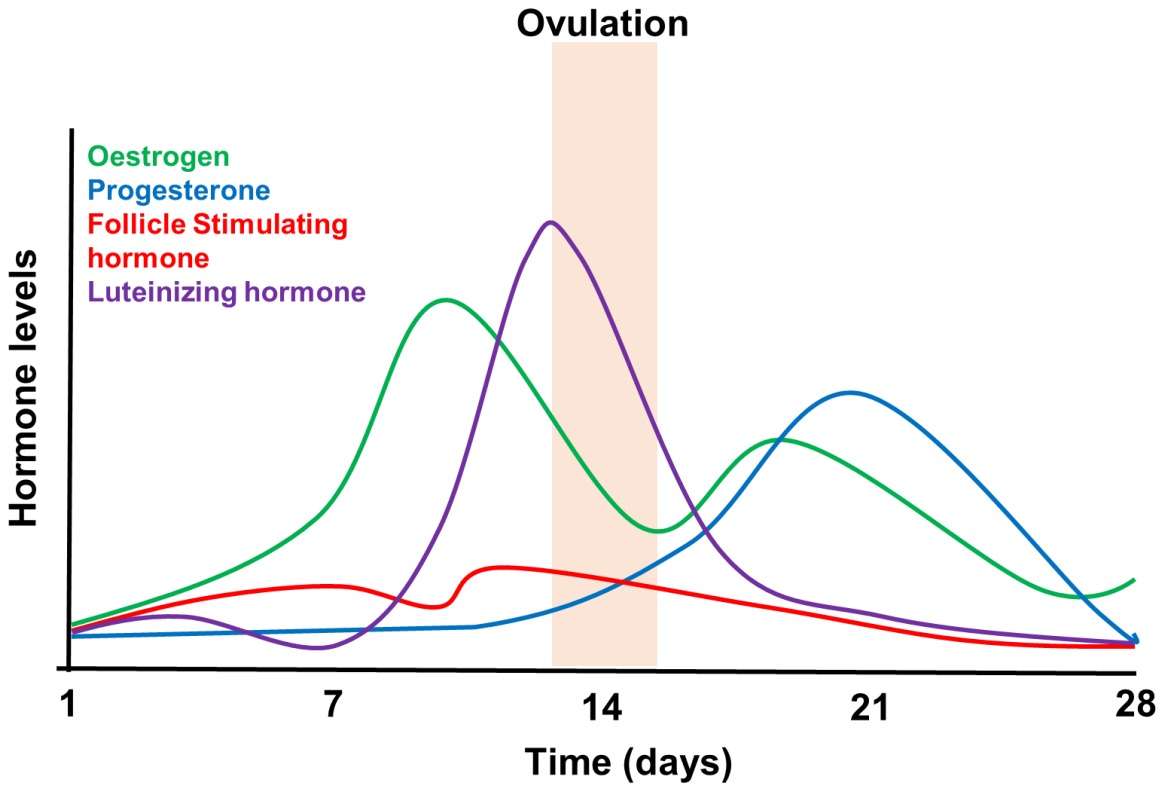


Figure 2.1 Diagram illustrating hormonal fluctuation throughout the menstrual cycle.

Menopause. The transition to menopause, referred to as peri-menopause, can last up to 8 years and is accompanied by irregular and less frequent menstruation. This is due to variable ovarian responses to increased FSH and fluctuating oestrogen levels with eventual loss of a normal reproductive cycle (Takahashi and Johnson, 2015, Roberts and Hickey, 2016). Menopause is defined by the World Health Organization (WHO) as the cessation of spontaneous menstruation for 12 months, typically occurring between 49 and 52 years, as a result of oestrogen reduction (WHO, 1996). The onset of menopause is associated with unfavourable modifications of many CV risk factors (Kannel et al., 1976, Matthews et al., 2009, Akahoshi et al., 1996). However, such risk factors, including abnormal lipid profile, BP and body composition, are also apparent with increasing age, it can therefore be difficult to separate the relative contribution of age and menopause to changes in CV risk. Recent studies have aimed to differentiate between the effect of age and/or menopausal status on CVD risk factors by comparing pre- and post-menopausal women of similar age. Post-menopausal women have poorer lipid profiles compared to pre-menopausal women differentiated by 5 years in age (Mandrup et al., 2017). Elsewhere, women who have been menopausal for just three years present with higher body fat percentage, higher diastolic BP and reduced high-density lipoprotein compared with pre-menopausal women (Nyberg et al., 2014). Furthermore, endothelial dysfunction is evident within one year of menopause (Moreau et al., 2012) with the authors attributing this decline to reduced oestrogen. Given the apparently accelerated effect of menopause on CVD risk factors and the higher incidence of CVD among women compared to men (Appelman et al., 2015), the menopause remains a critical event in facilitating our understanding of CVD risk in women. Nonetheless, the effect of menopause on cerebrovascular response remains largely unexplored. The following sections will outline current understanding of cerebrovascular and peripheral vascular changes during menopause.

2.1.1 The Cerebrovascular System

Despite only occupying 2-3% of total human body mass, the brain accounts for 20% of human metabolism and 14% of blood flow (Payne, 2016). The control of adequate cerebral blood flow to the brain is critical to normal brain functioning; insufficient blood flow (hypoperfusion) is responsible for brain damage through ischemic injury. Moreover, excessive cerebral blood flow (hyperperfusion) can lead to seizures, headaches, encephalopathy, while both can cause ischemic and haemorrhagic stroke (Fantini et al., 2016). Cerebral blood flow is regulated by metabolic demand, myogenic and neurogenic factors (Ainslie and Duffin, 2009). The myogenic response refers to the active behaviour of the vasculature to respond to changes in BP. It acts to regulate vessel diameter when necessary and facilitate a constant cerebral blood flow velocity. The neurogenic response remains the most poorly understood with cerebral sympathetic activity proving to be the most difficult parameter to interpret and measure (Payne, 2016).

The ability to measure cerebral blood flow velocity can provide valuable information of the functional status of the blood vessels that supply the brain. Transcranial Doppler (TCD) ultrasound is frequently used to measure cerebral blood flow velocity in humans. It is a safe, non-invasive, non-ionising portable and inexpensive method that is comparable between studies. In brief, TCD ultrasonography uses probes that emit ultrasonic beams that can be focused onto vessels of interest. The assessment of cerebral blood velocity is derived from the Doppler shift, created by the reflection of ultrasound waves from moving red blood cells within the blood vessel that are returned to the receiver unit in the Doppler probe. The Doppler shift refers to the difference between the transmitted and received ultrasound signals (Aaslid et al., 1986) with faster red blood cell movement associated with higher velocities. Knowledge of typical vessel depths, correct probe directions and mean blood flow velocities all affect the chances of collecting valid cerebral measurements (Arnolds and von Reutern, 1986) (Table 2.1).

Table 2.1 Typical patterns for identification of cerebral arteries.

Artery	Window	Depth (mm)	Direction	Mean Flow Velocity
MCA	Temporal	30 to 60	Toward probe	55 ± 12 cm/s
ACA	Temporal	60 to 85	Away	50 ± 11 cm/s
PCA	Temporal	60 to 70	Bidirectional	40 ± 10 cm/s

Abbreviations: MCA, middle cerebral artery; ACA, anterior cerebral artery, PCA, posterior cerebral artery.

During the menstrual cycle the highest cerebral blood flow velocities occur during the luteal phase of the menstrual cycle when both oestrogen and progesterone are elevated implying an influence of sex hormones on cerebral vessels (Brackley et al., 1999). Regional cerebral blood flow velocity depends on the vasodilation action of oestrogen (Toda et al., 2009) via endothelial-derived NO and prostacyclin pathways (Krause et al., 2006). Post-menopausal women have significantly lower cerebral blood flow velocity compared to pre-menopausal women (Slopien et al., 2003) and is also reduced in hypoestrogenic women (Greene et al., 1998). After menopause there is a negative correlation between serum 17b-estradiol level and pulsatility index of the internal carotid artery and the MCA (Battaglia et al., 1999). The administration of exogenous oestrogen referred to as hormone replacement therapy (HRT) increases cerebral blood flow velocity in menopausal women (Slopien et al., 2003). This improvement in cerebral blood flow velocity has been demonstrated in other clinical trials using varying dosages of HRT (Bain et al., 2004, Buchanan et al., 2007). Taken together, this evidence seems to demonstrate some role for oestrogen in the regulation of cerebral blood flow velocity.

Cerebrovascular reactivity. The brain is sensitive to changes in carbon dioxide (CO_2), with very large changes in cerebral blood flow occurring as CO_2 is altered (Payne, 2016).

As a result, reactivity of the cerebrovasculature to increases (hypercapnia) or decreases (hypocapnia) of CO_2 can be assessed. Cerebrovascular reactivity (CBV-R) provides an index of reactivity of the intracranial vessels in response to a stimulus i.e. through ventilator alterations and can be measured by a variety of means (Fierstra et al., 2013). The mechanisms by which CBV-R takes place involves changes in plasma pH caused by increased CO_2, which activate K^+ channels in the vascular smooth muscle and stimulate the relaxation of cerebral vessels (Jackson, 2005). Evidence has also suggested a role for NO in the vasodilatory response to hypercapnia with previous work showing that the dilatory response of cerebral vessels during hypercapnia is attenuated when a NO synthase inhibitor has been used (Smith et al., 1997).

Measuring the cerebrovascular response to changes in CO_2 concentration reflects an index of the ability of the cerebrovascular beds to dilate or constrict is termed cerebrovascular reactivity (CBV-R) (Ainslie and Duffin, 2009) and can be assessed in three ways. The earliest technique used was breath-holding, whereby participants would hold their breath to progressively increase CO_2. This technique is highly variable between individuals and is influenced by lung size and age (Fierstra et al., 2013). Another common technique used is rebreathing exhaled gas to increase in $PETCO_2$. This method requires minimal equipment with just an exhaled gas reservoir and gas sensors necessary and allows assessment of ventilatory response. Nevertheless, this method is limited by the ramp-like response to exhaled air with each breath causing an increase in $PETCO_2$, resulting in an unstable measurement of increased cerebral blood flow velocity. Lastly, exposing participants to external CO_2 ranging from 2-7% concentrations by means of a nonrebreathing face mask is favourable as it can induce a standardised hypercapnic stimulus (Fierstra et al., 2013). Due to the range of methods available, difficulties arise when comparing study results and interpreting findings.

Cerebrovascular reactivity to CO_2 has been reported to be similar between pre- and post-menopausal women (Mitsis et al., 2007), despite a small sample size and the inclusion of

women on HRT, the authors imply that oestrogen did not play a role in the CBV-R. In another study CBV-R, measured using the breath hold index, was reduced following the menopause (Matteis et al., 1998). The authors suggested this difference was potentially due to changes in hormone levels. Furthermore, interpretation of these findings are limited by the high variability and low reproducibility associated with the breath hold index test (Alwatban et al., 2018). CBV-R is reported to be enhanced by the use of HRT suggesting a role for oestrogen on the cerebrovasculature (Kastrup et al., 1998). Although insightful, the older participants included in this study were those admitted to hospital with minor illnesses which may have influenced the results. The effect of menopause on CBV-R therefore remains unclear as current findings are limited by differing methodology, small sample sizes, the inclusion of unhealthy participants and no actual quantification of oestrogen levels (Mitsis et al., 2007, Kastrup et al., 1998, Matteis et al., 1998).

Cerebral autoregulation. Cerebral autoregulation refers to the brain's ability to maintain a relatively constant blood flow despite changes in arterial blood pressure (BP). To avoid cerebral injury, cerebral autoregulation must take place. A rapid and unopposed drop in BP, and subsequently cerebral blood flow, can lead to dizziness and fainting. Alternatively, a similarly rapid rise in BP and cerebral blood flow can lead to increased mechanical stress on the smaller fragile cerebral blood vessels, which can be fatal. Indeed, brain injury occurs when autoregulatory mechanisms are lost (Euser and Cipolla, 2007, Novak et al., 1998a). The mechanisms involved in the intrinsic proficiency of cerebral vessels to maintain a constant cerebral blood flow velocity over a range of systemic BP levels is yet to be fully elucidated, yet most likely involves myogenic and/or autonomic mechanisms (Paulson et al., 1990, Aries et al., 2010, Strandgaard and Paulson, 1984). The primary driver involved in cerebral autoregulation is cerebral perfusion pressure; calculated as BP–intracranial pressure. As BP is constantly fluctuating as a result of daily tasks such as a change in posture, the cerebral autoregulatory system is under constant demand. Initial understanding of cerebral autoregulation implied that steady-state cerebral blood flow remained relatively

stable across a wide range of blood pressure i.e. 60-160 mmHg requiring reflex adjustment in cerebrovascular resistance in response to changes in blood pressure (Lassen et al. (1989). However, within-subject human data indicate that the cerebral blood flow mean arterial pressure (MAP) relationship is not flat through a broad range of MAP and instead is responsive to the severity an direction of change in perfusion pressure (Willie et al., 2014). This implies a much more passive relationship between cerebral blood flow and perfusion pressure than conventionally believed.

The myogenic reflex is the intrinsic ability of the vascular smooth muscle to respond to changes in pressure or mechanical load with a constriction in response to increased pressure, and dilation in response to decreases in pressure, referred to as the 'Bayliss effect' (Mellander, 1989). The cerebral vessels are also heavily innervated by neural sympathetic fibres that are also involved in the autoregulatory responses. Whilst transient changes in arterial BP occur throughout the cerebrovasculature and the extra-cranial vessels, the pial arterioles appear to be the most responsive to these changes (Willie et al., 2014).

Cerebral autoregulation has traditionally been assessed in response to transient decreases in BP induced by the sudden deflation of bilateral thigh cuffs after an inflation period to supra-systolic levels (Mahony et al., 2000) however this technique is not well tolerated and provides variability in BP responses (Willie et al., 2014). The sit-to-stand manoeuvre has been deemed tolerable by participants to induce changes in BP. Importantly though, this technique elicits an observable drop in BP and consequently, cerebral blood flow (Murrell et al., 2007, Serrador et al., 2005).

Lipsitz et al. (2000) compared cerebral autoregulation in mixed sex groups of young healthy adults and older adults and found that all older adults retained similar cerebral autoregulation capacity to the young participants, implying that cerebral autoregulation

remains intact with aging. When cerebral autoregulation was compared between older post-menopausal women and age matched men, women were found to have a greater ability to regulate cerebral blood flow velocity in response to a sit-to-stand protocol (Deegan et al., 2011). Given that all women were post-menopausal, this data rules out sex hormones as a mediator of maintained cerebral autoregulation. In support, endogenous sex hormones were shown to play no role in cerebral autoregulation responses to orthostatic stress in pre-menopausal women when tested at various times across the menstrual cycle (Claydon et al., 2006). Importantly, this study measured oestrogen and progesterone levels providing definitive cyclical changes. Alternative mechanisms for maintained cerebral autoregulation were explored by Edgell et al. (2012); during postural transitions, women exhibit a smaller increase in CO with lower stroke volume compared to age-matched men. This was thought to occur due to an absent compensatory increase in heart rate (HR), and as a result, an attenuated venous return. This suggests that cerebral autoregulation is dependent on autonomic responses distal to the cerebral system. In summary, cerebral autoregulation is complex and appears to be performed more efficiently in women compared to men. The reason for this is not entirely understood and further research is warranted to understand its behaviour across the female lifespan.

2.1.2 The Peripheral Vascular System and Menopause

Artery Function. Conduit artery function is greatly dependent on the integrity of the endothelium to maintain a balance between vasoconstriction and vasodilation through inhibiting and stimulating vascular smooth muscle cell proliferation and migration, thrombogenesis and fibrinolysis (Ross, 1999).The endothelium regulates vascular tone by releasing vasoconstrictors such as endothelin and angiotensin II in response to physical and chemical stimuli and endothelium-dependent vasodilators including prostacyclin, bradykinin and NO (Davignon and Ganz, 2004). Nitric oxide is of unique interest due to its ability to act as a potent vasodilator on the vascular system (Bauer and Sotnikova, 2010). NO is continuously synthesised from the amino acid L-arginine in endothelial cells by NO

synthase (NOS) (Lamas et al., 1992, Forstermann and Sessa, 2012). Once synthesised, endothelial NOS (eNOS) rapidly diffuses into the vascular smooth muscle cell where it binds to the enzyme guanylate cyclase resulting in an increase in cyclic guanosine monophosphate that induces smooth muscle relaxation and subsequent vasodilation (Green et al., 2004). Importantly, shear stress from increased blood flow upregulates NO production by activating calcium and potassium channels on the endothelial cell surface resulting in potassium efflux and calcium influx. Increased intracellular calcium causes eNOS to detach from the protein caveolin located in the caveolae stimulating further, the production of NO (Forstermann and Sessa, 2012, Sandoo et al., 2010).

Reduced NO bioavailability results in the initiation, progression and complications of atherosclerosis through numerous means including lipid damage, inflammatory response, vasoconstriction, platelet aggregation, smooth muscle cell proliferation and migration, leukocyte adhesion as well as oxidative stress (Chen et al., 2018). The bioavailability of NO in the coronary and peripheral arteries has been regarded as an indicator of prospective CV events and a marker of endothelial (dys)function. Endothelial function can be measured using the flow mediated dilation (FMD) technique (Celermajer et al., 1992). This technique is established as a surrogate marker of CVD (Thijssen et al., 2011a, Green et al., 2011a) and a predictor of future CV risk (Ras et al., 2013, Inaba et al., 2010).

Oestrogen stimulates prostacyclin and NO synthesis via oestrogen receptors at the endothelial cell surface (Smiley and Khalil, 2009) implying oestrogen may be an important contributor to artery health. Artery function has shown to be lower in men almost ten years earlier than their age-matched pre-menopausal female counterparts highlighting potential differing aetiology of CVD between sexes (Celermajer et al., 1994). Interestingly, in women a steep decline in artery function commences around the time of the menopause. Indeed, in pre-menopausal women, a higher FMD has been reported during the follicular phase when oestrogen levels are greater than in comparison to the luteal phase (Williams et al.,

2001). The effects of removal of oestrogen on endothelial function was explored by Jensen-Urstad and Johansson (2001), where brachial artery FMD was compared in pre- and post-menopausal women at 35 and 55 years of age respectively. Unsurprisingly, post-menopausal women showed a lower FMD when compared to the younger cohort which was attributed to changes in oestrogen. This was further expanded upon when brachial artery FMD was shown to be impaired, independent of traditional CV risk factors, in late peri-, early post-menopause and late-post-menopausal women compared to pre-menopausal controls (Moreau et al., 2012). Interestingly, late peri-menopause was not different to early post-menopause implying that the rapid deterioration in endothelial function may be related to prolonged oestrogen deficiency. Elsewhere, short-term HRT was shown to improve FMD in post-menopausal women which has strengthened evidence for direct oestrogenic effects on vascular endothelial function in post-menopausal women (Lieberman et al., 1994), however plasma oestrogen was not determined and so the explanation of these findings remain speculative. Nonetheless, this evidence does imply a protective role of oestrogen on endothelial function providing a plausible explanation for increased CVD post-menopause.

Increased age is associated with endothelial dysfunction, arterial stiffening and remodelling, impaired angiogenesis, defective vascular repair, increased prevalence of atherosclerosis and is an independent CVD risk factor (Dhingra and Vasan, 2012). Vascular ageing; associated with reduced NO production (Goubareva et al., 2007) is a natural phenomenon and although aging *per se* has detrimental effects on the vasculature. Specifically, the lack of oestrogen due to menopause may add an aggravating CVD risk factor in women, compared to arterial aging in men (Celermajer et al., 1994). Older women exhibit attenuated brachial FMD responses relative to younger women (Eskurza et al., 2006, Eskurza et al., 2004, Black et al., 2009). Aging has been associated with significant reductions in the direct oestrogen- mediated mechanisms of vascular relaxation (Wynne et al., 2004, LeBlanc et al., 2009). The lack of oestrogen responses in these studies was not related to age-

associated changes in the plasma levels of oestrogen or activity of ER, but rather to possible age-related changes in oestrogen-mediated signalling pathways in the vasculature. Modifications in the ratio between ERα and ERβ in older female mice are associated with the lack of protective effects of oestrogen on NO production and with a reversal in its antioxidant effect to a pro-oxidant profile (Novensa et al., 2011). Moreover, clinical studies have revealed that CVD risk factors in post-menopausal women were lower among women aged 50–59 years at HRT initiation (Manson et al., 2007, Sherwood et al., 2007). These studies clearly establish the complexity of oestrogen effects, which may be influenced by pathophysiological conditions including aging and subclinical CVD. It is accepted that the vascular protective effects exerted by oestrogens have been proposed as the major reason for reduced signs of vascular aging and CVD risk in premenopausal women, compared to men. Furthermore it appears that when natural oestrogen withdrawal occurs and a woman enters her climacteric stage, effects of sudden vascular aging become evident, leading to vascular dysfunction and increased risk of a cardiovascular event (Novella et al., 2012). At present to the best of our knowledge, the independent contribution of ageing and menopause on artery function has yet to be eluded.

Artery structure. Artery intima-media thickness (IMT) is an index of artery wall thickness typically measured at the common carotid artery non-invasively using B-mode ultrasound. The magnitude of IMT has shown to correlate well with major CV risk factors, disease prevalence and the severity of atherosclerosis in vascular beds (Engelen et al., 2013). Ultimately, the progression of IMT leads to a narrowing of the lumen diameter which can restrict blood flow which can detrimentally lead to CV events including myocardial infarction and stroke (Bots et al., 1997, Lekakis et al., 2000). Arterial stiffening is determined in large by the elastin to collagen ratio in artery walls. It can be measured by the 'gold-standard' pulse wave velocity (PWV) technique (Covic and Siriopol, 2015) and can relate to future CV events (Laurent et al., 2006). In general, artery stiffening is increased with age and can come about due to changes in collagen and elastin properties, arterial pressure and the

production of glycation end products (Thijssen et al., 2016). Taken together, IMT and artery stiffness are important considerations as makers of systemic vascular health.

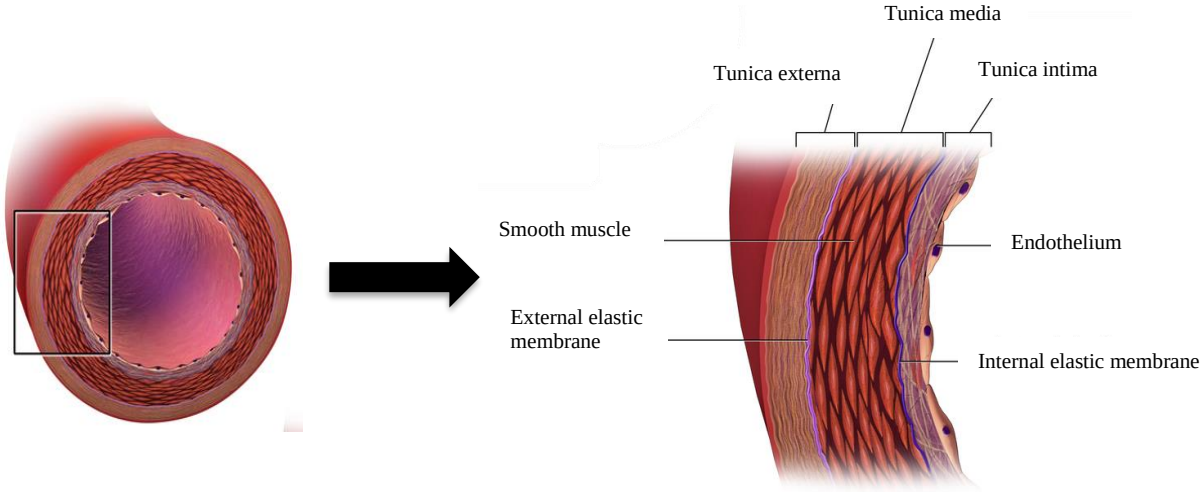


Figure 2.1.2 Diagram illustrating the structural layers of an artery.

Artery structure and menopause. Measurement of IMT has been shown to be greater in post-menopausal women compared to pre-menopause (Sutton-Tyrrell et al., 1998). Normative IMT values are lower for younger women compared to age matched men (Engelen et al., 2013), however in older subjects, normative values are the same for both sexes (Sinning et al., 2011). In agreement, Stamatelopoulos et al. (2012) reported similar carotid IMT (cIMT) values between recently post-menopausal women (<5years) and age matched men, implying that the menopause transition accelerates IMT progression to match that of male counterparts. Interestingly, hormone replacement therapy (HRT) studies have reported beneficial effects of oestrogen replacement on arterial stiffness, arterial compliance and carotid artery distensibility. In the Cardiovascular Heart Study (CHS), women over the age of 65 had smaller IMT when using HRT compared with age and sex matched controls (Jonas et al., 1996, Glisic et al., 2018), a finding later supported by the Rotterdam Study (Westendorp et al., 1999). In the Asymptomatic Carotid Atherosclerosis Progression Study, HRT, specifically oestrogen, has reportedly reduced or halted the progression of IMT in post-menopausal women over a 3 year period (Espeland et al., 1995).

Taken together these data imply that oestrogen exerts beneficial effects on artery wall thickening.

The mechanism by which exogenous oestrogen protects against the progression of IMT is unknown (Glisic et al., 2018). Recent evidence has suggested that the timing of exogenous oestrogen administrating may be an important consideration in benefitting vascular health (Clarkson et al., 2013). To explain this, data from animal studies indicate that the anti-atherogenic effect of oestrogen on the artery wall is abolished by a damaged endothelium suggesting that an in-tact endothelium is crucial for the beneficial anti-atherosclerotic effects of oestrogens on the artery wall (Holm et al., 1999, Hanke et al., 1999). In light of this, there appears to be a window of opportunity to protect against the age-associated IMT thickening exacerbated by the onset of menopause.

2.1.3 Physical activity and cardiovascular outcomes

Physical activity is an important mediator of CVD. Specifically, PA has been shown to benefit conduit artery (Black et al., 2009) and cerebrovascular function (Tyndall et al., 2013) in healthy adult populations, including older populations. Despite this, habitual PA levels are reduced with increased age (Townsend et al., 2015a). This was demonstrated by Jefferis et al. (2014) who reported few older adults meeting recommended PA guidelines and specifically, moderate-to-vigorous PA was lower in older women compared to similarly aged men.

Similar trends were reported in the US National Health and Nutrition Examination Survey (2003 and 2006) which contained accelerometer data for almost 7000 adults aged 20 to 79 (Plows et al., 2018). This data demonstrated low levels of PA across all ages and a decline in higher intensity activities with increasing age (Sparling et al., 2015). Evidently, PA

declines with age however, the independent effects of this on CV health is unclear. It is plausible that some ageing effects may be driven by or compounded by reduced PA levels or reductions in the intensity of PA performed, although more research is warranted to understand this further.

Physical activity has been deemed beneficial for human cerebrovascular health by potentially offsetting declines in cerebral tissue density (Colcombe et al., 2003) and increasing brain volume in ageing humans (Colcombe et al., 2006). To the best of our knowledge, no research has examined objectively measured habitual PA and its association with CBV-R and cerebral autoregulation. However, exercise interventions can provide insight into the possible role it can play. Endurance trained men have shown higher MCAv compared with sedentary aged-matched male counterparts, potentially through direct effects on endothelial eNOS activity (Ainslie et al., 2008). Elsewhere, resting MCAv and CBV-R were examined in young and older men and women following a 12-week progressive aerobic exercise intervention. While MCAv was unchanged, CBV-R was elevated independent of age indicating that healthy ageing does not affect the ability of the cerebrovasculature to adapt to exercise training (Murrell et al., 2013). This exercised induced elevation in CBV-R may provide an important physiological link in the relationship between fitness and cerebrovascular disease. Given that women live longer than men (Townsend et al., 2015b) and have an increased risk of cerebrovascular disease (Li and Singh, 2014), understanding the effects of lifestyle and modifiable risk factors, like PA, on cerebrovascular health is important and will may help inform which mediators should be targeted in future intervention design.

Physical activity has shown enhancement of brachial artery endothelial function in healthy (Black et al., 2009) and in overweight and obese post-menopausal women (Merino et al., 2013). Black et al. (2009) performed a 24-week exercise intervention that was progressed in intensity form 30% heart rate reserve (1-12 weeks), to 60% (13-24 weeks), in previously sedentary adults. The authors observed an attenuated age-related decline in brachial artery

FMD response in post-menopausal women only implying varying vascular responses to exercise between sexes. In contrast, 50 minutes of brisk walking per day for 8 weeks elicited no improvement to brachial FMD in healthy post-menopausal compared to similarly aged men (Pierce et al., 2011). The differing exercise intensity used in these studies may explain the contrasting findings regarding FMD. This evidence suggests the exercise intensity, and not the duration is potentially more important in yielding beneficial effects on vascular function. In summary, PA particularly at a moderate intensity, appears to benefit peripheral vascular function. A reduction in PA and/or it's intensity that accompanies increased age, may make the independent effects of age and/ menopause on the vasculature difficult to discern. Nonetheless, studies should aim to objectively measure PA to account for its role on peripheral and cerebrovascular senescence.

2.2 Pregnancy

Pregnancy is a dynamic process underpinned by huge maternal hormonal change and physiological adaptation to support a growing foetus. The CV system undergoes the most profound change of all systems due to increased demand to accommodate a growing foetus. This change begins during the fifth week of gestation and can last up to a year following delivery (Barakat et al., 2015). As a result, maladaptation of the CV system can arise which leads to short-term complications such as pre-eclampsia and gestational diabetes (Skow et al., 2017). These complications are also associated with increased risk of CVD in later life. Pre-natal exercise can positively influence both maternal and offspring CV health. The following sections will focus on female CV adaptation to pregnancy with specific focus on cerebral and peripheral vascular outcomes and will provide an overview of the impact of exercise training on these parameters. Lastly, the review outlines current evidence on the influence of exercise and the intrauterine environment on offspring health.

2.2.1 Cardiovascular Adaptation to Pregnancy

During a normal pregnancy, the heart increases in size by up to 30% (Regitz-Zagrosek et al., 2011) and HR increases by 10 to 20 beats.min^{-1} (Mahendru et al., 2014). Within the first 8 weeks of gestation systolic and diastolic blood pressure drops 5-10 mmHg below pre-pregnancy baseline. Then BP gradually decreases from trimester 1 to a nadir during the second trimester before increasing during the third trimester and returning close to preconception levels postpartum. Blood volume and CO increase by approximately 40% and peripheral vascular resistance is reduced due to the high flow, low resistance circuit in the utero-placental circulation (Figure 2.2.1). As blood volume increases, uterine blood flow increases significantly to allow perfusion of the intervillous spaces of the placenta and support foetal growth (Gongora and Wenger, 2015). Increased vasodilation is necessary to accommodate increased blood volume (Lopes van Balen et al., 2017) and is thought to be instigated by a decreased sensitivity to norepinephrine and angiotensin, increased eNOS bioavailability at the endothelium due to increased oestrogen levels, increased prostacyclin production and reduced aortic stiffness (Gongora and Wenger, 2015). Failure of the maternal vasculature to adequately adapt to any of the above can result in increased risk of CVD both during and beyond pregnancy (Hutcheon et al., 2011).

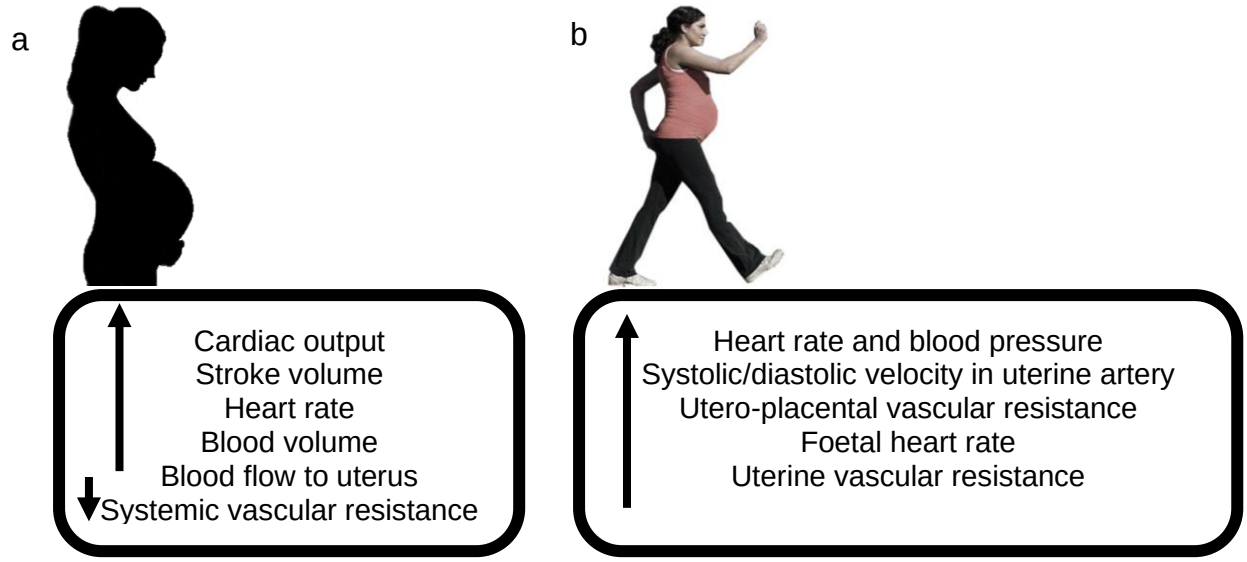


Figure 2.2.1 Cardiovascular adaptations that occur with pregnancy (a) and with exercise during pregnancy (b).

CV risk in pregnancy. The risk of CVD is elevated during pregnancy and increases with gestational age, the prevalence of diabetes, hypertension and obesity (Fraser et al., 2012). Hypertensive disorders are the leading cause of maternal and perinatal morbidity and mortality during pregnancy and occur in 5-10% of all pregnancies (Hutcheon et al., 2011). Gestational hypertensive disorders (GHDs) are believed to result from ischaemia of the placenta which in turn releases anti-angiogenic factors including vascular endothelial growth factor-1 and soluble endoglin into the maternal circulation. These factors are understood to induce features of pre-eclampsia including hypertension and fetal growth restriction (Powe et al., 2011). Importantly, the vascular endothelium relies on pro-angiogenic factors and so the release of such anti-atherogenic factors are a plausible cause of the endothelial dysfunction associated with pre-eclampsia (Powe et al., 2011). Moreover, the production of nitrite has shown to be compounded by pre-eclampsia which may exacerbate endothelial in the presence of GHDs (Seligman et al., 1994). However, it is not yet fully understood whether endothelial dysfunction is the cause or consequence of GHDs. Irrespective of the aetiology, development of GHDs have important consequences for long term CV risk (Irgens et al., 2001). Women who develop GHD have a 7-15% higher risk of

developing CVD in a subsequent pregnancy compared with a 1% chance for women with no pre-eclampsia in their first pregnancy (Irgens et al., 2001). Furthermore, women who develop pre-eclampsia have a three- to four- fold increased risk of developing chronic hypertension and a two-fold increased risk of ischemic heart disease and stroke (Schneider et al., 2012).

Gestational diabetes mellitus (GDM) affects 3%-8% of pregnancies (Lee et al., 2007) and is associated with obesity, maternal age of first pregnancy, high calorie diets and low levels of PA (Ramos-Leví et al., 2012). Of the pregnancies that develop GDM, an estimated 10% will develop T2DM soon after delivery while 20-60% of women will present with it in the 5-10 years after birth (Buchanan et al., 2012). GDM is a risk factor for the development of CVD given the common risk factors between both disease states including obesity, hypertension, and dyslipidaemia (Matheus et al., 2013). FMD has been evaluated in women with GDM, impaired glucose tolerance (IGT) and a control group in the third trimester of pregnancy. Women with IGT had FMD at 70% of the control group's FMD, while the GDM group had an FMD at 38% of the control group demonstrating the negative impact of GDM on endothelial function. Elsewhere, artery wall thickness has shown to be significantly greater in a group of women with GDM compared with a control group in the second trimester of pregnancy (Tarim et al., 2006). Following delivery, GDM has lasting effects for vascular risk with FMD impaired at 3 and 6 months PP compared to uncomplicated pregnancies (Anastasiou et al., 1998). Additionally, cIMT was measured in women with a history of GDM and without, 2 years after delivery (Volpe et al., 2008). The women with a history of GDM were significantly older, had higher BP; although within normal range, and higher waist circumference compared to those without a history of GDM. Those with a history had a significantly larger cIMT proposing that incidence of GDM has lasting CV consequence. Offspring born to mothers with GDM are more likely to be overweight or obese and have poor lipid profiles increasing their risk of CVD (Drake and Reynolds, 2010) and exhibit adverse vascular changes such as increased abdominal artery wall thickness

(Akcakus et al., 2007). Furthermore, offspring of women with GDM had a reportedly 8-fold increased risk of diabetes. At the age of 22 years, the prevalence ofT2DM was 21% in offspring of mothers with GDM, 11% in those whose mothers had pre-gestational T1DM and 4% in offspring of mothers with no diabetes (Clausen et al., 2008). The mechanisms by which offspring CVD risk is elevated are thought to arise following the altered capacity of the endothelium to release NO in the fetoplacental vasculature. This results in abnormal functioning of the L-arginine/NO signalling pathway leading to altered vascular function and changes in umbilical vessels blood flow from and to the foetus implying a pathological link between this in-utero programming and long term vascular function (Leiva et al., 2011). GDM clearly has negative consequences for both mother and infant in the immediate and long-term.

CV risk is also elevated in women who have low birth weight babies (<2500g) and/or deliver preterm (<37 weeks gestation) (Kessous et al., 2013, Smith et al., 2000a). Retrospective studies demonstrate that women who have delivered a baby weighing less than 2500g have 7-11 times more risk of death from CVD compared to women with babies weighing 3500g or more (Smith et al., 2000a, Smith et al., 2001) suggesting a link between maternal risk factors for CVD and fetal programming (Sattar and Geer, 2002). Women with a history of delivery before 37 weeks demonstrated twice the risk of coronary heart disease compared to end gestation delivery (Smith et al., 2000b). Furthermore, premature delivery, often accompanied by low birth weight, has elsewhere shown to result in higher BP and fasting glucose in adulthood (Irving et al., 2000). This is supported by work from Barker (1995) and Leon (1998) who have documented a link between birth weight and adult CV risk. The reason for elevated CVD risk for women who deliver preterm is partly dependent on the reason for early delivery (Goldenberg et al., 2008). Medically induced preterm delivery often arise due to pre-eclampsia, intrauterine growth restriction, maternal obesity or GHDs (Goldenberg et al., 2008). Spontaneous preterm deliveries typically arise from intrauterine infection, inflammation, uteroplacental ischemia or hemorrhage, stress or vascular disease

(Goldenberg et al., 2008, Romero et al., 2006). For medically induced and spontaneous premature delivery, CVD risk factors are already elevated which is understood to at least in part explain the subsequent risk of CVD following delivery (Tanz et al., 2017). For offspring, it is plausible that these risk factors compound the L-arginine/NO pathway in a similar way to that of GDM, altering vascular function exhibiting long-term consequences (Leiva et al., 2011, Leiva et al., 2016). Pregnancy undoubtedly warrants complex physiological interactions between the maternal and in-utero environment. Failure of this interaction to normally results in elevated CVD risk for mother and offspring both in the immediate and in the long-term.

2.2.2 Exercise and Pregnancy

Historically, women were advised to reduce activity levels during pregnancy and avoid any strenuous exertion for fear of imposing any threat to their foetus (Hammer et al., 2000). According to the most recent guidelines for PA during pregnancy (Mottola et al., 2018),PA during pregnancy leads to a decreased risk of pre-eclampsia, GDM, C-section, instrumental delivery, excessive weight gain, and a decreased severity of depressive symptoms and lumbopelvic pain. Garnaes et al. (2016) has demonstrated that the incidence of GDM in late pregnancy is lower in obese pregnant women who were offered exercise training compared to those that received standard maternal care. Moreover, maternal exercise may prove beneficial to the inter-utero environment and contribute to optimal foetal development via positive fetoplacental vascular adaptation (Gluckman et al., 2008), and may result in fewer offspring complications (ie, large for gestational age) (Khatun et al., 2013). Exercise during pregnancy also reduces CV risk in later life for both mother (Markus and Cullinane, 2001) and offspring (May et al., 2012, May et al., 2014, May et al., 2010, May et al., 2016). However, the specific effects of pregnancy exercise on maternal and offspring vascular health remain unclear.

2.2.3 Cerebrovascular system and Pregnancy

Cerebral blood flow. Cerebral blood flow velocity measured at the MCA decreases during pregnancy (Serra-Serra et al., 1997, Ikeda and Mori, 1990, Williams and Wilson, 1994), as a consequence of increased artery distensibility, and parallel reductions in arterial resistance (Belfort et al., 2001). Moreover, Zeeman et al. (2003) reported a decrease in cerebral blood flow velocity in late pregnancy but no change in either MCA or posterior cerebral blood vessel diameter measured by magnetic resistance imaging, confirming that changes to cerebral blood flow velocity occur due to vasodilation of the downstream resistance vessels to maintain a steady hemodynamic state. In addition to MCAv, the posterior cerebral artery (PCA) is of unique interest during pregnancy due to the propensity for oedema to form in the posterior cortex of the cerebral circulation during conditions such as pre-eclampsia (Cipolla, 2013). PCAv showed a significant reduction in women near term and not as early as the MCA, possibly due to less blood flow in the posterior circulation in the normal state (Zeeman et al., 2003). It is unclear if the time specific reductions in blood flow at the MCA and PCA are informative of the health status of a pregnancy. For example, if pre-eclampsia is associated with oedema in the posterior circulation, quantifying PCA velocity in women at risk of pre-eclampsia throughout pregnancy might provide insight to the severity and progression of the disease.

Cerebrovascular reactivity and pregnancy. There is a paucity of evidence detailing normal CBV-R response during a healthy pregnancy. To date, only one case study has measured CBV-R longitudinally throughout a healthy pregnancy by means of CO_2 reactivity (Steinback et al., 2015). The authors observed an improvement in CBV-R and attributed this gain to altered capillary blood gases (pH, HCO_3^-) because of increased ventilation and chronic respiratory alkalosis that accompanies a normal pregnancy. Although the authors have suggested that the observed increase in CBV-R during pregnancy might not be attributed to vascular adaptation *per se*, the study is limited by the inclusion of one case

control and more evidence is required to clarify these observations. During complicated pregnancy, CO_2 reactivity was reduced in pre-eclamptic pregnancy compared with normotensive pregnant women when assessed using an isometric handgrip test and 5% CO_2 inhalation, indicating a possible negative impact of hypertension on CBV-R (Riskin-Mashiah et al., 2001).

Cerebral autoregulation and pregnancy. Cerebral autoregulation adaptation to pregnancy is unclear, but it has been suggested that the neurological manifestation of pre-eclampsia is likely to be caused by impaired cerebral autoregulation leading to cerebral oedema and posterior encephalopathy syndrome. Cerebral autoregulation is impaired in severe pre-eclamptic pregnancies (n=3) (Oehm et al., 2003) and in the post-partum period following pre-eclampsia (Oehm et al., 2006). Nonetheless, cerebral autoregulation measured using TCD and TFA of respiratory-induced hemodynamic oscillations was unchanged in mid pregnancy in healthy and pregnancies at risk of pre-eclampsia compared to similarly aged non-pregnant controls (Janzarik et al., 2014) implying that cerebral autoregulation is only compounded in pregnancies with severe pre-eclampsia.

In a longitudinal study design, cerebral autoregulation was unchanged throughout a normotensive pregnancy in response to an isometric handgrip test (Bergersen et al., 2006). The normal boundaries of cerebral autoregulation lie between pressures of ~60-150mmHg (Cipolla et al., 2011). It has been speculated that pregnancy results in an extension of the upper and lower limits of cerebral autoregulation observed in an animal model during pregnancy (Chapman et al., 2013). The authors suggested this may occur to prepare and protect the maternal brain against possible acute and drastic fluctuations in blood pressure (Cipolla et al., 2011). While clarification of the normal cerebral autoregulation response to pregnancy is warranted, the effect of pregnancy exercise on parameters of cerebral autoregulation has yet to be defined.

Exercise, cerebral blood flow and pregnancy. The relationship between exercise and cerebral blood flow during pregnancy is largely unknown and studies to date are largely based on acute exercise responses. Studies attempting to explore its effects are limited by the measurement of the common carotid artery (CCA) as a proxy for cerebral blood flow velocity. Following a 3-minute cycling test at 36-40 weeks gestation, CCA mean blood flow velocity did not change, however the authors observed an increased systolic maximum velocity and a decreased end-diastolic maximum velocity in the CCA, which led to a significant increase in both the RI and PI (+5% and +36% respectively). An animal study reported an increase of 136% in CCA blood flow velocity in both exercising pregnant and non-pregnant sheep following an exhaustive exercise bout performed on a treadmill at 3 miles per hour and a gradient of 10% (Orr et al., 1972). The authors suggested that this increased CCA velocity, consistent with the increased CO, was likely a thermoregulatory response, although they did not exclude that cerebral blood flow velocity could have been increased with exercise. Similarly, CCA blood flow velocity progressively increased from rest to 80% maximal oxygen consumption (VO_{2max}) in 10 healthy non-pregnant human participants (Sato et al., 2011). The authors also observed a different response between the ICA and external carotid artery (ECA) velocities during exercise, with the change in ICA velocity negatively associated with the change in ECA velocity at high-intensity exercise. Since the ECA supplies blood to the neck and face, it was hypothesized that the increase in ECA velocity was attributed again, to thermoregulation. While these studies suggest some evidence of increased cerebral blood flow velocity immediately after exercise, the influence of chronic exercise participation on cerebral blood flow velocity during pregnancy has yet to be determined.

Exercise training, cerebrovascular reactivity and pregnancy. In healthy non-pregnant humans, CBV-R has shown to subtly increase following a 12-week aerobic exercise intervention at 30% and 70% VO_{2max} (Murrell et al., 2013). Moreover, an elevation in fitness has been shown to positively correlate with the change in CBV-R in stroke survivors

identifying a role for aerobic capacity in improving CBV-R (Ivey et al., 2011). The use of MRI in rats has shown a 22% increase in cerebral signaling responses to 10 % CO_2 inhalation following 30 days of voluntary exercise compared with sedentary controls (Swain et al., 2003). Taken together, these data suggest a role for exercise in improving CBV-R. Although the mechanism involved for improved CBV-R remains to be confirmed, it has been speculated that increased cerebral blood flow velocity is partly NO dependent (Peebles et al., 2007). Moreover it has been suggested that any improvements in systemic vascular function might also be reflected in the cerebral circulation (Ainslie et al., 2007), however this has yet to be explored during pregnancy.

2.2.4 Peripheral vascular system and pregnancy

Vascular function. Endothelial function is enhanced between 10-30 weeks gestation during normal pregnancy and thereafter, returns to pre-pregnancy levels (Savvidou et al., 2000). The mechanisms surrounding this enhancement are not yet fully understood, however it is believed to be accomplished mainly through a decrease in endothelial vascular responsiveness to vasoconstrictors such as angiotensin II and noradrenaline, and an increased availability of and susceptibility to NO (Lopes van Balen et al., 2017).

Flow mediated dilation (FMD) has been used extensively to assess artery function during pregnancy. FMD measured between 10-40 weeks gestation, was higher compared to non-pregnant controls (Savvidou et al., 2000). When assessed by trimester, FMD is progressively enhanced throughout pregnancy measured at the first second and third trimester compared to non-pregnant controls (Dorup et al., 1999). In a longitudinal study design, FMD was increased in the final trimester of pregnancy compared to non-pregnant controls (Faber-Swensson et al., 2004). Quinton et al. (2007) assessed FMD at five time-points during healthy uncomplicated pregnancy and recognised FMD to be variable

throughout pregnancy; it increased non-significantly until 32 weeks gestation however decreased significantly from 36 weeks highlighting the importance of gestational age when assessing FMD. A meta-analysis performed to establish reference values for FMD have reported that, in an uncomplicated pregnancy FMD is enhanced while women with a complicated pregnancy tend to have a lower FMD (Lopes van Balen et al., 2017). The limitation among these studies is the large variation in the quality of the FMD measurement and analysis. To our knowledge only 1 study (Torrado et al., 2015) has adopted the most recent methodological guidelines for FMD measurement (Thijssen et al., 2011a). Torrado et al. (2015) report enhanced endothelial function in pregnant women at 34 weeks in comparison to non-pregnant controls, however, this evidence does not report the adaptation to pregnancy prior to 34 weeks gestation. It is clear that pregnancy induces endothelial adaptation at the brachial artery and this seems to progress until late gestation. Nonetheless, no study has examined vascular function in different vascular beds at each trimester of pregnancy whilst adhering to best practice guidelines in FMD measurement, with appropriate control for shear and baseline diameter (Thijssen, Atkinson, Pyke).

Exercise and vascular function. Exercise induced improvements in endothelial function have been suggested to play a major role in reducing the risk of CVD in healthy and diseased non-pregnant adults due to increased vasodilatory responses and reduced vasoconstriction (Green et al., 2014). However the hemodynamic responses to exercise during pregnancy remain largely unknown (Davenport et al., 2016). Gilbert et al. (2012) documented a greater vasodilatory response to acetylcholine which stimulates the release of NO from the endothelium, in rats who exercised during pregnancy compared to a sedentary pregnant control group. To date, only one human study has examined the effect of exercise training during pregnancy on vascular function. The authors reported an increase in brachial FMD in women following 16 weeks of exercise training for 60 minutes three times per week at 50-60% max heart rate at 16-20 weeks gestation to until the end of their pregnancy (Ramirez-Velez et al., 2011). FMD was higher in the exercise group

compared to the control group implying exercise enhances FMD during pregnancy. Nonetheless, the authors did not account for the negligible changes in artery diameter which may have influenced the result and thus, the interpretation of their findings given that pregnancy is characterised by increased vasodilation and reduced vascular resistance (Torrado et al., 2015). A study from Weissgerber et al. (2011) measured brachial FMD at 34 weeks gestation in healthy pregnancy and quantified exercise levels using a 3-day activity diary. The authors reported no relation between exercise participation PA and FMD measured at 34 weeks gestation compared to inactive control. Taken together, the impact of exercise during pregnancy on vascular function is unclear and warrants further investigation.

Vascular structure. Using a prospective longitudinal design and a strict inclusion criterion of healthy nulliparous women, Iacobaeus et al. (2016) reported a decrease in cIMT across pregnancy which the authors suggest is due to passive distension. In a complicated pregnancy, the consensus regarding IMT is unclear. Women with GDM have presented with greater cIMT at 25 weeks of pregnancy compared with unaffected controls (Tarim et al., 2006) and an increased cIMT from the second to the third trimester (Yousefzadeh et al., 2012). Interestingly, in the latter study, cIMT did not change in the control group throughout the pregnancy suggesting that perhaps pregnancy does not alter IMT.

A normal pregnancy is associated with a decrease in central pressure and arterial wave reflection and no change in the PWV of the carotid-radial and carotid-femoral arterial pathways (Macedo et al., 2008, Smith et al., 2004). Similarly, despite controlling for MAP, no change in PWV has been observed during a normal pregnancy (Mahendru et al., 2014). Moreover, in a cross-sectional study comparing 23 non-pregnant controls to 193 pregnancies, PWV did not change between 11-41weeks (Macedo et al., 2008). Contrasting with these findings, longitudinal studies have reported a decrease in PWV in the second trimester of pregnancy and increased above baseline during the third trimester (Edouard et

al., 1998, Iacobaeus et al., 2016, Robb et al., 2009). Taken together, a decrease in peripheral vascular resistance associated with pregnancy affects arterial stiffness (Elvan-Taspinar et al., 2005), however the direction and timing of this change warrants further clarification.

Exercise and vascular structure. Although functional adaptation to exercise may rapidly occur at the start of an exercise training program, structural adaptations occur at a slower rate (Tinken et al., 2008). In general, artery structure may be altered due to an increase in luminal diameter and/or a reduction in IMT however, the impact of prenatal exercise on structural adaptations (diameter, IMTs, vessel wall composition) has not been studied directly in humans. Women with previous pre-eclampsia demonstrate a 21% larger cIMT in the PP period (6-12 months) compared with normotensive women. Exercise training in the PP reduced IMT by 9% in previously pre-eclamptic women, but still remained 11% larger than healthy pregnancies (Scholten et al., 2014). This suggests that exercise in the PP may improve the negative vascular remodelling in women who developed pre-eclampsia; however, a short duration exercise program (12 weeks) may not be sufficient to reverse the effects of vascular remodelling during pregnancy.

No differences have been reported for brachial artery diameter between active and inactive pregnancies (Ramirez-Velez et al., 2011, Weissgerber et al., 2011). Exercise training in non-pregnant populations may increase conduit artery size by up to 15% through outward artery remodelling and reduces blood flow resistance at rest (Green, 2009a, Green et al., 2011b). This remodelling is directly related to NO bioavailability as exercise training has been shown to increase NO production by approximately 50% in endothelial cells in non-pregnant adults (Green, 2009a). The exercise-related change in blood vessel diameter is similar to the normal increase in vessel diameter observed in healthy pregnancy (approx. 10% increase in diameter), which may account for the lack of change in vessels observed in active compared with inactive pregnant women (Green et al., 2011b). This may indicate

that there is a maximal ability for the blood vessels to dilate while maintaining function and BP, and that either pregnancy or exercise can result in a plateau at maximal vessel diameter.

To date only one study has compared brachial-ankle PWV in pregnant women who exercised regularly compared to sedentary pregnant women. In this study, values were similar between groups in the second trimester, when PWV is typically lowest, however those who exercised had a 10% lower PWV compared to those who didn't exercise at 1 month PP (Kawabata et al., 2012). Although the difference at PP was not viewed as clinically relevant, it does suggest that exercise assists in the recovery of the vascular system beyond pregnancy. This sparse evidence does provide rationale to continue investigating the impact of pre-natal exercise on arterial stiffness especially among those at risk of hypertensive disorders.

The intrauterine environment

Maternal health is an important determinant of offspring health during and beyond gestation. The following two sections will explore literature surrounding this phenomenon with a specific focus on the Barker Hypothesis and will outline current literature regarding the influence of maternal exercise on the intrauterine environment and its impact on offspring health.

2.2.5 The Barker Hypothesis

Early research from Barker et al. (1989) showed that low birth weight was inversely correlated with increased prevalence of coronary artery disease and early mortality in 100,000 men and women in the UK. Barker has since hypothesised that coronary heart disease is associated with specific patterns of disproportionate foetal growth that result from foetal under-nutrition during middle to late gestation (Barker et al., 1993). Transition from

embryo to foetus involves the division of cells resulting in tissue growth. The timing of cell division differs depending on the tissue, and the process for each tissue requires adequate nutrients and oxygen. Where cell division is slowed due to a lack of these components, growth can become disproportionate (Barker, 1995). Low birth weight is a consequence of disproportional intrauterine growth as a result of brain sparing as the foetus prioritises circulating glucose for the growing brain before other tissues (Miller et al. 2016). In addition, these adaptations made by the foetus in the uterus may program insulin resistance and high blood pressure which may lead to CVD and diabetes in adult life (Hocher, 2014). While Barker's hypothesis is primarily focused on the impact of maternal nutrition on foetal programming and coronary heart disease, much less is known about how maternal exercise levels impact foetal origins of disease.

2.2.6 Maternal Exercise and Offspring Cardiovascular Health

Previous authors have suggested a protective effect of exercise during pregnancy against future offspring CVD development (Huikuri et al., 1999, Gluckman et al., 2008). Recent research has confirmed the importance of the pre-natal environment on foetal CV health (May et al., 2010, May et al., 2014, May et al., 2012). During the 2nd and 3rd trimesters, the parasympathetic and sympathetic nervous system are maturing which leads to a decreased foetal heart rate and an increased foetal heart rate variability. These changes appear to represent a maturation of brainstem, central and autonomic nervous system. This maturation process is particularly important as it reduces the prevalence of atherosclerosis, a precursor of endothelial dysfunction (Huikuri et al. 1999). Maternal exercise increases circulating levels of catecholamines that cross the placenta into the foetal compartment and are essential for foetal development, chronic exposure to catecholamines, including norepinephrine during gestation, may positively influence cardiac autonomic control (May et al., 2010). Data from May et al. (2010) has illustrated that at week 36 of gestation,

exercising during pregnancy results in a decreased foetal heart rate and an increase in heart rate variability. This may contribute to a maturation of the autonomic nervous system and brainstem.

As mentioned earlier, the physiological adaptation to pregnancy is critical for maternal and offspring health (Leiva et al., 2011). Maladaptation of the maternal system to pregnancy may lead to pregnancy related complications including GHDs and GDM which in the long-term, increases the risk of maternal CVD (Hutcheon et al., 2011). This CVD risk is also thought to translate to offspring vascular health increasing the susceptibility of offspring endothelial dysfunction (Leiva et al., 2011). It therefore would seem imperative to optimise maternal health for the benefit of maternal and offspring CV risk. ~~Recently,~~ Newcomer et al. (2012) has examined the impact of maternal aerobic exercise during pregnancy on offspring endothelial function at birth using a swine sample (n=8). This study demonstrated for the first time that maternal exercise during pregnancy enhanced endothelial cell function of the thoracic aorta. Newcomer et al. (2012) has thus highlighted the need for more research to be done with regard to the effects of pregnancy exercise on human offspring endothelial health.

2.3 Summary

In summary, the integrity of the female vascular system is greatly challenged throughout the lifespan due to fluctuating oestrogen levels. These fluctuations occur in a cyclical manner from the age of menarche until menopause when oestrogen is significantly reduced, and for some women this is preceded by pregnancy where oestrogen is elevated for a period of time. For some women, these key life events are accompanied by changes to the CV risk profile and altered cerebral and peripheral vascular function (Maturana et al., 2007, Hunter, 1990, Regitz-Zagrosek et al., 2011). The maternal adaptation appears to have a profound impact on the intrauterine environment, such that a pathological pregnancy

translates to offspring health resulting in immediate and long-term consequences for their risk of CVD. In light of this, optimising maternal health would seem pivotal to CV health and can be achieved through modifiable lifestyle factors such as exercise. Maternal exercise is currently recommended during pregnancy and based on current literature, appears to be an important mediator of CV risk for both mother and child.

Chapter 3: How do Age and Menopausal Status affect the Peripheral and Cerebrovasculature in Healthy Females?

3.1 Introduction

Age is an independent determinant of CVD (Santos et al., 2017) and is accompanied by the rise of traditional CV risk factors, including, BMI, BP, and cholesterol levels (de Kat et al., 2017), as well as a decline in peripheral vascular function. Vascular function is further reduced in post-menopausal compared to pre- and peri-menopausal women (Moreau et al., 2012) and men matched for age (Celermajer et al., 1994). It has been suggested that the menopause-induced reduction of oestrogen is thought to be responsible for diminished NO bio-availability at the endothelium (Moreau et al., 2012), as well as increased oxidative stress (Santo Signorelli et al., 2006), sympathetic nerve activity (Matsukawa et al., 1998) and circulating vasoconstrictors (Khatun et al., 2013).Through these mechanisms, menopause may also have negative consequences on the cerebrovasculature, however these effects are yet to be fully determined.

Cerebral blood flow is reduced by ~5% per decade of age (Ainslie et al., 2008, Fisher et al., 2013, Stoquart-ElSankari et al., 2007). Cerebrovascular disease increases with ageing (Beam et al. 2018), and it has been inferred that the menopause may underpin the higher incidence of cerebrovascular disease in women compared to men (Wong et al., 2016, Davey, 2017). However, there is paucity of high quality data and agreement on the additive effect of the menopause and aging on the cerebrovasculature. For example, MCAv was comparable between pre- and post-menopausal women (Matteis et al., 1998), and unchanged in women following surgical menopause (Penotti et al., 2002). Yet, the pulsatility index of the MCA and ICA was significantly greater in post-compared to pre-menopausal women of similar age (Penotti et al., 1996). The effect of menopause on CBV-R is also conflicting having been reported as both lower (Matteis et al., 1998, Kastrup et al., 1998) and unchanged (Mitsis et al., 2007), in post-menopausal women compared to pre-menopause. The contrasting findings between studies, different measurement techniques, small sample sizes and insufficient inclusion criteria prevent any meaningful consensus being reached on the effect of menopause on the cerebrovasculature. There is greater

agreement that cerebral autoregulation is unaffected by menopause (Deegan et al., 2011). Nonetheless to our knowledge no study has comprehensively assessed cerebrovascular function in a large cohort of post-menopausal women, to do so would expand current knowledge regarding cerebrovascular health in post-menopausal women.

Exercise training increases cardiorespiratory fitness , improves peripheral vascular function (Black et al., 2009, Swift et al., 2011), increases MCAv (Bailey et al., 2016) and improves CBV-R in post-menopausal women (Leslie and Briggs, 2016). Along with aging, PA and cardiorespiratory fitness, may therefore be critical confounders in any study which aims to assess vascular outcomes in post-menopausal women. Yet, no study has attempted to objectively quantify the difference in PA and cardiorespiratory fitness in conjunction with cerebral and peripheral vascular function between pre and post-menopausal women. Therefore, the primary aim of this study was to investigate the effect of menopause on cerebrovascular function, as well as peripheral vascular function and structure, with simultaneous measurement of PA and cardiorespiratory fitness. To the author's knowledge, this is the first study to comprehensively assess both cerebrovascular and peripheral vascular outcomes and PA and fitness in the same cohort. It was hypothesised that all vascular outcomes, will be reduced in post-menopausal women compared to pre-menopausal women.

3.2 Material and Methods

3.2.1 Participants

Females (n=100) aged between 18-70 years were recruited. Participants were non-smokers, had no history of CVD and were not on any form of medication. Pre-menopausal (PRE-M) women were defined as eumenorrheic having a consistent menstrual cycle for at least 3-months and were not on any form of hormone-based contraception. Post-menopausal (POST-M) women were recruited based on having no menstrual cycle for at least 12 consecutive months and were not previously or currently taking any form of hormone replacement therapy. Each participant provided written consent before taking part

in the experimental procedure. The research study was ethically approved by the Liverpool John Moores School of Sport and Exercise Science Research Committee (Reference: 16/SPS/022) and adhered to the Declaration of Helsinki.

3.2.2 Experimental Procedure

Participants visited the laboratory on 2 occasions having abstained from exercise for 24 hours and alcohol for 12 hours as well as any food/caffeine/stimulants 6 hours prior to the experiment. Participants completed a battery of CV and cerebral assessments in the following sequence; IMT, FMD, PWV, CAR, CBV-R, and cerebral autoregulation, followed by 7 days of PA and sedentary behavior monitoring. A cycling based maximal cardiorespiratory fitness test was scheduled within 7 days of the vascular measurements based on participant's preference. All visits were completed at the same time of day (between 8-11am) in a temperature-controlled environment (20-22°C). Eumenorrheic women completed the laboratory visit within the first seven days of their menstrual cycle (Liu et al., 2016).

3.2.3 Anthropometrics, physical activity and sedentary behaviour

Anthropometry and Body Composition. Stature and weight were recorded to the nearest 0.1unit using a stadiometer and digital scales respectively. BMI was calculated as weight in kilograms divided by stature in metres squared (kg/m^2). Body fat (BF) percentage was estimated using bioelectrical impedance analysis (Tanita BC-420MA, Tanita Corp., Tokyo, Japan).

Cardiorespiratory Fitness Test. A ramp-based cycling protocol was used to determine maximum oxygen uptake (VO_{2max}). Participants cycled until volitional exhaustion or, until subjects were no longer able to maintain a pedal speed of at least 50 rpm. Oxygen uptake

(VO_2) and CO_2 were measured breath-by-breath, via an online gas analysis system (Jaeger Oxycon Pro, Viasys Health Care, Warwick, UK). Heart rate was monitored continuously using short-range telemetry (Polar, Kempele, Finland). Participants completed a 5-minute warm up at a self-selected resistance. The test began with 50W of resistance and increased by 30W every two minutes. During the final 20 seconds of each stage, participants were asked to rate their exertion, using the rating of perceived exertion (RPE) scale (Borg, 1970), and HR was recorded by the researcher. Criteria for participants reaching their maximal capacity was achieving a respiratory exchange ratio of >1.15, a heart rate >199bpm and/or a rating of perceived exertion of 20 on the Borg RPE Scale (Billat et al., 2017). The VO_2 data was exported in 10 second averages for the duration of the test to an Excel file and plotted on a graph with time on the x-axis and VO_2.ml.kg.min on the y-axis. The highest value over 30 seconds was extracted as the VO_{2max} value.

Physical Activity. Physical activity was monitored using a tri-axial accelerometer (Actigraph wGT3x-BT). Participants wore the accelerometer on their right hip for 7 consecutive days. Participants removed the device for sleeping, contact sports and water-based activities and recorded the times the device was worn on a diary sheet provided. Non-wear time was defined as 90 consecutive minutes of zero counts.min^{-1} (Choi et al., 2011). Inclusion criteria for analysis were ≥10 hours of wear time per day, for a minimum of 4 days, including one weekend day (Trost et al., 2005). The Actilife software, version 6.2 (ActiGraph, Pensacola, Florida) was used to download the data to a computer. Raw acceleration data was converted to 60s-epoch activity count data (counts·min^{-1}). PA intensity was determined using the following cut points (Sasaki et al., 2011): light (≤2689 counts.min-1), moderate (≤6166 counts.min-1), and vigorous (>6167 counts.min-1). Activity data were exported and handled in Excel (Microsoft) and total time (minutes) spent in light, moderate and vigorous was calculated.

Sedentary Behaviour. Sedentary time was objectively measured using an activPAL activity

monitor (activPAL micro, PAL Technologies Ltd., Glasgow, UK) worn continuously for seven days on the middle anterior of the right thigh. Monitors were enclosed in a rubber sleeve and attached by the researcher to the skin using a waterproof transparent seal (Tegaderm Roll, 3M). The monitor quantified the time spent sitting, lying, standing and walking per day. A sedentary bout was defined as no activity registered for at least 60 seconds (Healy et al., 2008).The raw data was downloaded from the monitor using the activPAL proprietary software (version 7.2, 32) from which data were exported to Excel (Microsoft, UK). Seconds of sedentary time during waking hours were totalled and converted to minutes.

3.2.4 Cerebrovascular Measurements

Middle Cerebral Artery Velocity (MCAv). Middle cerebral artery velocity was measured at rest using a 2MHz pulsed transcranial Doppler (TCD) ultrasound system (DWL, Compumedics, Germany) from the MCA. The subject was fitted with a headband which supports an ultrasound probe on each side of the head. Ultrasound gel was applied to the temporal window (just above the zygomatic arch) and to the probes allowing an optimal signal to be obtained. To isonate the correct vessel, specific criteria were followed with the mean and peak MCAv values above 50cm.sec^{-1} and 80cm.sec^{-1} respectively and depth set between 40-60mm (Willie et al., 2011). Care was taken to stabilise the probes ensuring a stable angle of isonation in line with best practice guidelines (Ainslie and Duffin, 2009, Giller et al., 1998). Simultaneously, arterial blood pressure was monitored non-invasively using a Finometer photoplethysmography (Finometer pro, Finapres Medical Systems, Netherlands) which was carefully fitted on either the right hand's middle or index finger as per manufacturer's recommendations. Real time MCAv and BP were recorded online and displayed in LabChart Pro version 7 (ADInstruments, Australia).

The weighted mean MCAv was calculated from the peak envelope of the velocity trace (1/3

systolic + 2/3 diastolic), which accounts for the relative time spent in each phase of the cardiac cycle (Skow et al., 2013). Data were expressed as cerebrovascular conductance (CVC), which was calculated as MCAv divided by mean arterial pressure (1/3SBP + 2/3DBP). Unilateral, or when obtainable, bilateral MCAv was recorded during all cerebral tests.

Cerebrovascular Reactivity (CBV-R). Cerebrovascular reactivity to perturbations in $PaCO_2$ were measured using a hypercapnic rebreathing protocol (Skow et al., 2013). Participants were instrumented as above with the addition of a rebreathing apparatus consisting of a mouthpiece, nose clip, a bacteriological filter and a three-way valve to allow switching of airflow between room air and a pre-filled Douglas bag containing a hypercapnic gas mixture of 5% CO_2, 21% O_2 balanced with nitrogen. Breath-by-breath CO_2 was sampled using a calibrated gas analyser (Ml206, ADInstruments) and the pressure of end-tidal carbon dioxide ($PETCO_2$) was recorded online (LabChart) and corrected for the daily barometric pressure.

A one-minute baseline recording was followed by a period of voluntary hyperventilation (1 breath per second) coached by the researcher until a reduction in $PETCO_2$ to <20Torr. Once achieved, the valve on the Douglas bag was switched so participants inhaled the 5% CO_2 mixture. Simultaneously, participants were instructed to return their respiratory rate to normal whilst breathing the 5% CO_2 mixture for 3-minutes. Data was exported from LabChart Pro (ADInstruments Australia).

Baseline $PETCO_2$ and MCAv were calculated as the mean of the 1-min prior to hyperventilation, while MCAv and $PETCO_2$ data during 5% CO_2 breathing was collected as 10-sec averages for the entire 3 minute period. Absolute MCAv and relative changes were plotted against $PETCO_2$ for each 10-sec of 5% CO_2 of breathing and reported as absolute and relative CBV-R sensitivity (cm/s per mmHg). CBV-R was subsequently quantified by linear regression (R^2 value). Relative MCAv was calculated as the difference between

baseline and 5% CO_2 MCAv divided by baseline MCAv (([5% CO_2 MCAv-baseline MCAv]/ baseline MCAv) x 100%) (Skow *et al.,* 2013).

Simultaneously, during the baseline and CO_2 breathing measurements, arterial diameter and blood velocity of the left CCA were acquired at least two centimeters below the point of bifurcation using high resolution ultrasound Images were acquired in accordance with methodological guidelines (Thomas et al., 2015). Data were used to determine the response of the CCA to elevations in $PETCO_2$ by averaging 30-sec of baseline diameter and comparing that to the diameter during the last 30-sec of 5% CO_2 breathing. The ultrasound measurements were completed as described above. Data were used to determine the response of the left CCA to elevations in $PETCO_2$ by calculating the difference between 30-sec average of baseline data to that obtained during the last 30-sec of 5% CO_2 breathing.

Cerebrovascular Autoregulation. Changes in BP and MCAv were assessed using a squat to stand procedure in order to induce transient changes in ABP. Participants performed squat-stand maneuvers at 0.10 Hz (5 second squat- 5 second stand) whilst breathing normal atmospheric air for a duration of 6 minutes with $PETCO_2$ monitored throughout. MCAv, $PETCO_2$, and MAP were extracted from LabChart every 0.1 seconds across the 6-minute period. The relationship between changes in MCAv and arterial BP was assessed via the transfer function analysis (TFA) in accordance with standardized guidelines (Claassen et al., 2016). Transfer function analysis was performed using MATLAB (2010b; MathWorks-Inc., Natick, MA) in order to calculate associated power (gain) and timing (phases) over three different frequencies; very low (0.02 – 0.07 Hz), low (0.07 – 0.20 Hz) and high (0.20 – 0.50 Hz) (Claassen et al., 2016). TFA also produces an estimated reliability of the relationship between the two signals (coherence) (Triedman and Saul, 1994). Data sets with a coherence value of <0.4Hz were excluded from data analysis. High frequency and very low frequency range data were excluded from analysis based on the frequency of the squat-stands used.

Carotid Artery Reactivity

The carotid artery reactivity (CAR) test. In a supine position, carotid artery diameter and blood flow velocity response were measured via ultrasound. A one-minute baseline measurement was recorded, then participants were instructed to immerse their left hand up to the wrist into a bucket of icy water (1-5ºC) for 3 minutes. For the duration of this test, participants were encouraged to breathe normally (avoid breath holding/hyperventilation) and keep as still as possible, without speaking (van Mil et al., 2017). Post immersion carotid artery data was assessed at 10-second intervals using the custom designed edge-detection and wall tracking software (Dicom Encoder) from which peak diameter change (maximum dilation/constriction) and area under the curve for the diameter change during CPT (CAR_{AUC}) was calculated. The peak diameter change refers to dilation or constriction, and the direction of this change was determined by a positive or negative CAR_{AUC} (i.e. dilation or constriction respectively). The technique shows a good correlation between the carotid artery diameter response (an extracranial blood vessel) and coronary artery flow response in asymptomatic healthy adults indicating the carotid artery to be a valuable site in assessing vascular health (van Mil et al., 2017). Taken together, this test can provide information on coronary artery vasodilator function and could be a valuable link to evaluating systemic vascular health with the menopause and aging in women.

3.2.5 Cardiovascular measurements

Carotid, Femoral and Brachial Intima-Media Thickness. Following 20 minutes of rest in a supine position, the left CCA was imaged using high-resolution B-mode ultrasound (Terason u-smart 3300, Teratech, Burlington, MA, USA) 5 mm proximal to the artery bulb (Polak et al., 2011). Participants lay with neck slightly extended facing the contralateral side to allow for optimal longitudinal imaging of the far-wall intima media interface from three angles (approximately 45°, 90° and 135°). Each image was recorded by the same sonographer for 30-40 seconds. Images were optimised to ensure clear contrast between

the artery walls and lumen with a distinct IMT visualised on the far wall defined as the distance between two echogenic lines represented by the lumen-intima interface and media adventitia interface of the artery wall. The IMT was also acquired at the left femoral artery 3 to 5 cm distal to the bifurcation of the femoral artery and left brachial artery 5 to 10 cm above the elbow and using the same criteria as for CCA. Due to data quality issues, brachial IMT was only obtainable for N=64 women.

Recordings were analysed offline using the edge detection software Carotid Studio v4.3.1 (Cardiovascular Suite, Quipu srl, Pisa, Italy) with a frame rate of 25 frames per second. Following calibration, an optimal region of interest that included both vessel walls with a minimum length of 1cm was selected by the researcher. Based on the quality of the scan, a 5-15 second time frame was chosen for analysis. The automated software produced an edge detection output of the near and far-wall media-adventitia interface during each cardiac cycle using a pixel-density algorithm. Continuous calculations by the software produced an average IMT and artery diameter recorded within the operator selected time duration. This was repeated for all three angles and an average of the three angles was calculated. This method has been shown to be valid and reproducible (Bruno et al., 2014, Bianchini et al., 2010). The extracted data were also used to calculate wall-to-lumen ratio (IMT/Lumen) at each arterial site which corrects for differences in baseline diameter.

Arterial Stiffness. Carotid-femoral PWV was assessed using a semi-automated device and software (SphygmoCor, AtCor Medical, Sydney, Australia) in the supine position. Firstly, three brachial ABP measurements were taken in succession (Dinamap V100, GE Medical Systems, Germany), with an average systolic (SBP) and diastolic (DBP) calculated and entered into the software. A single applanation tonometer probe was used to capture a proximal (carotid artery) and distal (femoral artery) pulse, recorded over 10 cardiac cycles. The QRS complex was measured simultaneously using electrocardiography (ECG). The time between the R wave of the ECG trace and the foot of the proximal waveform is

subtracted from the time between the R wave and the foot of the distal waveform to obtain the pulse transit time. To determine the distance used for PWV, the distance from the proximal measurement site to the suprasternal notch was subtracted from the distance between the distal measurement site and the suprasternal notch using an anthropometric measuring tape. PWV was automatically calculated by dividing the distance between the two arterial recording sites by transit time to provide an index of arterial stiffness. PWV measurements were made in triplicate and an average was calculated and used in data analysis.

Brachial and Femoral Flow Mediated Dilation. Left brachial and left femoral artery diameters were assessed simultaneously via high resolution 2D duplex ultrasound (Terason u-smart 3300, Teratech) with a 10-12 MHz linear array probe. B-mode images were obtained and optimised, and the probe was held in the same position for the duration of the test. After 1 minute of baseline measurement, occlusion cuffs, connected to a rapid inflator (Hokanson, Bellevue, WA), placed around the left thigh, proximal to the patella, and the left forearm, distal to the humeral epicondyle, were inflated to a suprasystolic pressure of 220mmHg for 5 minutes. Arterial images were recorded for a further 3 minutes post cuff deflation in accordance with best practice guidelines (Thijssen et al., 2011a).

Brachial FMD (bFMD) and femoral FMD (fFMD) data were analysed by custom designed edge-detection and wall tracking software (Dicom Encoder), of which the reproducibility and validity have been demonstrated elsewhere (Woodman et al., 2001). An optimal region of interest was selected by the sonographer, on the basis of the quality of the distinction between the artery walls and lumen. The vessel walls and blood velocity are traced in B-mode frames via pixel density and frequency distribution algorithm. The software automatically calculated the relative diameter change, time to peak (following cuff release) and shear rate area-under-the-curve (SR_{AUC}). The peak artery FMD was defined as the peak percentage change in artery diameter from baseline to during 3-minutes post cuff

release. Although the initial region of interest selection was operator-determined, the remaining analysis was independent of operator bias. FMD data was analysed with covariate control for baseline artery diameter (adjusted FMD) allowing FMD to be scaled for changes in artery diameter (Atkinson and Batterham, 2013).

3.2.6 Statistical analysis

All data were analyzed using statistical software (SPSS Version 24.0, IBM Corporation, Somers, NY, USA), A univariate general linear model was used to analyze the differences between: i. PRE-M and POST-M women; ii. A sub-group of 20 pre- (N=10) and post-menopausal women (N=10) that were menopausal for <5 years (Moreau et al., 2012). Women in the sub-analysis were identified as the 10 oldest pre-menopausal women (Late-PRE-M), and women who were menopausal for <5 years (Early-POST-M). This analysis acknowledges the challenge of differentiating between the relative contributions from two parallel and intimately linked processes i.e. ageing and menopause and attempts to eliminate the role of ageing on the vascular measures. Statistically significant differences were followed up with the least significant difference approach to multiple comparisons where appropriate. Data are presented as mean [95% confidence intervals]. Data in tables and figures are presented as mean (SD). Statistical significance was assumed at $p<0.05$.

3.3 Results

3.3.1 Participant Characteristics

Women were POST-M for 6.5±4.3 years based on the time from their last menstrual period. POST-M women had a higher SBP (126 [123, 130 mmHg] $p<0.001$) and DBP (72 [70, 74 mmHg] $p<0.001$) compared to PRE-M women (Table 3.1). POST-M women had a higher body fat (34 [31, 36 %] $p=0.09$) compared to PRE-M (31 [28, 34%] $p=0.09$) however this did not reach statistical significance. Body mass and BMI were similar between PRE-M and POST-M women ($p=0.26$ and 0.87 respectively).

In the sub-analysis, the time since the last menstrual cycle for Early-POST-M women was 2.6±1.3 years (Table 3.2). The Late-PRE-M were significantly younger (45.9 [44, 48 years]) than the Early-POST-M group (50.4 [48, 52 years] p=0.003) but there were no differences in body mass (p=0.48), BMI (p=0.63), body fat (p=0.49), SBP (p=0.85) or DBP (p=0.75) between the groups.

Physical activity, sedentary behaviour and cardiorespiratory fitness

POST-M women had a significantly lower $\dot{V}O_{2max}$ (24.8 [22.6, 26.9 ml·kg·min]) compared to PRE-M (34.7 [32.6, 36.9 ml·kg·min] p<0.001). Daily vigorous PA was significantly lower for POST-M women (5 [3, 7 min/d]) compared to PRE-M women (9 [7, 11 min/d] p=0.01). POST-M women engaged in less sedentary time (490 [461, 520 min/d] p=0.09) compared to PRE-M women (525 [497, 553 min/d] p=0.09) however this did not reach statistical significance. There were no differences between groups for light (p=0.40), moderate PA (p=0.24), average daily PA (p=0.81) or accelerometer wear time (p=0.64, Table 3.1). Women in the Late-PRE-M group performed a greater amount of light PA (354 [292, 415 min/d]) than the Early-POST-M group (264 [199, 329 min/d] p=0.05). There were no differences between groups for $\dot{V}O_{2max}$, moderate PA (p=0.25), vigorous PA (p=0.92), average daily PA (p=0.12) or sedentary behaviour (p=0.11). Late-PRE-M women had significantly greater accelerometer wear time (858 [785, 930 min/d]) compared to Early-POST-M women (743 [616, 819 min/d] p=0.04). Nonetheless there were no significant differences in percentage of wear time at each PA intensity between groups (Table 3.2).

Table 3.1 Participant characteristics between PRE- and POST-M women.

Characteristic	PRE-M	POST-M	p-value
N	50	50	-
Time from menopause (y)	-	6.5±4.3	-
Age (y)	33.2±9.1	58.5±5.5*	**<0.001**
Body mass (kg)	68.6±1.9	65.4±1.9	0.25
BMI (kg/m^2)	25±6	25±4	0.87
BF (%)	31.2±8.6	34.1±8.0	0.09
VO_{2max} (ml.kg)	34.8±8.6	24.8±6.2*	**<0.001**
SBP(mmHg)	109±8	126±15*	**<0.001**
DBP (mmHg)	65±7	72±7*	**<0.001**
Physical Activity			
Light PA (mins)	285±74	299±88	0.40
% Wear Time	34±7	35±9	0.88
Moderate PA (min/d)	55±22	49±24	0.24
% Wear Time	7±3	6±3	0.94
Vigorous (min/d)	10±9	5±7*	0.01
% Wear Time	1±1	1±1	0.98
Average daily PA (min/d)	350±78	353±94	0.81
Average daily wear time (min/d)	832±140	846±135	0.64
Average daily sedentary time (min/d)	524±80	490±100	0.09

Values are mean ± SD. Abbreviations: PRE-M; pre-menopause, POST-M, post-menopause, BMI; body mass index, BF; body fat, VO_{2max}; maximal oxygen consumption, PA; physical activity, SBP; systolic blood pressure, DBP; diastolic blood pressure. Significance is denoted by *p<0.05.

Table 3.2 Participant characteristics between Late-PRE-M and Early-POST-M women.

Characteristic	Late-PRE-M	Early-POST-M	p-value
N	10	10	-
Time from menopause (y)	-	2.6 1.3	-
Age (y)	45.9±3.1	50.4±2.8*	**0.003**
Body mass (kg)	68.1±6.3	65.8±8.1	0.48
BMI (kg/m^2)	25±3	26±3	0.63
BF (%)	33.6±5.9	35.2±5.0	0.49
VO_{2max} (ml.kg)	31.6±6.0	28.2±7.6	0.29
SBP (mmHg)	114±10	115±13	0.85
DBP (mmHg)	69±8	70±8	0.75
Physical Activity			
Light PA (min/d)	354±80	264±104*	**0.05**
% Wear Time	41±8	35±10	0.41
Moderate PA (min/d)	43±18	55±23	0.25
% Wear Time	5±2	8±4	0.38
Vigorous (min/d)	9±8	9±8	0.92
% Wear Time	1±1	1±1	0.98
Average daily PA (min/d)	406±85	328±118	0.12
Average daily wear time (min/d)	858±101	743±118*	**0.04**
Average daily sedentary time (min/d)	519±55	445±108	0.11

Values are mean ± SD. Abbreviations: BMI; body mass index, BF; body fat, VO_{2max}; maximal oxygen consumption, PA; physical activity, SBP; systolic blood pressure, DBP; diastolic blood pressure. Significance is denoted by *p<0.05.

3.3.2 Cerebrovascular Measurements

POST-M women had a significantly lower baseline MCAv (61.3 [57, 66 cm/s] p=0.002) and CVC (0.53 [0.45, 0.66 $cm \cdot s^{-1} \cdot mmHg^{-1}$] p<0.001) compared to PRE-M women (Table 3.3). There were no differences between PRE-M and POST-M for $PETCO_2$ (p=0.99), absolute (p=0.52) and relative MCA CBV-R (p=0.18), r^2 value (p=0.55), or CCA diameter response to the CBV-R test (p=0.64). In the sub-analysis comparing Late-PRE-M and Early-POST-M, there were no differences in baseline MCAv (p=0.91), CVC (p=0.07), $PETCO_2$ (p=0.23) absolute (p=0.79) and relative CBV-R (p=0.99), r^2 value (p=0.53), or carotid artery diameter response to the CBV-R test (p=0.25) (Table 3.4). There were no differences between PRE-M and POST-M women for the cerebral autoregulation parameters of normalised gain (p=0.56) and phase (p=0.73) measured in the low frequency (Table 3.3). Similarly, there were no differences between Late-PRE-M and Early-POST-M women for normalised gain (p=0.77) and phase (p=0.18) measured in the low frequency (Table 3.4).

Table 3.3 Cerebral hemodynamic differences between pre- and post-menopausal women.

	PRE-M	**POST-M**	**p-value**
$PETCO_2$ (mmHg)	36.2±3.2	36.2±3.6	0.99
MCAv (cm/s^{-1})	72.0±14.9	61.3±15.4*	0.002
CVC ($cm.s^{-1}.mmHg^{-1}$)	0.79±0.33	0.53±0.32*	**<0.001**
MAP (mmHg)	80±7	90±9*	**<0.001**
Hypercapnic CBV-R test			
$PETCO_2$ (mmHg)	44.9±2.1	44.5±3.5	0.97
Carotid Diameter (cm)	6.46±0.01	6.64±0.01	0.18
Carotid Diameter (cm) (last 30 seconds)	6.49±0.06	6.37±0.15	0.64
CBV-R (r^2)	0.82±0.08	0.81±0.09	0.55
Absolute CBV-R ($cm{\cdot}s{\cdot}mmHg^{-1}$)	3.76±1.48	3.51±1.92	0.52
Relative CBV-R ($cm{\cdot}s{\cdot}mmHg^{-1}$)	4.84±1.99	5.47±2.17	0.18
Cerebral Autoregulation	***Low frequency range***		
$PETCO_2$ (mmHg)	38.2±2.5	38.0±2.1	0.98
MAP	80±9	89±7	0.10
Normalised gain (%)	1.35±0.37	1.30±0.40	0.56
Gain (cm.s/mmHg)	0.98±0.22	0.72±0.22*	**<0.001**
Phase (degrees)	22.61±14.77	23.79±13.22	0.73
Coherence	0.62±0.12	0.67±0.12	-

Values are mean ± SD. Abbreviations: PRE-M; pre-menopause; POST-M; post-menopause; MCAv; middle cerebral artery velocity, CVC; cerebrovascular conductance, MAP; mean arterial pressure, CBV-R; cerebrovascular reactivity, $PETCO_2$; end tidal carbon dioxide. Significance is denoted by *p<0.05.

Table 3.4 Cerebral hemodynamic differences between Late-PRE-M and Early-POST-M women.

	Late-PRE-M	**Early-POST-M**	**p-value**
$PETCO_2$ (mmHg)	36.3±3.3	41.9 ± 13.6	0.23
MCAv (cm/s^{-1})	68.95±6.04	68.18±16.32	0.91
CVC ($cm.s^{-1}.mmHg^{-1}$)	0.82±0.12	0.56±0.41	0.07
MAP (mmHg)	83±9	88±8	0.62
Hypercapnic CBV-R test			
$PETCO_2$ (mmHg)	45.1±2.1	45.0±3.0	0.99
Carotid Diameter (cm)	6.37±0.17	6.28±0.17	0.72
Carotid Diameter (cm) (last 30 seconds)	6.45±0.24	6.86±0.25	0.25
CBV-R (r^2)	0.83±0.07	0.80 ± 0.11	0.53
Absolute CBV-R (cm·s/mmHg-1)	4.03±1.56	4.31 ±2.58	0.79
Relative CBV-R (cm·s/mmHg-1)	5.43±2.51	5.45 ± 2.36	0.99
Cerebral Autoregulation	***Low frequency range***		
$PETCO_2$ (mmHg)	37.1 1.1	37.4 2.0	0.97
MAP	84±3	88±5	0.58
Normalised gain %	1.35±0.29	1.31±0.28	0.77
Gain (cm.s/mmHg)	0.92±0.17	0.83±0.19	0.35
Phase (degrees)	24.92±12.65	14.74±11.87	0.18
Coherence	0.67±0.12	0.62±0.08	-

Values are mean ± SD. Abbreviations: PRE-M; pre-menopause; POST-M,; post-menopause; MCAv; middle cerebral artery velocity, CVC; cerebrovascular conductance, MAP; mean arterial pressure, CBV-R; cerebrovascular reactivity, PET CO2; end tidal carbon dioxide.

Carotid artery reactivity

CAR was significantly lower in the POST-M women (0.7 [-0.7, 2.1%)] compared to PRE-M women (3.0 [1.5, 4.4 %] p=0.03) (Table 3). In the sub-analysis, CAR was not different between Early-POST-M (0.81 [-2.94, 4.56 %]) and Late-PRE-M (0.77 [-2.98, 4.51 %] p=0.99) (Table 3, Figure 3.1).

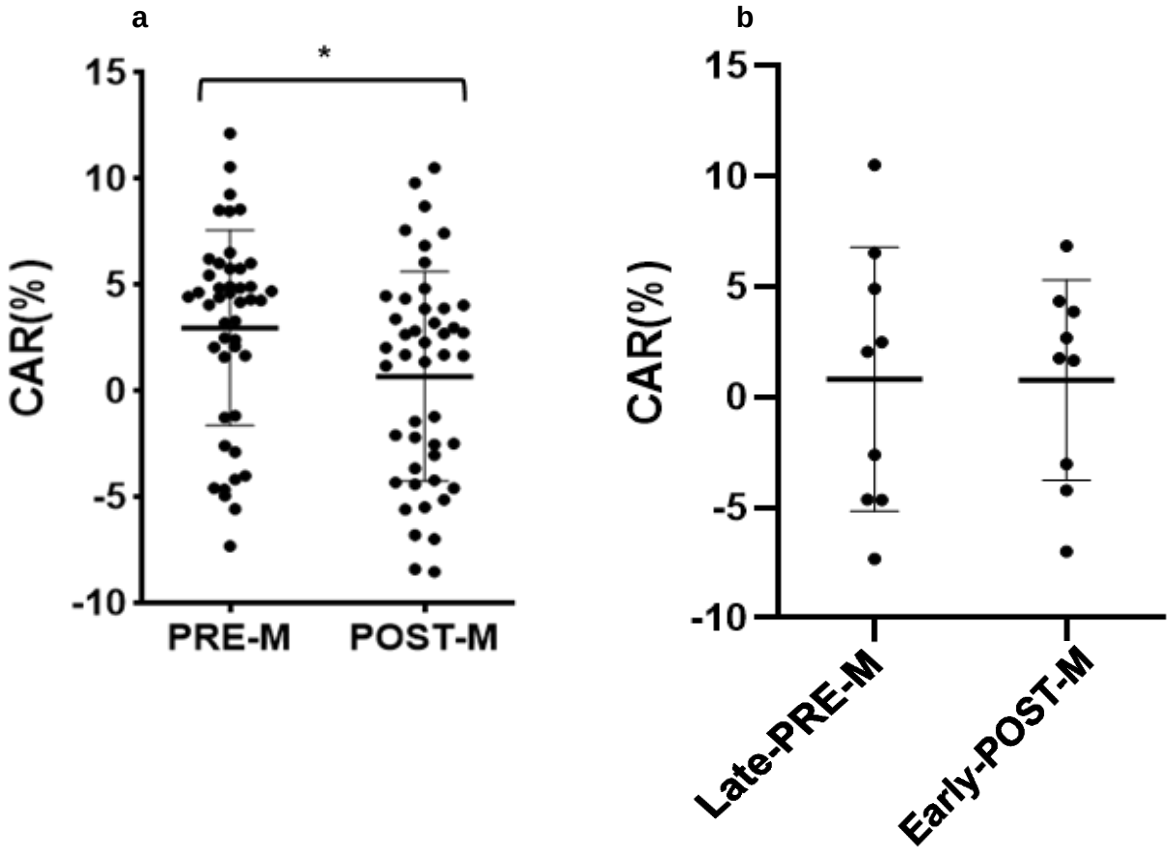


Figure 3.1 Carotid artery reactivity for PRE- and POST-M women (a) and women in the sub-analysis (b). Carotid artery reactivity for PRE- and POST-M women (a) and women in the sub-analysis (b). CAR is significantly lower is POST-M women compared with PRE-M women (a). A greater number of POST-M women demonstrate a constriction of the carotid artery during the CPT (*p=0.03). Carotid artery reactivity is not different between the Late-PRE-M and Late-PRE-M group (p>0.05) (b).

3.3.3 Peripheral Vascular Function

3.3.3.1 Brachial Femoral FMD and PWV

The POST-M women had a significantly lower brachial FMD (4.1 [2.9, 5.2 %]) compared to PRE-M women (6.4 [5.4, 7.5%] p=0.004) (Figure 3.2). The time to peak (TTP) was significantly higher in POST-M women (65 [57, 74 seconds]) compared to PRE-M women (46 [39, 54 seconds] p=0.002). There were no differences between groups for baseline diameter (Dbase) (p=0.62), peak diameter (Dpeak) (p=0.75), or shear rate area under the curve (SR_{AUC}) (p=0.10, Table 3.5). POST-M women had a significantly lower femoral FMD (2.8% [1.9, 3.6 %]) compared to PRE-M women (5.8 [4.9, 6.7 %] p<0.001) (Figure 3.2). There were no differences between PRE- and POST-M groups for femoral TTP (p=0.15), Dbase (p=0.19), Dpeak (p=0.72), or SR_{AUC} (p=0.76) (Table 3.5). PWV was significantly higher for POST-M women (6.87 [6.5, 7.3 m/s]) compare to PRE-M women (5.45 [5.1, 5.8 m/s] p<0.001) (Figure 3.3).

In the sub analysis, brachial TTP was faster for Late-PRE-M women (66 [50, 82 seconds]) compared to the Early-POST-M group (37 [30, 49 seconds] p=0.01). There were no significant differences between Late-PRE-M and Early-POST-M women for bFMD (p=0.58), SR_{AUC} (p=0.09), Dbase (p=0.73), or Dpeak (p=0.53). Early-POST-M women had a significantly lower fFMD (2.1 [0.8, 3.5 %]) compared to Late-PRE-M women (4.1 [2.7, 5.5 %] p=0.049) (Figure 3). There were no differences between groups for TTP (p=0.69), Dbase (p=0.80), Dpeak (p=0.86) or SR_{AUC} (p=0.99). Allometric scaling did not alter the FMD responses reported for brachial or femoral arteries (Table 3.5 and 3.6). PWV was not different between Early-POST-M and Late-PRE-M (p=0.84, Table 3.6).

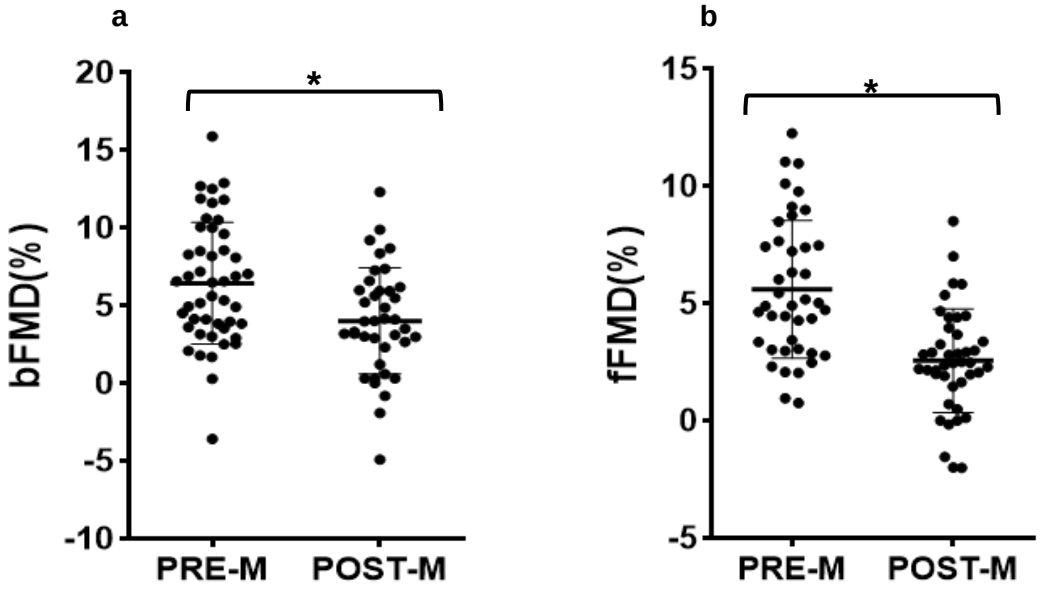


Figure 3.2 Brachial (a) and femoral (b) FMD for PRE and POST-M women. Brachial FMD is significantly lower in POST-M compared to PRE-M women (*p=0.004). Similarly, Femoral FMD is significantly lower in POST-M compared to PRE-M women

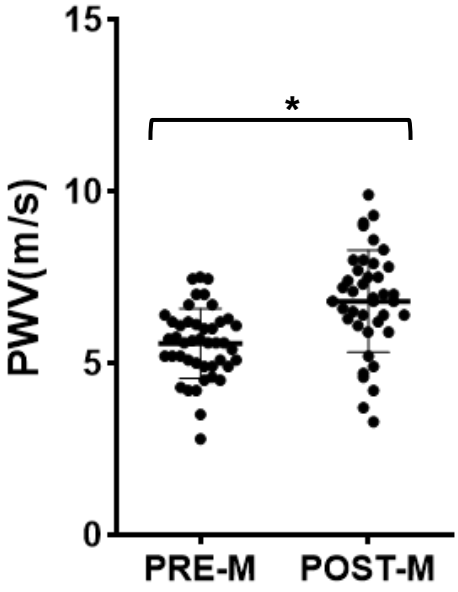


Figure 3.3 Pulse wave velocity is significantly higher from PRE- to POST-M (*p<0.001)

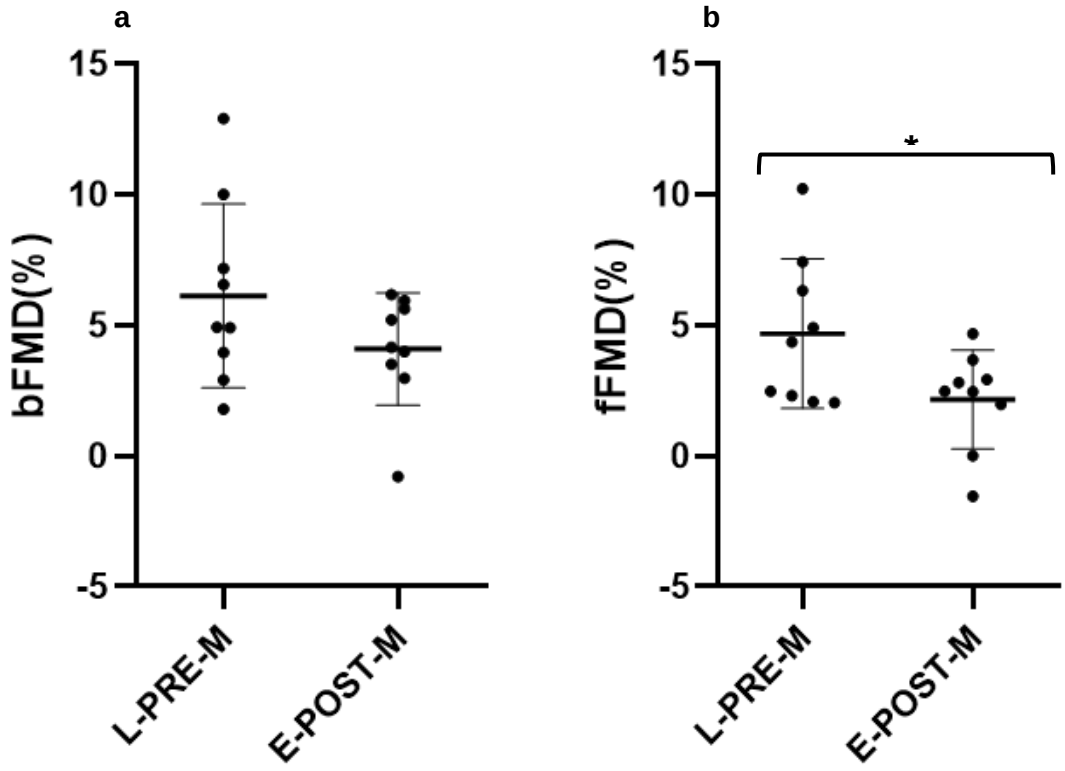


Figure 3.4 Brachial (a) and femoral (b) FMD for Late-PRE- and Early-POST-M women. Brachial FMD was not different between Late-PRE-M and Early-POST-M women (p=0.58) while femoral FMD was reduced in Early-POST-M women (*p=0.049).

3.3.4 Carotid and Peripheral Vascular Structure

3.3.4.1 Intima Media Thickness

POST-M had a greater IMT at the carotid (0.70 [0.68, 0.73 mm] p<0.001), brachial (0.38 [0.36, 0.41mm] p=0.001) and femoral arteries (0.49 [0.46, 0.53 mm] p<0.001) compared to PRE-M women (Table 3.5, Figure 3.5). POST-M women also had a higher IMT-to-lumen ratio at the carotid (0.10 [0.10, 0.11] p<0.001), brachial (0.10 [0.09, 0.11] p=0.001) and femoral arteries (0.06 [0.07, 0.08] p<0.001) compared to PRE-M women. Carotid artery diameter was greater in POST-M women (6.85 [6.7, 6.9 mm]) compared to PRE-M women (6.6 [6.5, 7.7 mm] p=0.014).

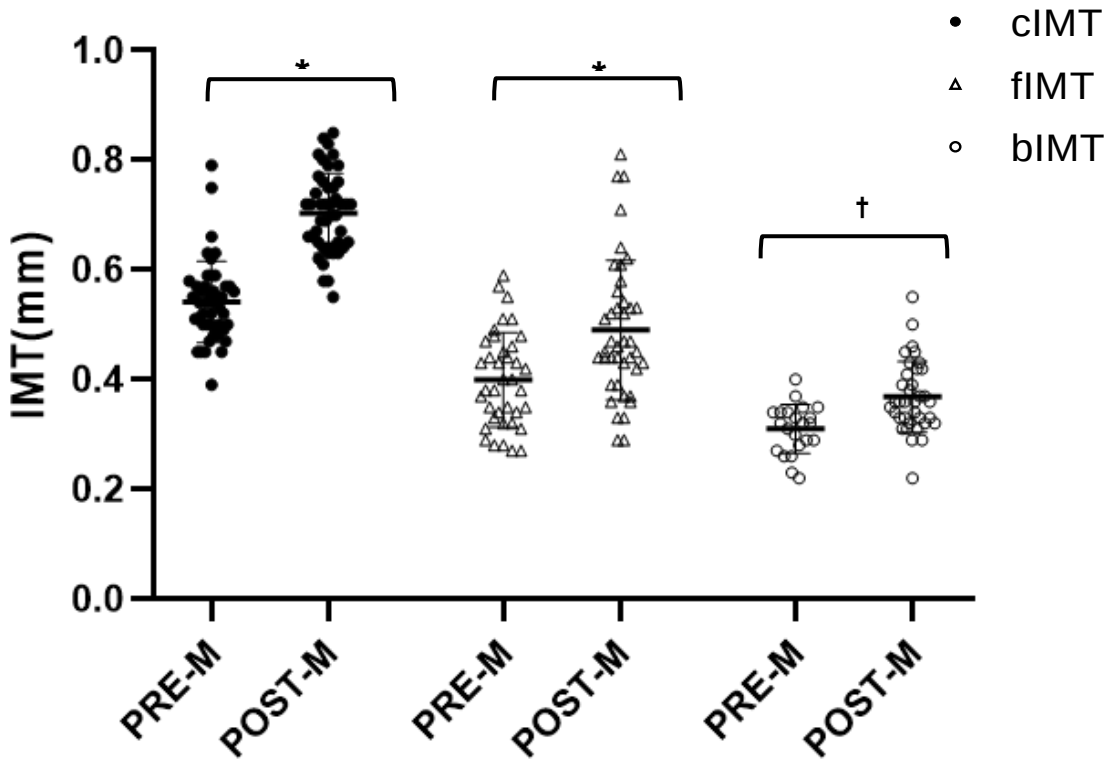


Figure 3.5 IMT at the carotid, brachial and femoral arteries are significantly higher in PRE- compared to POST-M women (*p<0.001, †p=0.001).

Early-POST-M women had a significantly larger cIMT (0.67 [0.63, 0.63 mm]) compared to Late-PRE-M women (0.59 [0.54, 0.65 mm] p=0.03). There was a trend for a larger brachial IMT in the Early-POST-M group (0.34 mm [0.30, 0.37 mm]) compared to the Late-PRE-M group (0.30 [0.27, 0.33 mm] p=0.09). There were no differences between groups for femoral IMT (p=0.43) (Table 3). Early-POST-M women had a significantly greater carotid IMT/lumen (0.10 [0.09, 0.11]) compared to Late-PRE-M women (0.89 [0.09, 0.10] p<0.001). A trend was observed towards a larger brachial IMT/lumen artery in the Early-POST-M group (0.09 [0.08, 0.10]) compared to the Late-PRE-M group (0.08 [0.06, 0.09] p=0.05). There were no differences between groups for femoral IMT/lumen (p=0.62). There were no differences between groups for carotid artery diameter (p=0.71). Brachial and femoral artery diameters are referred to in the FMD section.

Table 3.5 Vascular data between PRE- and POST-M women.

		PRE-M (N=48)	POST-M (N=41)	p-value
Pulse Wave Velocity (m/s)		5.45±0.99	6.87±1.40*	**<0.001**
FMD	**Brachial Artery**			
	Baseline artery diameter (mm)	3.40±0.42	3.41.34±0.43	0.62
	Peak artery diameter (mm)	3.61±0.45	3.54±0.51	0.75
	Time to Peak (secs)	46±23	65±32*	**0.002**
	SR_{AUC} ($x10^3$)	16.8±7.7	20.0±10.9	0.10
	FMD (%)	6.4±3.9	4.1±3.4*	**0.004**
	Adjusted FMD (%)	6.4±0.5	4.2±0.6*	**0.007**
IMT *(N=64)*	Lumen artery diameter (mm)	3.84±0.71	3.82±61	0.92
	IMT (mm)	0.31±0.04	0.38±0.08*	**0.001**
	IMT/Lumen	0.08±0.14	0.10±0.02*	**0.001**
	Femoral Artery			
FMD	Baseline artery diameter (mm)	5.81±0.81	6.40±0.91	0.19
	Peak artery diameter (mm)	6.14±0.82	6.23±0.90	0.72
	Time to Peak (secs)	60±33	71±36	0.15
	SR_{AUC} ($x10^3$)	20.3±16	19.3±13.2	0.76
	FMD (%)	5.8±4	2.8±2.3*	**<0.001**
	Adjusted FMD (%)	6.0±0.4	2.5±0.4*	**<0.001**
IMT	Lumen artery diameter (mm)	6.11±0.07	6.40±0.93	0.15
	IMT (mm)	0.39±0.09	0.49±0.11*	**<0.001**
	IMT/Lumen	0.07±0.01	0.06±0.01*	**0.01**
	Carotid Artery			
IMT	Lumen artery diameter (mm)	6.59±0.40	6.85±0.53*	**0.01**
	IMT (mm)	0.54±0.07	0.70±0.08*	**<0.001**
	IMT/Lumen	0.08±0.01	0.10±0.01*	**<0.001**
Reactivity	CAR (%)	3.0±4.6	0.7±4.9*	**0.03**

Values are mean ± SD. Abbreviations: PRE-M; pre-menopause, POST-M; post-menopause, $SR_{AUC;}$ shear rate area under the curve, FMD; flow mediated dilation, IMT; intima media thickness, IMT/lumen; IMT to lumen ratio, CAR; carotid artery reactivity. Significance is denoted by *p<0.05.

Table 3.6 Vascular data between women before menopause and women <5 years post menopause.

		Late-PRE-M (N=10)	Early-POST-M (N=10)	p-value
Pulse wave velocity		5.87 ± 0.73	5.79 ± 1.05	0.84
FMD	**Brachial artery**			
	Baseline artery diameter (mm)	3.51±0.41	3.42±0.61	0.73
	Peak artery diameter (mm)	3.72±0.42	3.62±0.72	0.53
	Time to Peak (secs)	37±17	66±27*	**0.01**
	SR_{AUC} ($x10^3$)	14.1±6.3	20.9±9.4	0.09
	FMD (%)	5.3±2.6	4.7±1.2	0.58
	Adjusted FMD (%)	6.1±1.3	4.0±0.7	0.30
IMT	Lumen artery diameter (mm)	4.12±1.01	3.74±0.67	0.44
	IMT (mm)	0.30±0.04	0.34±0.30	0.09
	IMT/Lumen	0.08±0.02	0.09±0.01	**0.05**
	Femoral artery			
FMD	Baseline artery diameter (mm)	6.03±0.61	6.20±0.53	0.80
	Peak artery diameter (mm)	6.08±0.62	6.24±0.51	0.86
	Time to Peak (secs)	63±48	71±39	0.69
	SR_{AUC} ($x10^3$)	17.3±8.8	17.3±11.2	0.99
	FMD (%)	4.1±1.9	2.1±1.9*	**0.05**
	Adjusted FMD (%)	5.3±1.1	2.2±1.0*	**0.05**
IMT	Lumen artery diameter (mm)	5.94±0.10	6.53±0.55	0.17
	IMT (mm)	0.46±0.08	0.49±0.10	0.43
	IMT/Lumen (mm)	0.08±0.02	0.08±0.02	0.62
	Carotid Artery			
IMT	Lumen artery diameter (mm)	6.61±0.30	6.72±0.54	0.71
	IMT (mm)	0.59±0.07	0.67±0.09*	**0.03**
	IMT/Lumen (mm)	0.09±0.01	0.10±0.01*	**0.04**
Reactivity	CAR (%)	0.8±6.0	0.8±4.5	0.99

Values are mean ± SD. Abbreviations: $SR_{AUC;}$ shear rate area under the curve, FMD; flow mediated, IMT; intima media thickness, IMT/lumen; IMT to lumen ratio, CAR; carotid artery reactivity. Significance is denoted by *, $p<0.05$.

3.4 Discussion

This study aimed to describe the peripheral and cerebrovascular structural and function differences between PRE-M and POST-M women with simultaneous assessment of PA and cardiorespiratory fitness. The findings of this study demonstrate that cerebral blood flow, central and peripheral vascular function decrease and artery wall thickness increase following the menopause. These changes occur with simultaneous reductions in vigorous PA and cardiorespiratory fitness. Nonetheless, only femoral FMD and carotid IMT changes were observed in early menopausal (<5 years) suggesting direct effects of oestrogen decline and not ageing on large artery structure and function. Taken together, our data suggests that menopause related vascular changes are site specific, with larger arteries that are more susceptible to atherosclerosis impacted early in the menopausal transition.

Cerebral blood flow measured via MCAv, was lower in PRE- compared to POST-M women however, this did not occur in the early years of menopause suggesting that declines in cerebral blood flow occur due to ageing (Ainslie et al., 2008). Nevertheless, CBV-R did not differ between PRE-M to POST-M, or in the sub-group analysis; in line with previous findings (Mitsis et al., 2007). Reduced CBV-R is more commonly associated with populations with overt disease (Strandgaard and Paulson, 1984, Markus and Cullinane, 2001) and is usually maintained in healthy individuals (Schwertfeger et al., 2006) however, the immediate and long term effect of menopause on CBV-R without the inclusion of hormone replacement therapy (Penotti, 2002) has been largely unreported. In contrast to a previous study, our findings suggest that cerebrovascular function is maintained during and following the menopause in healthy women with no CVD risk factors or overt CVD (Matteis et al., 1998). This may be explained by differential methods used to assess cerebral autoregulation with our study adhering to recommended guidelines for assessing cerebral autoregulation (Claassen et al., 2016) while the Matteis study used the breath hold index. In summary, cerebrovascular function appears unaltered with menopause despite reductions in cerebral blood flow velocity.

A novel aspect of the current study relates to the assessment of CAR via the cold pressor test. This was included given the role of the carotid artery as an extra cranial vessel delivering blood flow to the brain (Faraci et al., 1987). In addition, the CAR test reflects coronary artery dilatory responses (van Mil et al., 2017) which may be indicative of coronary artery disease and CV events (Schachinger et al., 2000). Thus, taken together CAR may provide insight into the interaction between central and cerebral vessels as well as central and peripheral vessels. Our data suggest CAR is lower in POST-M compared to PRE-M but may not be directly associated with the menopause *per se* since the CAR response was similar between Late-PRE-M and Early-POST-M women. Interestingly, given the extracranial role of the carotid artery, the CAR data does not reflect the intact CBV-R and autoregulatory responses between PRE-M and POST-M women. It does however support the age-related reductions in cerebral blood flow velocity as well as peripheral artery vascular function (brachial and femoral arteries) observed in the current study. The dilation (or constriction) during the CAR test is influenced by sympathetic nerve activity (Berkenboom and Unger, 1990), which also increases with age (Rehman and Masson, 2001). This age-associated increase in sympathetic nerve activity may explain the reduced CAR in POST-M women. Alternatively, the diminished response at the carotid artery may also reflect endothelial dysfunction in line with that observed at the brachial artery as a consequence of ageing.

We also included an assessment of femoral artery endothelial function in the current study to obtain a systemic view of the vascular system with age and menopause in females but also because, like the carotid artery, the femoral artery is susceptible to atherosclerosis (Thijssen et al., 2008). Importantly, we show a decline in femoral artery endothelial function which is evident early in the menopausal transition, suggesting this dysfunction may be due to menopause transition rather than ageing. This could be explained by differences in the contribution of oestrogen to vasodilation in the femoral artery via nitric oxide or oestrogen receptors located in the endothelial and smooth muscle cells (Moreau et al., 2003). Alternatively, the impairment could be explained by the reduction in PA observed from pre to post-menopause in the current study. It is known that artery size influences the shear stimuli

and functional responses of an artery (Thijssen et al., 2011d). An acute prolonged sitting intervention is associated a reduction in femoral but not brachial FMD (Thosar et al., 2014). It is plausible that chronic reductions in PA could exacerbate this response further resulting in longer-term reduced femoral artery function, however this requires further clarification. Taken together, the CAR test reflects reduced systemic endothelial function between PRE-M and POST-M women that is seemingly age related. In contrast, the impairment in femoral artery endothelial function appears to be a consequence of reduced oestrogen and/or physical inactivity. Our data supports the concept of site-specific arterial adaptation highlighting the need to assess multiple vascular sites in order to gain a comprehensive understanding of systemic hemodynamics.

Artery wall thickening at the carotid, femoral and brachial arteries were evident with ageing in this study. Previous research has shown that significant carotid artery remodelling post-menopause, independent of atherosclerotic risk factors and metabolic variables (Muscelli et al., 2009). Our data support this, suggesting that remodelling occurs early in menopause period and is more pronounced at the carotid compared with brachial and femoral arteries. Increased BP, which exerts a greater distending force on the artery wall is considered an important contributor to carotid thickening (Tanaka et al., 2001) but BP increases did not occur in the early menopausal period in the current study. The carotid artery is susceptible to atherosclerosis due to its large artery diameter and the greatest shear rate in comparison to the brachial and femoral arteries (Wu et al., 2004). According to our data, this susceptibility is increased in early menopause and may be related to the elastic nature of the artery wall (Binder et al., 1996). Oestrogen has the ability to increase elastin/collagen ratio and attenuate collagen deposition in aortic smooth muscle cells in vitro (Chironi et al., 2003, Natoli et al., 2005). Oestrogen reduction may therefore have an opposing effect on the connective tissue component of the artery wall leading to increased stiffness and altered structure (Westendorp et al., 1999). In contrast, the brachial and femoral arteries are muscular in nature with thicker medial layers and more smooth muscle cells compared with elastic arteries (Sturek et al., 2007). Increases in IMT in healthy adults are thought to be due to smooth muscle cell

hypertrophy within the medial layer, and as such, muscular arteries may have more plasticity (Dinenno et al., 2001). This may explain the slower progression of IMT in the periphery compared to the carotid artery. Moreover, according to previous research, maintaining moderate-vigorous PA in early menopause may have influenced several putative factors known to modulate smooth muscle cells in the arterial wall including sympathetic-adrenergic activity, circulating ANG II and endothelin-1, and locally released vasoactive factors such as nitric oxide (Kingwell, 2000, Espeland et al., 1995, Majmudar et al., 2000). It would therefore be reasonable to suggest that a reduction in this moderate to vigorous PA could compound these vasoactive factors. Interestingly, POST-M had a lower level of vigorous PA and simultaneously, a higher brachial and femoral IMT in comparison to PRE-M women. Previous findings have shown exercise to protect against IMT development in ageing men and women (Moreau et al., 2006). It is understood that exercise protects against IMT development by releasing local vasoactive factors at the endothelium to counteract vasoconstrictors associated with artery stiffening and wall thickening (Kingwell, 2000). Acute increases in blood flow and a resulting elevation in shear stress (Buchanan et al., 2012) is known to increase nitric oxide production (Buchanan and Xiang, 2005). The withdrawal of this stimulus may therefore contribute to IMT development in the periphery. Taken together, the heterogenous progression of IMT across multiple arteries may be explained by differential artery wall properties and the influence of PA in attenuating peripheral IMT development. Overall these data imply for the first time, that menopausal status plays a role in the regulation of carotid artery structure and femoral artery function. Remaining outcomes such as CAR, brachial and femoral structures and brachial FMD appear to be more heavily influenced by ageing. This may influence future study designs protocols where ageing and/or menopause are being investigated.

This study, for the first time, examined important confounders of CVD including body composition, aerobic capacity, PA and sedentary behaviour in conjunction with a large battery of CVD risk markers in order to gain a more complete understanding of the influencers of CVD risk in women. Despite 84% of women in this cohort surpassing PA guidelines of 150

min/week of moderate to vigorous PA, cardiorespiratory fitness declined from PRE-M to POST-M. This may be due to the significant reduction in vigorous PA in POST-M women, as vigorous PA has been shown to significantly impact cardiorespiratory fitness. Consequently, in our cohort of healthy women, achieving PA guidelines was insufficient to prevent the decline CV risk markers, in agreement with existing research (Akbartabartoori et al., 2008). Future research should look to identify a dose response to vigorous PA and CV risk factors among older women in an attempt to preserve confounders of CVD.

Strengths and Limitations

Firstly, this study is strengthened by a large sample size and the inclusion of a vast range of peripheral vascular and cerebrovascular outcomes that are novel risk factors for CVD compared to traditional use of fitness and BMI. These techniques have been combined with other traditional confounders of CVD risk including body composition, aerobic capacity, objectively measured PA and sedentary behaviour that have been typically assessed in isolation in this cohort. The wide age range has facilitated three separate analysis to be performed providing novel insight between PRE-M and POST-M women with ageing and Late-PRE-M and Early-POST-M women. For the first time in this cohort, we have applied allometric scaling to the brachial and femoral arteries during our FMD analyses to account for changes in baseline diameter.

A key limitation, as with all cross-sectional study designs is that we cannot make causal inference from the data. Additionally, 84% of the cohort included in this study met PA guidelines of 150 minutes MPA per week which may not be representative of the general female population and may have influenced our findings. The results of our sub-analysis must be interpreted with caution based on the small sample size, however we were able to show significant statistical differences in this analysis. We acknowledge that we were unable to age match the Late-PRE-M and Early-POST-M groups and as such we have been unable to wholly remove the influence of age in this comparison.

3.5 Conclusion

To conclude, menopausal women have lower cerebral blood flow velocity, central and peripheral vascular function and higher artery wall thickness in comparison to pre-menopausal women. These decrements are not all present in early menopause suggesting that the duration of time from menopause and increased age may have greater effect on these parameters than the event of menopause. Finally, our data provides some evidence to suggest that post-menopausal vascular decline may be at least partly driven by changes in vigorous PA or cardiorespiratory fitness, however this hypothesis must be confirmed with future longitudinal research designs.

Chapter 4: Effect of Regular Moderate Intensity Aerobic Exercise on the Peripheral and Cerebral Vasculature during Pregnancy: A Pilot Study.

4.1 Introduction

Pregnancy is associated with significant CV adaptation including increased CO, blood volume, vasodilation and decreased MAP (Gongora and Wenger, 2015). Increased vasodilation is critical for adequate delivery of oxygen and nutrients to the foetus and is achieved in part, via increased production of vasoactive substances such as NO at the vascular endothelium in response to elevated and sustained levels of oestrogen (Boeldt and Bird, 2017). For this to occur, the integrity of the endothelium is pivotal (Melzer et al., 2010). However, underlying endothelial injury may prevent proper adaptation to the due to the increased demand on the maternal CV system, and is believed to underpin the pathophysiology of several peripheral (Srivaratharajah and Abramson, 2018) and cerebrovascular diseases (Treadwell et al., 2008) that affect pregnant women.

Pregnancy can be accompanied with cerebrovascular complications including various types of stroke and haemorrhage (Cipolla et al., 2011, Sloan and Stern, 2003). Although the incidence rate of cerebrovascular disease during pregnancy is low (11-26 per 100 000 deliveries), this is expected to rise due to the increased age of women giving birth and obesity (Novak et al., 1998b). Nonetheless, cerebrovascular adaptation during an uncomplicated pregnancy is not well understood. It is thought that cerebral blood flow velocity is reduced during pregnancy, likely as a result of reduced vascular resistance (Belfort et al., 2001, Serra-Serra et al., 1997, Williams and Wilson, 1994). To date, just one case study has shown increased CBV-R during an uncomplicated pregnancy (Steinback et al., 2015). Moreover, the upper and lower limits of cerebral autoregulation have been shown to be extended during pregnancy in an animal model, possibly to protect the cerebrovasculature against ischemic brain injury in response to pregnancy related changes in BP (Chapman et al., 2013). Whilst further research is warranted, taken together, this limited data suggests cerebrovascular function may be upregulated during pregnancy.

To the author's knowledge, there is a paucity of evidence examining multiple measures of vascular function and structure in pregnancy. While vascular function is understood to be improved in pregnancy (Savvidou et al., 2000), to date only one study has shown enhanced brachial vascular function and artery size, alongside reductions in carotid IMT and aortic stiffness during pregnancy compared with healthy non-pregnant controls (Torrado et al., 2015). Nevertheless, these parameters were only assessed in the final trimester of pregnancy and do not provide a comprehensive insight to the central and peripheral vascular response to pregnancy. A longitudinal approach with measurements of systemic vascular function is warranted and may be of clinical value given endothelial dysfunction, characterised by a decrease in peripheral vascular function, can arise during pregnancy and is implicated in the pathogenesis of GDM and pre-eclampsia (Cockell and Poston, 1997, Myatt and Webster, 2009).

Exercise has shown a positive effect on cerebral (Murrell et al., 2013) and peripheral vascular function (Black et al., 2009) in otherwise healthy females. Furthermore, exercise has been shown to improve brachial artery function to a greater extent than non-exercisers during pregnancy (Ramirez-Velez et al., 2011) and importantly, is associated with reduced risk of pregnancy-related complications (Davies et al., 2003). Exercise may be a viable means to reduce pregnancy related vascular complications (Mosca et al., 2011), yet the normal cerebral and peripheral vascular adaptations to exercise during pregnancy require further investigation. Therefore, the primary aim of this study was to define the normal systemic vascular adaptation to an uncomplicated pregnancy. Secondly, we aimed to determine the impact of a 6-month pregnancy exercise intervention on maternal vascular adaptation.

4.2 Material and Methods

4.2.1 Participants

Twenty-one singleton pregnant participants were recruited at local pre-natal clinics. Women were eligible if they were in the first trimester of pregnancy, non-smokers for at least 6

months, had no history of CVD, GDM or pre-eclampsia, were not on any form of medication, participated in structured exercise less than twice/week and had a BMI <35kg/m^2. All participants provided written consent before taking part in the experimental procedure. The research study was ethically approved by the National Health Service Liverpool Central Research Ethics Committee and adhered to the Declaration of Helsinki.

4.2.2 Experimental Procedure

Participants were required to visit the temperature controlled (20-22°C) laboratory on 3 occasions having abstained from exercise for 24 hours and alcohol for 12 hours as well as any food/caffeine/stimulants 6 hours prior to the experiment. Each visit corresponded with the end of each trimester (T1, T2, and T3) and were repeated at the same time of day for each visit. Participants completed a battery of CV and cerebrovascular assessments in the following order; neurovascular coupling (NVC), IMT, FMD, PWV, CBV-R and cerebral autoregulation. The Astrand submaximal cycling test was performed to estimate cardiorespiratory fitness ($VO2_{peak}$). Following each visit, participants recorded 7 days of objective PA and sedentary behavior monitoring. At the initial visit (T1), participants chose to take part in a partially supervised exercise (EX) intervention or to participate as a control (CONT). Those in the control group received conventional care only.

4.2.3 Anthropometrics, physical activity and sedentary behaviour

Anthropometry and Body Composition. Stature and body mass were recorded to the nearest 0.1cm using a stadiometer and digital scales respectively. BMI was calculated as body mass in kilograms divided by stature in metres squared (kg/m^2). Skinfold thickness measurements were performed at the biceps, triceps and subscapular landmarks in line with the International Standards for Anthropometric Assessment Manual to calculate body fat percentage (BF%) (Marfell-Jones M). All measurements were obtained using Harpenden Callipers with a 0.2 mm dial graduation and a measuring range of 0-80 mm. The calliper dial was viewed at 90° to avoid errors of parallax. Two measurements were taken and if the difference was greater than 7.5% a third measurement was performed (Kannieappan, 2013). The final measurements were recorded to the nearest 0.1 mm and were reported as the average of

two measurements or the median of three (if three measurements were obtained). To measure the arm circumference, the participant was asked to stand with her arms relaxed at her side. The midpoint between the most superior and lateral point of the acromion border and the most proximal and lateral border of the head of the radius was determined. Using the cross-hand technique, the arm circumference was measured at this point, ensuring it was taken at eye level, and with constant tension applied to the tape. With the tape still around the midpoint of the arm, a mark was made on the most anterior point of the biceps (just above measuring tape) and the most posterior point of the triceps (just below measuring tape) area to assist in locating the biceps and triceps skinfold landmark. To measure skinfold thickness, the indicator on the callipers was zeroed. The thumb and index finger were held parallel and used to grasp the skinfold, ensuring the skin was rolled from side to side to remove any muscle. The callipers were placed at 90° to the skin, one centimetre distal to the marked skinfold site with the measurement taken after two seconds. The following equation was used to calculate BF%:

BF% = 12.7 + 0.457 x *triceps SFTM* + 0.352

X *subscapular SFTM* + 0.103

X *biceps SFTM* – 0.057 x *height* + 0.265

X *arm circumference*;

Where skin fold thickness measure (SFTM) were measured in mm, and arm circumference and height were measured in cm (Kannieappan, 2013).

Physical activity and sedentary behaviour. Physical activity and sedentary behaviour were monitored as described in Chapter 3, section 3.2.3.

4.2.4 Cardiovascular measurements

Carotid, and femoral IMT, artery stiffness, brachial and femoral FMD measurements were performed as described in Chapter 3, section 3.2.4. Due to the difficulty obtaining the of brachial IMT measurement in Chapter 3, it was omitted from this experimental procedure.

4.2.5 Cerebrovascular Measurements

Neurovascular Coupling (NVC). Participants lay in a semi-recumbent position instrumented as above with the addition of a rebreathing apparatus consisting of a mouthpiece, nose clip, a bacteriological filter and a three-way valve to allow for $PETCO_2$ monitoring. On one side of the cerebral headband, the posterior cerebral artery (PCA) was isonated in a posterior direction from the anterior temporal window at a depth of 60-70mm as described elsewhere (Willie et al., 2011). On the other side, the MCA was secured following isonation as described above. A 120s baseline was recorded with participant's eyes open, followed by 120s of eyes closed to act as a familiarization phase. Participants were then asked to open and close their eyes in 30 second intervals for a period of 5 minutes as prompted by the researcher. During 'eyes open' participants were instructed to fixate on a red dot in the center of a moving black and white checkered image (Figure 4.1). The video was display on an iPad (Apple) and held ~7cm away from their eyes to reduce the effects of external light. The same side for MCA and PCA were isonated for repeated measurements to ensure consistency. The visual stimulation used provides a strong mechanistic model of neurovascular coupling (Phillips et al., 2016) by increased neural activation of the occipital lobe.

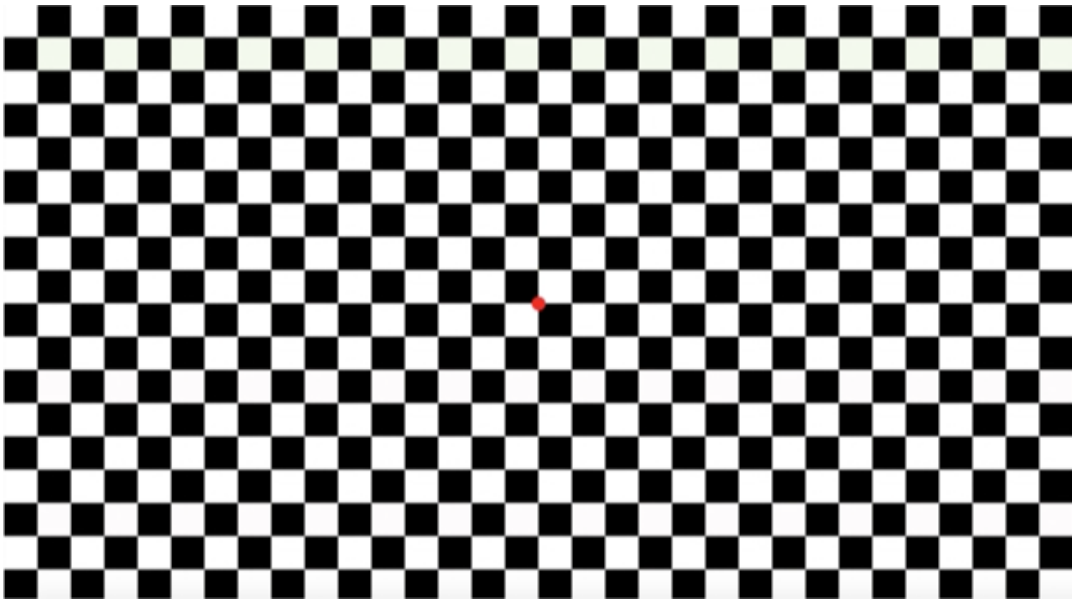


Figure 4.1 Screenshot of video used during the eyes open phase of neurovascular coupling. Participants were instructed to focus on the red dot in the middle of the screen, while the black and white squares flash for each 30 second 'eyes open' cycle.

Data was anaylsed following recommended guidelines using an automated software (Phillips et al., 2016). First analysis of NVC was performed by visually inspecting recordings on LabChart for noise in the signals and artefacts. Interpolation was used to replace any short segements and any larger segments were excluded. Data were extracted from LabChart (Figure 4.2) on a beat-to-beat basis (peak, minimum and mean MCAv & PCAv along with BP, $PETCO_2$ and HR). Only the 5 cycles of 30 seconds eyes-open-eyes-closed are extracted for analysis. Data was placed into a separate spreadsheet where it was checked for correct coding (i.e. O = eyes open, C = eyes closed) and formated correctly to be compatible with the recommended automated MATLAB (Mathworks-Inc., Natick, MA) script. Data was run through the MATLAB script where the software combines all the cycles into one average contour for each physiological recording of each eyes-open-eyes-closed cycle. Time to peak response, absolute and percentage change in the PCAv were used to quantify the NVC response for the purpose of this thesis. Middle cerebral artery velocity, CBV-R and CA measurements were performed as described in Chapter 3, section 3.2.5.

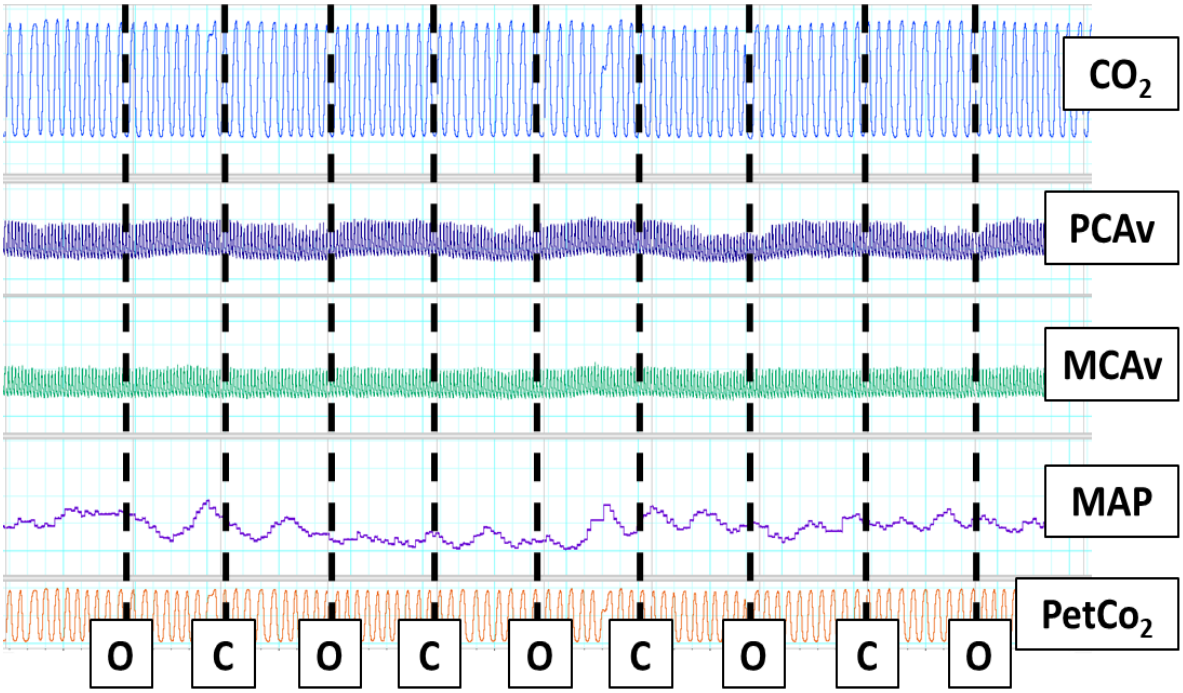


Figure 4.2 Example recording of one participants response during a neurovascular coupling assessment. Channels shown; carbon dioxide (CO_2), posterior cerebral blood flow velocity (PCAv), middle cerebral artery blood flow velocity (MCAv), mean arterial pressure (MAP) and end tidal pressure of carbon dixoxide ($PETCO_2$) during the eyes open (O) and eyes closed (C) cycles. Note the increase-decrease in PCAv throughout the cycles whilst MCAv remains unchanged.

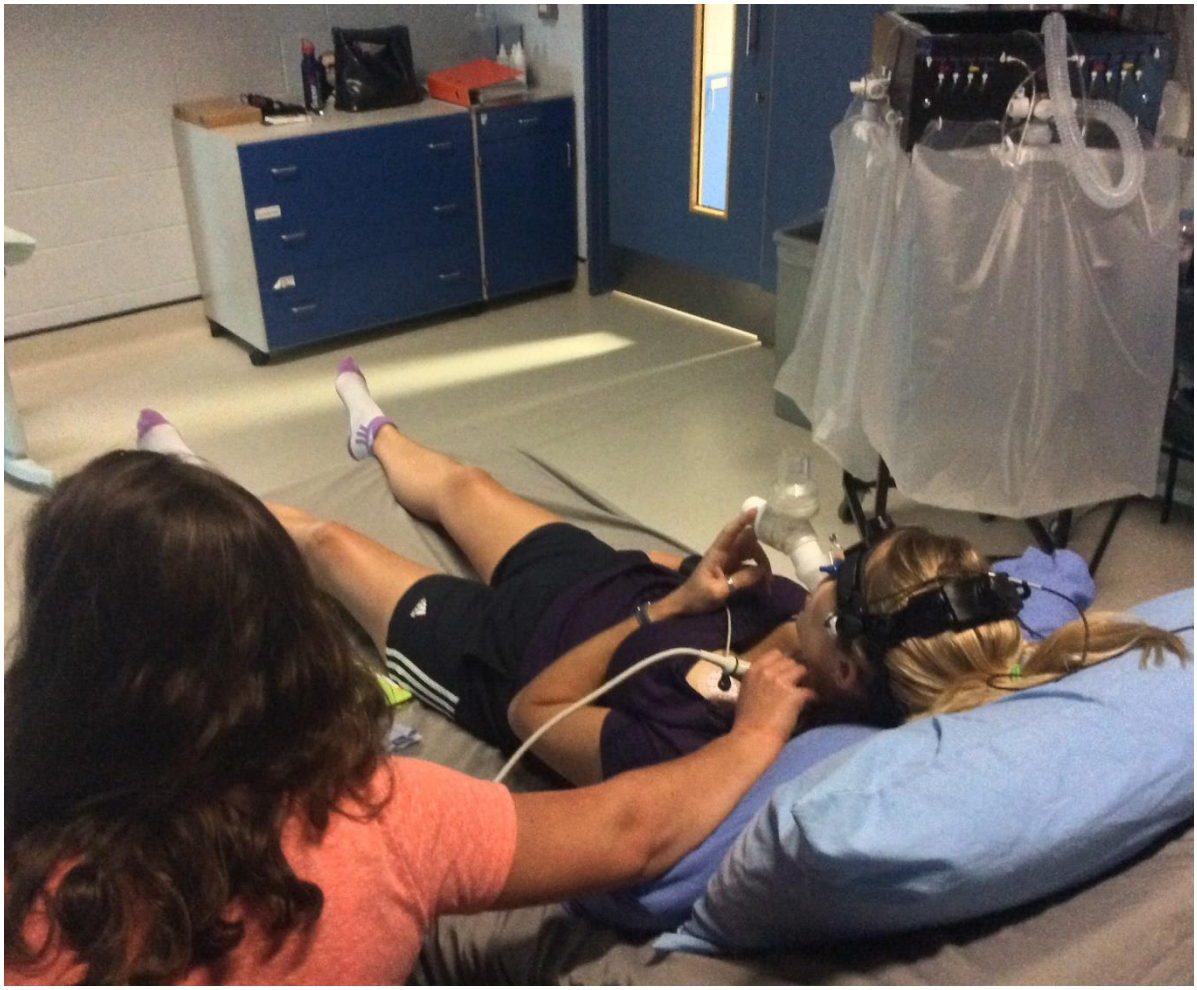


Figure 4.3 Illustrates participant set up for cardiovascular reactivity test. The participant is wearing a cerebral headband and a mouthpiece ready for connection to a pre-filled douglas bag containing a hypercapnic gas mixture of 5% CO_2, 21% O_2 balanced with nitrogen. Simultaneously, the carotid artery is being scanned using ultrasound.

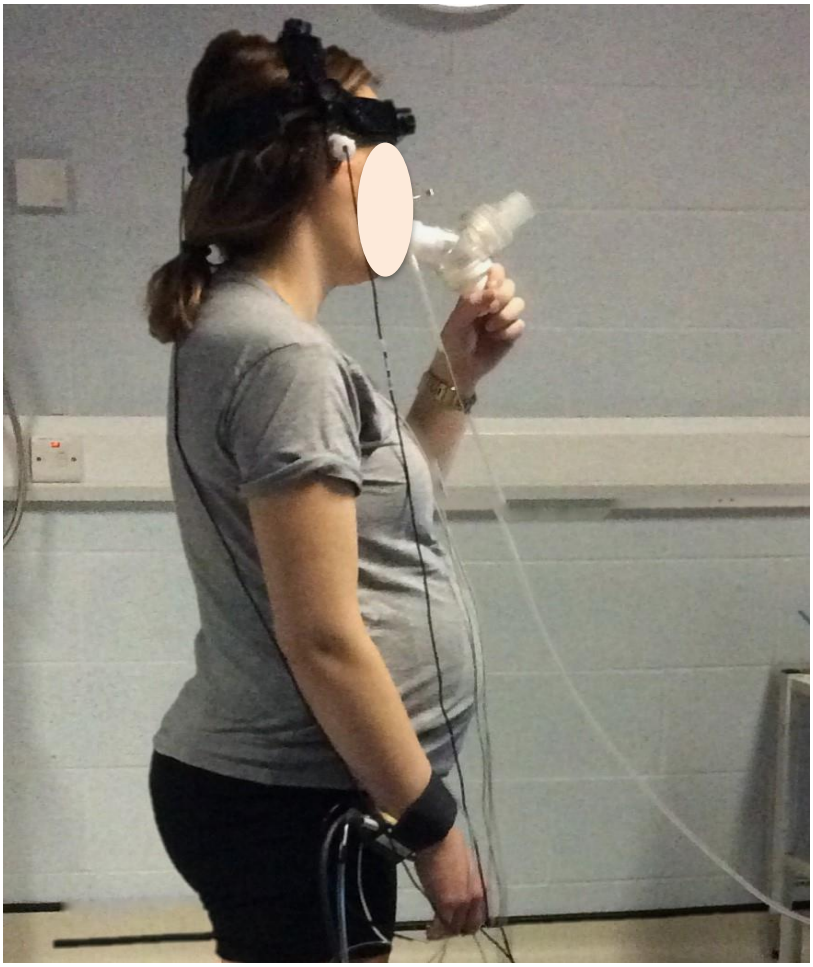


Figure 4.4 Participant equipped with cerebral headband, mouthpiece and finometer secured at the right wrist to perform squat stand protocol.

Cardiorespiratory Fitness Test. Participants performed a submaximal cycling test to estimate VO_{2max} as previously described (Astrand and Ryhming, 1954). In brief, participants cycled at a self-selected resistance and pace for 2-minutes to be familiarised to the cycle ergometer. HR was monitored continuously throughout the test using short-range telemetry (Polar, Kempele, Finland) and noted at the end of each minute alongside RPE. The test began with participants cycling with a load of 60 Watts (W) for a period of 6 minutes. If mean HR was between 125-170bpm in the sixth minute the test was stopped, otherwise, the test continued for a further 3 minutes with an increased resistance of 30W until the criterion HR was met (Astrand and Ryhming, 1954). Participant's VO_2 was estimated using the Astrand-Rhyming nomogram based on mean HR (corrected for age) in the final minute of the test and the mechanical load at that point (Astrand, 1960). The absolute and relative VO_2 was calculated

and reported.

4.2.6 Exercise Intervention

Participants began the exercise intervention after completing PA and sedentary behavior monitoring in T1. The intervention commenced with 3x 15 minutes continuous exercise sessions at the beginning of T2, 3x 30 minutes by the end of T2 and 4x 30 in T3 in line with RCOG pregnancy exercise guidelines (Bell and Dooley, 2006). The intervention comprised of aerobic exercise (60-70% MHR; where MHR was calculated using the Karvonen formula of 220-age), and was performed using a bike, treadmill, cross-trainer, rower or a combination of these as chosen by the participant. One session per week was supervised by the researcher at the Liverpool John Moores University premises and the remaining sessions were completed at a Liverpool City Council gym for which memberships were provided. Participants wore a Polar Bluetooth Heart Rate Monitor during all sessions and HR was recorded via a personal Polar Beat account which was remotely accessible to the researcher. Compliance was verified by totaling the number of sessions attended by each participant divided by the minimum number of sessions achievable in that time (Aries et al., 2010) and is reported as a percentage. Adherence to the exercise was checked by verifying the heart rate recorded for each session by the Polar monitor. A session was adhered to if HR was equal to or greater than 60% MHR (Paulson et al., 1990).

4.2.7 Statistical analysis

Participant characteristics were compared using an independent *t-test*. Cerebrovascular parameters, FMD, IMT, and PA were analysed using a linear mixed model. Significant interactions and main effects were followed up using least significant difference pairwise comparisons. Where a main effect of time is reported, data is presented as the accumulated increase, or decrease during pregnancy with T2 and T3 increases or decrease displayed in brackets. Analysis was conducted using Statistical Package for Social Sciences (Version 25; SPSS Inc., Chicago, IL). Data are presented in the text as mean (95% confidence interval) unless otherwise stated, with exact *p* values.

4.3 Results

4.3.1 Participant Characteristics

Baseline characteristics (Table 1) on entry to the study were similar in terms of age (p=0.48), BMI (p=0.76), BF (p=0.26), estimated absolute (p=0.90) and relative VO_2 (p=0.54), SBP (p=0.31) a DBP (p=0.87). Of the 21 women enrolled in the study, 3 withdrew (2 from the EX group) due to time commitment, and 1 (CONT) laboured prior to the final measurement. Body mass increased (10.7 kg [5, 17 kg]; main effect of time p=0.004). Similarly, BMI increased (4 kg/m^2 [2, 6 kg/m^2]; main effect of time p=0.007). There were no intervention or intervention*time interactions for body mass or BMI. There were no main effects of time, intervention or intervention*time for SBP, DBP, MAP, absolute or estimated relative VO_2 (p>0.05, Table 4.1).

Table 4.1 Descriptive characteristics of participants in the exercise and control group at the end of trimester 1, 2 and 3.

	Exercise group			**Control group**			***Two Way Anova***		
Trimester	**T1**	**T2**	**T3**	**T1**	**T2**	**T3**	***Time***	***Intervention***	***Time*Intervention***
Body mass (kg)	62.0±8.4	69.5±8.6	73.3±9.7	66.6±7.7	73.0±7.3	76.5±6.6	**0.01**	0.24	0.99
BMI (kg/m^2)	23±3	26±3	27±4	24±3.	26±3	28±3	**0.01**	0.87	0.96
Body fat (%)	22.3±2.2	24.6±3.7	25.4±4.2	23.5±3.7	25.1±3.7	25.9±4.2	0.11	0.95	0.97
SBP (mmHg)	99±6	102±7	104±6	103±12	105±13	104±7	0.68	0.36	0.69
DBP (mmHg)	60±8	61±6	66±6	60±6	60±6	62±7	0.17	0.38	0.64
Absolute VO_2 (ml.kg)	2.3±0.6	2.5±0.5	2.5±0.4	2.5±0.7	2.5±0.7	2.5±0.7	0.91	0.95	0.75
Estimated VO_2 (ml.kg)	35.3±10.2	34.0±8.9	32.7±7.5	36.7±12.3	33.3±11.4	30.9±10.4	0.38	0.77	0.81

Values are mean ± SD. Abbreviations: T1; trimester 1, T2; trimester 2, T3; trimester 3, BMI; body mass index, SBP; systolic blood pressure, DBP; diastolic blood pressure, VO_2; oxygen consumption. **Bold numbers** represent significant main effect ($p<0.05$).

Exercise Intervention and Physical Activity Levels

Overall compliance to the EX intervention was 78%, (T2; 84% and T3, 68%) and all sessions attended were adhered to. The EX group engaged in significantly less sedentary time; (456 min/d [411, 501 min/d] compared to the CONT group (523 min/d [485, 562 min/d]; main effect of intervention p=0.03), but there was no time or time*intervention effects for sedentary behavior. There were no main effects for time, intervention or time*intervention for light, moderate, vigorous and total PA, or accelerometer wear time (p>0.05, Table 4.2).

Table 4.2 Physical activity and sedentary behaviour for participants in the exercise and control group and the end of trimester 1, 2 and 3.

	Exercise group			**Control group**			***Two Way Anova***		
Trimester	**T1**	**T2**	**T3**	**T1**	**T2**	**T3**	***Time***	***Intervention***	***Time* Intervention***
Sedentary time (min/d)	464±101	445±85	458±132	534±110	553±72	484±103	0.70	**0.03**	0.49
Step count	5626±1304	6051±2315	6141±1965	6072±2254	5017±1513	5136±1822	0.89	0.33	0.45
Light PA (min/d)	335±79	346±60	315±97	322±88	299±76	301±57	0.75	0.27	0.74
LPA % Wear Time	39±7	42±5	38±8	40±12	36±9	39±8	-	-	-
Moderate PA (min/d)	29±16	27±19	30±19	30±9	25±13	24±24	0.71	0.78	0.81
MPA % Wear Time	3±2	3±2	4±2	4±2	3±2	3±3	-	-	-
Vigorous (min/d)	1±2	1±2	1±2	1±2	0±1	1±1	0.76	0.58	0.69
Vigorous PA % Wear Time	0±0	0±0	0±0	0±0	0±0	0±0	-	-	-
Total PA (min/d)	364±92	373±75	345±106	355±100	323±83	326±66	0.75	0.30	0.77
Avg. daily wear time (min/d)	847±91	824±78	814±92	822±89	848±118	771±76	0.30	0.59	0.59

Values are mean ± SD. Abbreviations: T1; trimester 1, T2; trimester 2, T3; trimester 3, PA; physical activity, LPA; light physical activity, MPA; moderate physical activity, Avg; average. **Bold numbers** represent significant main effect (P<0.05).

4.3.2 Cerebrovascular Function

Middle cerebral artery blood flow velocity. MCAv reduced significantly during pregnancy (-8 cm/s^{-1} [-14, -2 cm/s]; main effect of time p=0.02) (Figure 4.5). There was no intervention (p=0.62) or time*intervention effect (p=0.49) for MCAv. There was a trend for a higher MCA-CVC in the EX group (0.91 $cm.s^{-1}.mmHg^{-1}$ [0.80, 1.0 $cm.s^{-1}.mmHg^{-1}$] compared to CONT group (0.79 $cm.s^{-1}.mmHg^{-1}$ [0.71, 0.87 $cm.s^{-1}.mmHg^{-1}$] main effect of intervention p=0.07). There was no time or time*intervention effect for MCA-CVC (p>0.05).

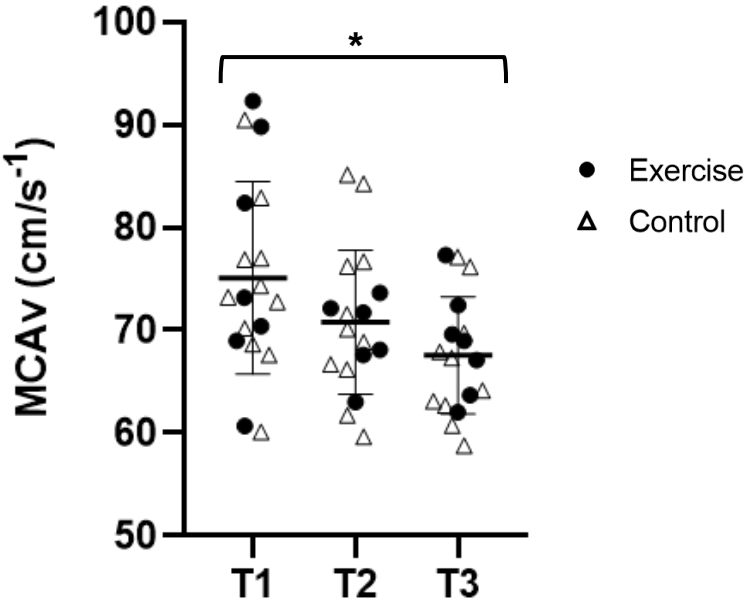


Figure 4.5 Middle cerebral artery velocity during pregnancy. MCAv declined during pregnancy data for trimester 1, 2 and 3 which. *main effect for time; p<0.05.

Cerebrovascular Reactivity. During pregnancy, there was no time, intervention or time*intervention effect for the response of the carotid artery to increased CO_2 (p>0.05, Table 5). There was no time, interaction or time*interaction effect for basal $PETCO_2$, linearity between MCAV and $PETCO_2$, absolute CBV-R, relative CBV-R or MAP response during the CBV-R test (p>0.05, Table 4.3).

Cerebral Autoregulation. There was a trend for normalised gain in the low frequency domain to increase during pregnancy by (1.6% [1.5, 1.7%]; main effect of time p=0.08) (Figure 4.6). There was no intervention (p=0.22) or time*intervention effect (p=0.71). There was a trend for an effect of time for increased phase in the low frequency (13.9° [29°, 39°]; main effect of time p=0.06). There was no intervention or time*intervention effect for normalised gain (p>0.05, Table 4.4).

Neurovascular coupling. PCAv was greater in the EX group (43.7 cm/s^{-1} [40.4, 47.0 cm/s^{-1}] compared to the CONT group (39.2 cm/s^{-1} [36.5, 41.9 cm/s^{-1}]; main effect of intervention p=0.04). There was no time or time*intervention effect for PCAv (p>0.05). There was no time, intervention or time*intervention effect for NVC response (p>0.05) (Table 4.3).

Table 4.3 Cerebral blood flow, neurovascular coupling and cerebrovascular reactivity data for participants in the exercise and control group at the end of trimester 1, 2 and 3.

	Exercise group			**Control group**			***Two Way Anova***		
Trimester	**T1**	**T2**	**T3**	**T1**	**T2**	**T3**	***Time***	***Intervention***	***Time* Intervention**
PCAv (cm/s^{-1})	43.7±9.1	45.5±10.6	42.0±5.3	43.5±9.3	38.3±4.9	35.8±4.8	0.15	**0.04**	0.44
NVC response (%)	13±6	14±8	15±9	14±9	17±8	18±4	0.48	0.37	0.85
MCA CVC ($cm.s^{-1}.mmHg^{-1}$)	0.92±0.14	0.93±0.28	0.87±0.28	0.84±0.13	0.87±0.13	0.74±0.11	0.46	0.07	0.84
MAP (mmHg)	84±10	79±18	83±17	89±11	84±8	90±12	0.42	0.16	0.96
PET CO_2 (mmHg)	31.9±1.8	33.8±1.9	32.8±1.5	33.0±1.9	32.7±2.1	32.8±0.9	0.82	0.58	0.56
CO_2 Reactivity test									
Carotid diameter (cm)	0.64±0.01	0.66±0.02	0.66±0.01	0.64±0.01	0.66±0.02	0.67±0.01	0.19	0.89	0.72
Carotid diameter (cm) (last 30 seconds)	0.66±0.02	0.67±0.02	0.67±0.02	0.66±0.01	0.67±0.02	0.68±0.01	0.51	0.74	0.96
Relative CBV-R ($cm \cdot s/mmHg^{-1}$)	4.9±1.4	4.9±1.4	4.7±1.4	4.3±1.2	3.9±0.9	4.3±1.4	0.92	0.23	0.47
Absolute CBV-R ($cm \cdot s/mmHg^{-1}$)	4.7±1.4	4.4±1.1	4.7±1.5	4.3±1.2	4.1±1.0	3.6±1.1	0.71	0.11	0.58
CBV-R (r^2)	0.9±0.1	0.9±0.1	0.8±0.2	0.9±0.1	0.9±0.1	0.8±0.1	0.23	0.85	0.98

Values are mean ± SD. Abbreviations: T1; trimester 1, T2; trimester 2, T3; trimester 3, MCAv; middle cerebral artery velocity, PCAv; posterior cerebral artery velocity, CVC; cerebrovascular conductance, MAP; mean arterial pressure, $PETCO_2$; end-tidal carbon dioxide CBV-R; cerebrovascular reactivity. **Bold numbers** represent significant main effect ($P<0.05$).

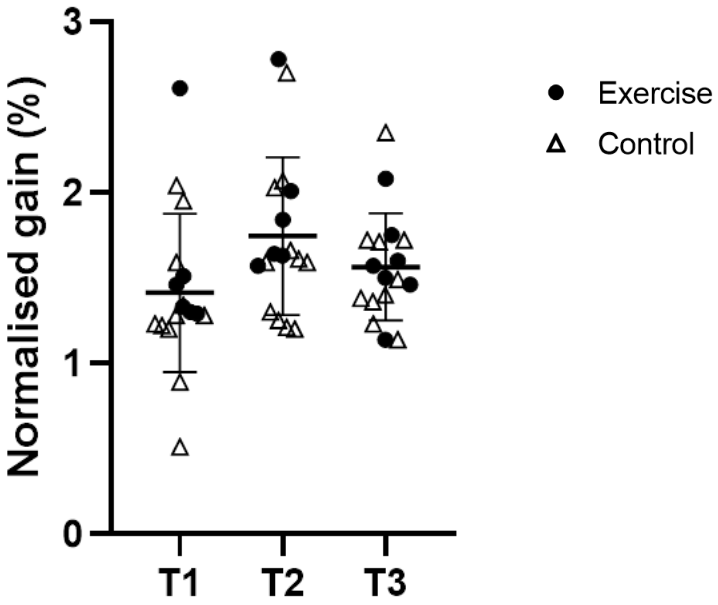


Figure 4.6. Normalised gain during pregnancy. Normalised gain increased in T2 and reduced in T3, although T3 remains higher than baseline (main effect of time; p=0.08).

Table 4.4 Autoregulation data for participants in the exercise and control group at the end of trimester 1, 2 and 3.

Exercise group				**Control group**			***Two way Anova***		
Trimester	**T1**	**T2**	**T3**	**T1**	**T2**	**T3**	***Time***	***Intervention***	***Time*Intervention***
MAP	99±11	98±10	98±9	101±8	98±10	98±9	0.67	0.81	0.96
$PETCO_2$	32.0±2.8	34.7±1.5	34.6±1.4	33.8±2.0	35.0±1.1	35.8±0.8	0.85	0.68	0.66
Gain ($cm.s^{-1}/mmHg^{-1}$)	1.2±0.3	1.3±0.3	1.0±0.1	0.9±0.3	1.1±0.3	1.0±0.3	0.13	**0.04**	0.34
Phase (degrees)	21.1±10.6	39.6±8.4	34.7±9.1	32.9±13.2	32.8±12.9	47.1±29.4	0.06	0.26	0.10
Normalised gain (%)	1.6±0.5	1.9±0.5	1.6±0.3	1.3±0.4	1.8±0.5	1.6±0.4	0.08	0.22	0.71
Coherence	0.66±0.07	0.58±0.16	0.62±0.10	0.54±0.14	0.63±0.09	0.61±0.09	-	-	-

Values are mean ± SD. Abbreviations: T1; trimester 1, T2; trimester 2, T3; trimester 3. **Bold numbers** represent significant main effect ($P<0.05$).

4.3.3 Peripheral Vascular Function

4.3.3.1 Brachial and Femoral FMD

Brachial artery diameter increased during pregnancy (0.03cm [0.01, 0.06 cm]; main effect of time p=0.03) (Figure 4.7). There was no intervention (p=0.24) or time*intervention effect for brachial artery diameter (p=0.69). There was no time, intervention or time*intervention effect for bFMD, scaled bFMD, TTP or SR_{AUC}. Femoral artery FMD decreased during pregnancy (3.5% [-6.5, -0.5%]; main effect of time p=0.03) (Figure 4.8). Allometric scaling to account for baseline artery diameter did not change this result (main effect of time p=0.02). There was no intervention or time*intervention effect for fFMD or scaled fFMD (p>0.05). There was no time, intervention or time*intervention effect for femoral artery diameter, TTP or SR_{AUC} (p>0.05, Table 4.5).

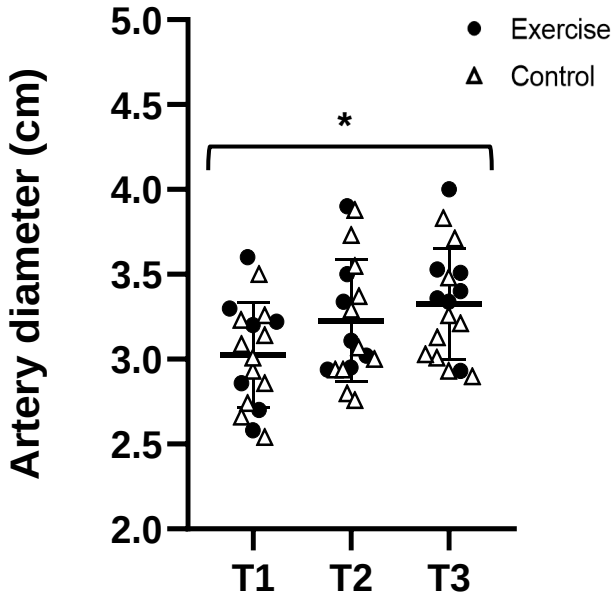


Figure 4.7 Baseline brachial artery diameter during pregnancy. Brachial diameter increased during pregnancy. *main effect of time; p<0.05.

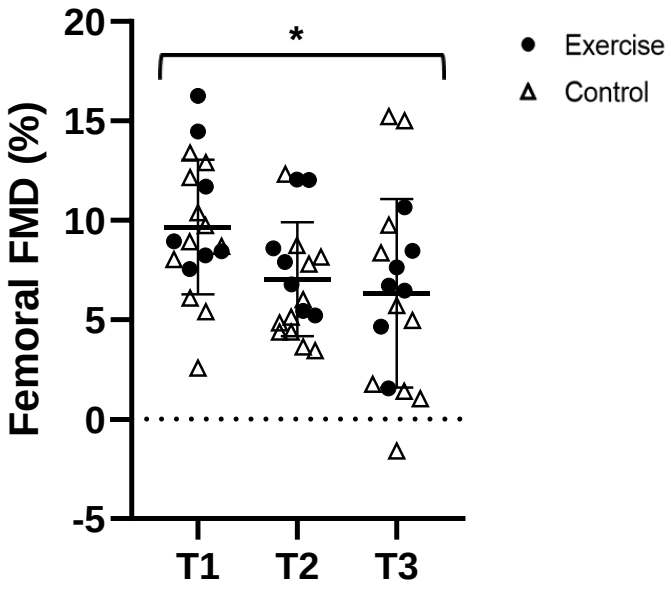


Figure 4.8 Femoral artery FMD during pregnancy. fFMD decreased progressively during pregnancy. *main effect of time; $p<0.05$.

Table 4.5 Brachial and femoral artery function for exercise and control group at the end of trimester 1, 2 and 3.

	Exercise group			Control group			*Two way Anova*		
Trimester	**T1**	**T2**	**T3**	**T1**	**T2**	**T3**	***Time***	***Intervention***	***Time*Intervention***
Brachial Artery									
Artery diameter (cm)	0.31±0.04	0.32±0.03	0.35±0.04	0.30±0.03	0.32±0.04	0.32±0.03	**0.03**	0.24	0.69
Peak artery diameter (cm)	0.33±0.04	0.35±0.03	0.39±0.04	0.32±0.03	0.35±0.04	0.35±0.04	**0.02**	0.16	0.29
Time to Peak (secs)	44±11	37±13	63±21	66±27	49±23	49±10	0.18	0.39	0.10
SR_{AUC} ($x10^3$)	17.2±10.0	15.8±8.2	23.0±9.3	20.9±6.8	19.3±10.9	22.9±6.0	0.17	0.33	0.74
FMD (%)	7.1±3.4	7.6±4.0	10.8±5.0	8.1±2.9	7.6±2.3	6.8±3.8	0.60	0.30	0.16
Adjusted FMD (%)	7.1±2.1	7.5±2.2	10.8±2.4	7.9±1.9	7.6±1.8	6.6±2.0	0.62	0.27	0.14
Femoral Artery									
Artery diameter (cm)	0.52±0.04	0.54±0.05	0.54±0.05	0.51±0.04	0.54±0.05	0.54±0.05	0.19	0.91	0.92
Peak artery diameter (cm)	0.58±0.04	0.59±0.05	0.57±0.05	0.56±0.04	0.57±0.04	0.57±0.06	0.72	0.51	0.83
Time to Peak (secs)	64±19	68±44	105±41	65±34	55±26	62±27	0.15	0.06	0.17
SR_{AUC} ($x10^3$)	18.0±5.0	13.7±3.8	15.1±5.3	23.3±12.8	16.2±7.2	16.5±4.9	0.15	0.15	0.75
FMD (%)	10.8±3.4	8.3±2.9	6.6±2.4	8.9±3.3	6.3±2.7	6.2±5.9	**0.03**	0.19	0.84
Adjusted FMD (%)	11.2±1.4	7.6±1.5	6.5±1.4	7.4±1.8	6.1±1.8	7.8±1.8	**0.02**	0.18	0.88

Values are mean ± SD. Abbreviations: SR_{AUC}; shear rate area under the curve, FMD; flow mediated dilation. †$p<0.05$ for condition.

4.3.4 Carotid and Peripheral Vascular Structure

4.3.4.1 Intima Media Thickness

There was no time, intervention or time*intervention effect for cIMT (p>0.05, Table 4.6). Carotid artery diameter increased during pregnancy by (0.33mm [0.07, 0.58 mm]; main effect of time p=0.04). There was no interaction or time*intervention effect for carotid diameter (p>0.05). Carotid IMT:lumen decreased during pregnancy (0.005 [-0.01, 0.001]; main effect of time p=0.046). There was no intervention or time*intervention effect for cIMT:lumen (p>0.05). There was no time, intervention or time intervention effect for fIMT, femoral diameter or fIMT:lumen (p>0.05).

4.3.5 Pulse Wave Velocity

There was a trend for a reduced PWV during pregnancy (5.01 m/s [2.8, 7.2 cm/s]; main effect of time p=0.09, Table 4.6). However, PWV was significantly greater in the EX group (5.3 m/s [1.6, 8.9 m/s] compared to the CONT group (4.7m/s [3.0, 6.5 m/s]; main effect of intervention p=0.04) (Figure 4.9). There was no time*intervention effect for PWV (p=0.68).

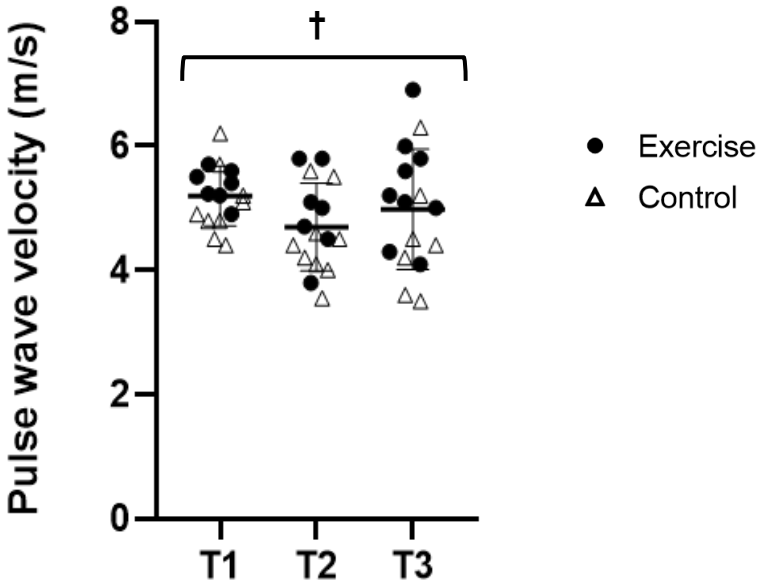


Figure 4.9 Pulse wave velocity during pregnancy. PWV reduced in T2 and increased to near baseline in T3 (†main effect of intervention, p=0.04).

Table 4.6 Carotid, femoral artery structure and pulse wave velocity for exercise and control group at the end of trimester 1, 2 and 3.

	Exercise group			**Control group**			***Two way Anova***		
Trimester	**T1**	**T2**	**T3**	**T1**	**T2**	**T3**	***Time***	***Intervention***	***Time*Intervention***
Pulse wave velocity (m/s)	5.36±0.27	4.96±0.71	5.51±0.88	5.07±0.57	4.49±0.67	4.67±1.29	0.09	0.04	0.68
Carotid Artery									
Lumen artery diameter (mm)	6.90±0.43	7.08±0.56	7.18±0.33	6.78±0.32	7.10±0.49	7.14±0.39	**0.04**	0.72	0.90
IMT (mm)	0.47±0.06	0.49±0.06	0.47±0.05	0.51±0.04	0.49±0.04	0.49±0.04	0.54	0.73	0.29
IMT/Lumen	0.07±0.01	0.07±0.01	0.06±0.00	0.08±0.01	0.07±0.01	0.07±0.01	**0.046**	0.19	0.21
Femoral Artery									
Lumen artery diameter (mm)	5.40±0.18	5.51±0.79	5.72±0.53	5.35±0.51	5.69±0.37	5.78±0.59	0.10	0.69	0.80
IMT (mm)	0.40±0.06	0.41±0.04	0.41±0.09	0.37±0.07	0.39±0.07	0.40±0.05	0.64	0.41	0.97
IMT/Lumen	0.07±0.01	0.08±0.01	0.07±0.02	0.07±0.01	0.07±0.01	0.07±0.01	0.96	0.55	0.84

Values are mean ± SD. Abbreviations: IMT; intima media thickness. *p<0.05 for time.

4.4 Discussion

The primary aim of this study was to define the normal systemic vascular adaptation to an uncomplicated pregnancy. The data confirms that MCAv declines during pregnancy, conduit arteries are enlarged and cerebral autoregulation is altered. Secondly, we aimed to determine the impact of a 6-month pregnancy exercise intervention on maternal vascular adaptation. We show evidence of a gestational increase in diameter of the carotid and brachial arteries alongside a reduction in femoral artery function. The uptake of exercise during pregnancy in line with the RCOG guidelines did not impact peripheral or cerebral vascular structure or function.

In this study, MCAv declined during pregnancy in line with normative data (Belfort et al., 2001). The reason for a reduction in MCAv has been attributed to reductions in SBP (Williams and Wilson, 1994) and downstream vasodilation of resistance vessels to help maintain a stable hemodynamic state (Zeeman et al., 2003, Belfort et al., 2001). Although we did not observe any changes in SBP, we did observe a significant increase in carotid artery diameter. As an extracranial vessel, an increase in carotid artery diameter would result in reduced vascular resistance and blood flow velocity and may therefore be responsible for reduced MCAv (Baltgaile, 2012).

This is the first human study to measure dynamic cerebral autoregulation longitudinally during pregnancy. In this study, there was a trend towards increased normalised gain and phase during pregnancy. Normalised gain represents the dampening effect of cerebral autoregulation on the magnitude of BP oscillations. Gain rises with increasing BP frequencies and when increased, is indicative of diminished dynamic cerebral autoregulation efficiency (Brackley et al., 1998). In the context of our findings, this suggests that there is a trend towards increased BP oscillations in pregnancy that

results in a greater magnitude between BP and MCAv, such that cerebral autoregulation efficiency is reduced. The second cerebral autoregulation parameter, phase, describes the synchronicity of oscillations of BP and MCAv, and a greater phase indicates more efficient cerebral autoregulation whereby MCAv and BP waveforms are in sync with one another (Brackley et al., 1998). In the context of our findings this implies that despite a greater magnitude between MCAv and BP, MCAv response time is improved to maintain synchronised wave forms and ensure intact cerebral autoregulation during pregnancy. In the non-pregnant state, the normal boundaries of cerebral autoregulation lie between pressures of ~60-150mmHg (Cipolla et al., 2011). It has been speculated that pregnancy results in an extension of the upper and lower limits of cerebral autoregulation observed in an animal model during pregnancy (Chapman et al., 2013). The authors suggested this may occur to prepare and protect the maternal brain against possible acute and drastic fluctuations in blood pressure (Cipolla et al., 2011). Importantly, pregnancy is a critical time for blood pressure regulation that can be perturbed by the onset of gestational disease such as pre-eclampsia. It is therefore plausible that the observed increase in phase and normalised gain in this study may also reflect a protective mechanism, however more research is warranted to confirm this.

The brachial and femoral arteries exhibited heterogenous responses to pregnancy. In line with previous findings, brachial artery diameter increased (Sierra-Laguado et al., 2006) while no change to femoral artery diameter was observed. To the author's knowledge, this is the first study to investigate femoral artery adaptation to a healthy uncomplicated pregnancy. We did observe a decline in the function of the femoral but not the brachial artery. This may be explained by the effect of increased body mass on the vascular beds. Previous authors have reported the femoral artery to be strongly correlated with BMI in comparison to the brachial artery (Thijssen et al., 2011b). Given that all women increased BMI, this may have compounded the fFMD response.

Nonetheless, this finding highlights the complexity of the vascular tree illustrating the importance of taking a systemic view to further understand the vascular adaptation to pregnancy.

Moderate intensity aerobic exercise during pregnancy comprising of 45-60 minutes duration performed four times per week has improved aerobic fitness during pregnancy (Bayliss et al., 1895).The EX group in this study demonstrated no improvement in estimated VO_2, however the reduction in estimated VO_2 was greater in the CONT group (-4.38±1.08ml.kg) compared to the EX group (-1.7±1.30ml.kg). This implies that the current RCOG pregnancy exercise guidelines (Bell and Dooley, 2006) were sufficient to maintain, but not improve aerobic fitness. These findings may be clinically relevant given that an increase of 1 MET in maximal aerobic capacity; the equivalent of 3.5ml.kg.min, results in a 13% risk reduction of all-cause mortality (Kodama et al., 2009). Although this may not be critical during pregnancy, this is important for long-term health given that over the reproductive life course, women may experience multiple pregnancies. A failure to regain any pregnancy induced losses in aerobic capacity between pregnancies may have a significant impact on long-term cardiorespiratory fitness and therefore morbidity and mortality. This reaffirms the importance of promoting exercise during pregnancy to prevent a decline in fitness and to protect maternal health during subsequent pregnancies and in the long-term.

Limitations

There are a number of limitations that are noteworthy. Firstly, this was a small sample pilot study with intervention allocation based on participant choice, likely influenced by motivation to exercise. Secondly, despite adhering and complying with the intervention, no increase in PA levels were observed except for daily step count. This may be a result of the inability of the hip worn accelerometer to detect non-ambulatory exercise including cycling or swimming which were preferred activities towards the end

of pregnancy. Furthermore, despite a rigorous screening process targeting previously inactive women and ensuring women were doing <2 structured exercise sessions per week, participants had active lifestyles with many women in both groups accumulating 150 minutes of MPA in T1. This combined with the low dose of exercise prescribed by the RCOG may explain the lack of exercise effects on most measures. Recently updated pregnancy exercise guidelines recommend 150-minutes of moderate intensity PA each week. Importantly, this should include a variety of aerobic and resistance exercise to benefit health (Mottola et al., 2018). In light of this, our study is limited by the inclusion of aerobic exercise only with resistance exercise possibly yielding differential results however, this warrants further investigation. Alternatively, normal changes to the vasculature during pregnancy may be so profound, they may have masked any small effects the exercise intervention may have had.

Implications

Pregnancy is underpinned by extensive systemic vascular adaptation to accommodate foetal growth. The observed alteration in parameters of cerebral health in response to uncomplicated pregnancy, provides new insight into the gestational adaptation of cerebrovascular function, closely monitoring changes to these parameters may be important in order to determine those at increased risk of cerebral events during pregnancy. Previously inactive healthy women should be made aware that beginning exercise in pregnancy that gradually increases in duration until delivery is safe and does not interrupt the necessary CV adaptation that arises. The benefit of doing this includes a preservation of cardiorespiratory fitness; a mediator of CV health and should be promoted in clinical and community settings.

4.5 Conclusion

To conclude, cerebral blood flow velocity declines during pregnancy, enlarged artery diameters and altered cerebral autoregulation. Furthermore, pregnancy results in an increase in systemic conduit artery size evident at the carotid, brachial and femoral arteries. These vascular changes were not impacted upon by adhering to RCOG pregnancy exercise guidelines.

Chapter 5: Effect of Aerobic Exercise during pregnancy on Offspring Vascular Structure and Maternal Quality of Life.

5.1 Introduction

In Chapter 4 we investigated the effect of prenatal exercise on cerebral and peripheral vascular function. We have shown that exercise training does not significantly alter the peripheral or cerebrovascular adaptation that arises in pregnancy. There is also emerging evidence within the literature that prenatal health is associated with childhood or adulthood obesity, diabetes and hypertension, thus connecting the intrauterine environment to the onset and development of CVD (Gluckman et al., 2008). Unfavourable intrauterine environments including maternal undernutrition, early, and late-onset intrauterine growth restriction have been linked with abdominal aortic IMT (Skilton et al., 2005, Skilton et al., 2006, Jackson, 2005). Carotid artery IMT provide prognostic information on future CVD risk (Celermajer et al., 1992, O'Leary et al., 1999) and neonatal IMT may in fact be influenced by an unfavourable uterine environment, which an inactive pregnancy could contribute too.

The positive effects of maternal exercise have been shown to program the intrauterine environment and have been linked to reduced body fat percentage (Greenland et al., 2000), lower heart rate and increased heart rate variability (May et al., 2014, May et al., 2010). Animal studies have also shown gestational exercise to enhance endothelial cell function at the femoral artery in immature offspring (Newcomer et al., 2012), and reduce vascular smooth muscle function in adult offspring (Kanaley et al., 2001, Blaize et al., 2015). However, to our knowledge no study has investigated the effect of gestational exercise on offspring vascular outcomes in humans and thus will be investigated for the first time in this study.

Maternal exercise can also benefit mothers in the post-partum (PP) period and is recognised as a means of reducing the odds and severity of PP depression (Davenport et al., 2018), low back pain, and reduces recovery time following delivery

(Jenkins, 2018). To the authors knowledge, no research has evaluated QOL in previously inactive women following compliance to RCOG exercise guidelines (Bell and Dooley, 2006). Taken together, exercise during pregnancy can program foetal health and may generate positive vascular changes to the offspring, while also positively influence maternal QOL PP. Therefore, the aim of this study was to investigate the impact of maternal aerobic exercise in healthy pregnant women on birth outcomes and offspring carotid IMT. The secondary aim was to report the effect of maternal exercise on post-partum quality of life (QOL).

5.2 Material and methods

5.2.1 Participants

Twenty-one singleton pregnant participants were recruited at local pre-natal clinics. During the study, 3 women withdrew due to time (EX, n=7; CONT, n=11). Eighteen had a vascular scan performed and 18 mothers completed the QOL. Women were eligible to participate if they were in the first trimester of pregnancy of a singleton pregnancy, non-smokers for at least 6 months, had no history of CVD, gestational diabetes or pre-eclampsia, were not on any form of medication, participated in structured exercise less than twice/week and had a BMI <35kg/m^2. All participants provided written consent before taking part in the experimental procedure. The research study was ethically approved by the National Health Service Liverpool Central Research Ethics Committee and adhered to the Declaration of Helsinki.

5.2.2 Experimental Procedure

Following consent, all participants attended the research laboratory in the Research Institute of Sport and Exercise Science at Liverpool John Moores University at the end of T1 where baseline characteristics were obtained. Participants chose to take part in a partially supervised exercise intervention during T2 and T3 or to be a control. Within

12-weeks of birth, offspring artery structure was measured and PP QOL was assessed via questionnaire. Clinical notes were used to inform of offspring characteristics and delivery information.

5.2.3 Exercise Intervention

Participants in the exercise intervention were previously inactive having participated in no structured exercise for at least 3 months. The exercise intervention was completed as per the information in Chapter 4, Section 4.3.2.

5.2.4 Maternal Anthropometry

Maternal stature and weight were recorded to the nearest 0.1cm using a stadiometer and digital scales respectively at the end of T1. BMI was calculated as weight in kilograms divided by stature in metres squared (kg/m^2).

5.2.5 Offspring Characteristics

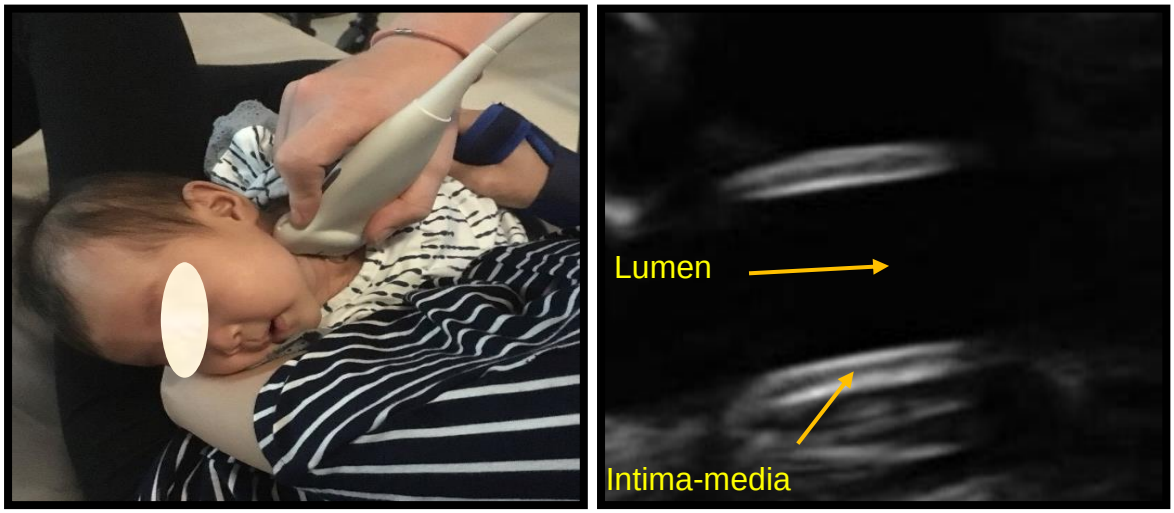
Offspring characteristics including sex, gestational age at birth, birth weight and delivery method including vaginal, C-section, ventouse and forceps were collected from midwives using patient notes.

5.2.6 Offspring Carotid Intima-Media Thickness

Offspring carotid IMT was measured within 12 weeks of delivery. The common carotid artery was imaged using high-resolution B-mode ultrasound (Terason u-smart 3300, Teratech, Burlington, MA, USA) 5 mm proximal to the artery bulb (Polak et al., 2011). Offspring were held by a parent where the neck was slightly extended and where possible, facing the contralateral side to allow for optimal longitudinal imaging of the far-wall intima media interface at one angle only due to the small neck space available

to scan. Images were recorded for 30-40 seconds and optimised to ensure clear contrast between the artery walls and lumen with a distinct IMT visualised on the far wall defined as the distance between two echogenic lines represented by the lumen-intima interface and media adventitia interface of the artery wall. All offspring underwent a carotid artery scan, however, due to quality issues, 1 scan (EX group) was omitted from the analysis.

From the cIMT recording, the best 6-8 seconds of clear IMT and artery walls was selected for analysis using IMT v3.0 edge detection software. An optimal region of interest, including both vessel walls with a minimum length of 1cm, was selected by the sonographer and the software produced a frame-by-frame edge detection output of the near and far wall and far-wall media-adventitia interface. The distance from the line of the far wall (lumen-intima interface) and line of the media adventitia interface was defined as IMT. Each frame was checked by the same sonographer, to ensure the diameter and IMT had been registered correctly, and any tracking mistakes made by the software were deleted. Continuous calculations by the software gave an average of the IMT and diameter recorded over the selected 6-8 second period. IMT-to-lumen ration was calculated by dividing the IMT result by the lumen diameter. Importantly, this method is reliable and operator-independent, demonstrating high levels of precision and accuracy for estimating conduit artery diameter and wall thickness (Thijssen et al., 2011c).



A ***B***

Figure 5.1 Carotid ultrasound scan being performed on a baby at 6 weeks old ***(A)*** and recorded ultrasound image of carotid artery with the lumen and intima-media identified ***(B)***.

5.2.7 Maternal Quality of Life

Post-partum mothers completed the World Health Organization Quality of Life Questionnaire (WHOQOL-BREF) (WHO, 1998) within 12 weeks of delivery, at the same visit to which offspring IMT was measured. The WHOQOL-BREF is a generic, self-report questionnaire that contains 26 items, and each item represents one facet. The facets are defined as those aspects of life considered to have contributed to a person's QOL. Among the 26 items, 24 of them make up 4 domains; physical health (seven items); psychological health (six items); social relationships (three items); and environment (eight items); with two remaining constructs that measure overall QOL and general health (Skevington et al., 2004). The questionnaire was analysed by each domain and questions 3, 4 and 26 were reversed according to guidelines for the WHOQOL-BREF (WHO, 1998). Each domain score was calculated by taking the mean of all items within one domain and multiplying by a factor of four as per administrative

guidelines. Missing values were replaced by the appropriate mean score for the domain to which the item belonged as recommended by the WHO guidelines. The WHOQOL-BREF has been validated elsewhere for use in the post-partum period (Webster et al., 2010). One participant (EX group) was removed for the analysis due to having an injury which was unrelated to the exercise intervention.

5.2.8 Statistical analyses

All data were analyzed using statistical software (SPSS Version 24.0, IBM Corporation, Somers, NY, USA), with significance accepted as $p<0.05$. An independent samples t-test was used to identify any differences between EX and CONT group baseline characteristics, offspring IMT and postpartum QOL. Data are presented in the text as mean (95% confidence interval) unless otherwise stated, with exact *p* values.

5.3 Results

Overall, 7 women completed the exercise training while 11 opted to be in the control group. Overall compliance to the exercise intervention was 78%, (T2; 89% and T3, 67.6%) and all participants adhered to the exercise.

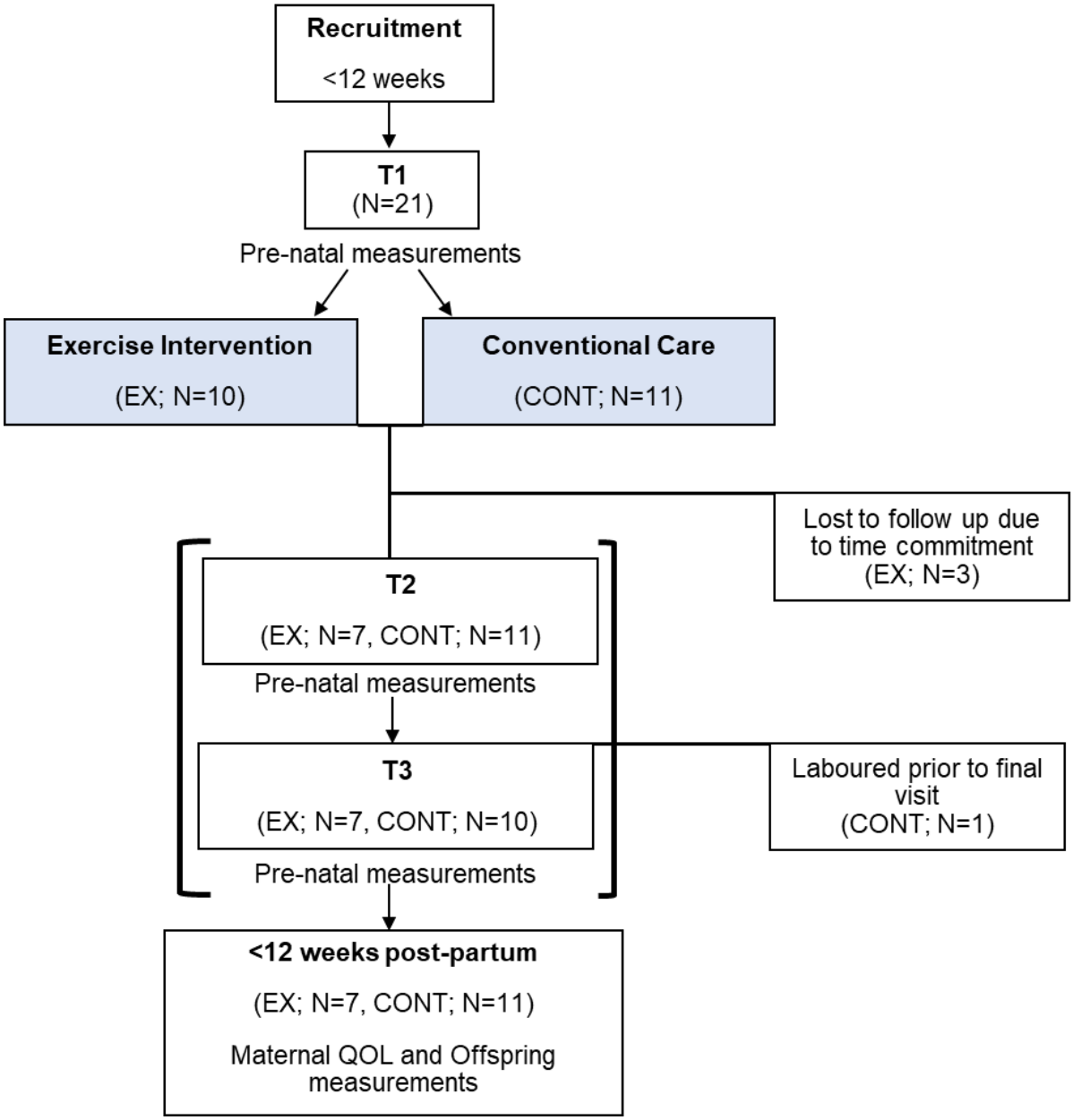


Figure 5.2 Schematic of the experimental procedure used. Participants were recruited within 12 weeks of pregnancy for vascular assessments. Participants chose to join an exercise intervention or continue with conventional care and made two subsequent visits at the end of T2 and T3 for vascular measurements. Within 12 weeks post-partum, maternal QOL and offspring vascular measurements were obtained.

5.3.1 Maternal Characteristics

T1 participant characteristics for EX and CONT groups are presented in Table 5.1. Upon enrolment to the study (i.e. T1), there were no differences between groups for maternal age, body mass, BMI, SBP, or DBP ($p>0.05$, Table 5.1).

Table 5.1 Maternal participant characteristics on enrolment to the study.

Characteristic	Exercise group (N=7)	Control group (N=11)	p-value
Age (yrs)	33±4	33±3	0.48
Body mass (kg)	62.0±8.4	66.6±7.7	0.87
BMI (kg/m^2)	23±3	24±3	0.76
SBP (mmHg)	99±6	103±12	0.31
DBP (mmHg)	60±8	60±6	0.87

Values are mean ± SD. Abbreviations: BMI; body mass index, SBP; systolic blood pressure, DBP; diastolic blood pressure.

5.3.2 Birth Outcomes

Offspring characteristics of EX and CONT mothers are presented in Table 5.2. Gestational age born ($p=0.72$) and birth weight ($p=0.50$) were comparable in the EX and CONT group. No birth defects, or adverse events were reported during delivery. In the EX group, 3 babies were born by normal delivery, 2 by C-section (elective), 1 by ventouse and 1 by rotational forceps delivery. In the CONT group, 6 were born by normal delivery, 4 by C-section (3 elective, 1 emergency), and 1 induced to normal delivery. This equated to 26% C-section in the EX group and 36% in the CONT group.

5.3.3 Offspring Intima Media Thickness

There were no differences between groups for the time point at which offspring IMT was measured (EX, 8±3 wks; CONT, 6±2 wks; $p=0.24$). Offspring of exercising mothers had a smaller, although non-significant cIMT (0.38, 0.55 mm; $p=0.27$) and

cIMT/Lumen (0.13, 0.18; p=0.19) compared with the control group (0.44, 0.54 mm; 0.15, 0.20 respectively). Lumen diameter was greater although non-significant in the exercise group (2.8, 3.0 mm; p=0.82) compared to the control group (2.6, 3.2mm; Figure 5.2).

Table 5.2 Offspring characteristics at birth and carotid artery measurements post-partum.

	Exercise group (N=7)	**Control group (N=11)**	**p-value**
Male gender	3	6	-
Gestational age at birth (wks)	39.7±1.1	39.3±1.4	0.72
Weight (kg)	3.5±0.5	3.5±0.4	0.50
Week of IMT measurement PP	8±3	6±2	0.24
IMT (mm)	0.45±0.06	0.49±0.07	0.27
Lumen (mm)	2.92±0.13	2.88±0.40	0.81
IMT/Lumen	0.15±0.02	0.17±0.04	0.19

Values are mean ± SD. IMT; intima media thickness.

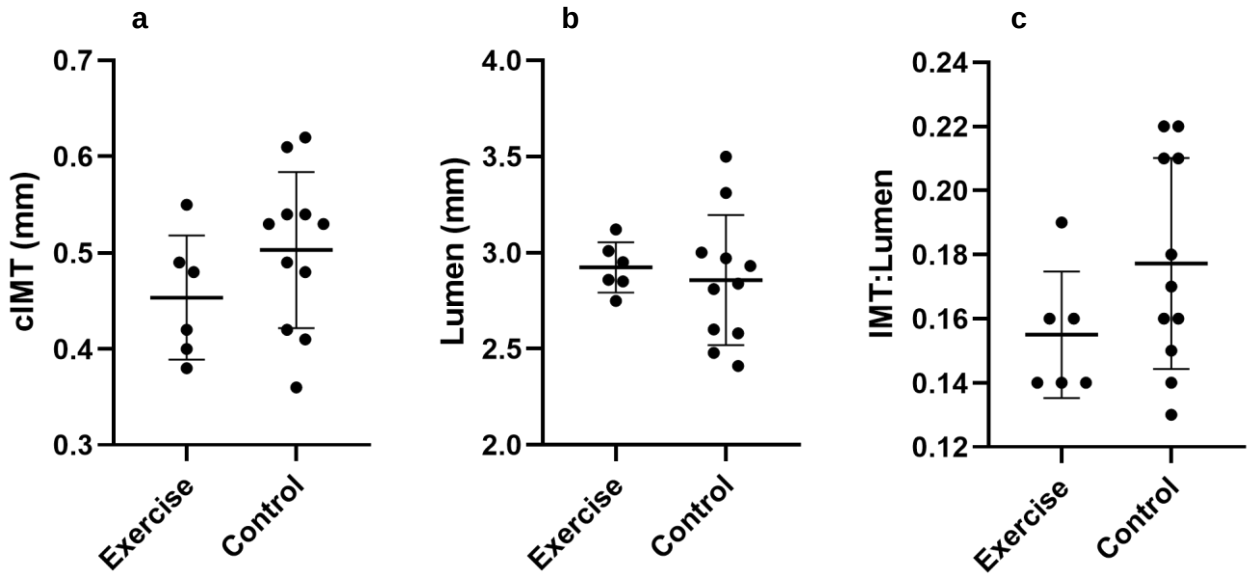


Figure 5.3 Offspring vascular outcomes data. Offspring cIMT (a) and IMT-to-lumen (c) are lower in offspring of exercising mothers compared to the control group, while the lumen diameter is larger (b) in exercisers compared to the control group.

5.3.4 Maternal QOL

WHOQOL-BREF are presented in Table 5.3. There were no significant differences between groups for the physical, environmental, psychological, social domain, overall QOL or general health score ($p>0.05$).

Table 8 Maternal quality of life recorded in the post-partum.

QOL domains	Exercise group (N=6)	Control group (N=11)	p-value
Overall QOL	5±1	5±0	0.51
Overall health	4±1	5±1	0.14
Physical	76±13	76±8	0.92
Psychological	72±6	76±5	0.19
Social	75±11	71±17	0.63
Environmental	82±9	86±8	0.28

QOL; quality of life

5.4 Discussion

The aim of this study was to investigate, the impact of gestational exercise in previously inactive pregnant women on offspring birth weight, and for the first time offspring vascular health. Secondly, we aimed to report the effect of gestational exercise on and maternal PP QOL. The results of this study indicate no significant effect of a moderate intensity aerobic exercise intervention on offspring cIMT, birth weight or PP QOL.

The offspring in the EX group had a lower cIMT and a larger lumen diameter compared to the CONT group, however whilst these differences did not reach statistical significance, the data suggests a need for further investigation in a larger cohort. We acknowledge a small sample size in this pilot study, which is likely to have impacted our ability to reach statistical significance. To the author's knowledge, previous research has investigated vascular function and not structure in animal offspring of exercising mothers and has demonstrated an ability to program offspring artery function. Porcine maternal aerobic exercise training, comprising of treadmill running 5-days a week at 60-85% maximum heart rate, improves offspring thoracic aorta endothelial cell function measured in vitro at 48hrs after birth (Newcomer et al., 2012). Importantly the current study has multiple differences in terms of study design, methods and exercise intervention that may partly explain the differential findings.

Firstly, Newcomer et al. (2012) used an invasive measurement of endothelial cell function compared to the non-invasive structural assessment used in this study. Vascular function responds rapidly to exercise and precedes structural changes (Binder et al., 1996, Green, 2009a, Tinken et al., 2008), implying that perhaps vascular function may be more indicative of foetal vascular adaptation to gestational exercise. Secondly, the measurements in this study were taken within 12 weeks of delivery compared to <48 hrs (Newcomer et al., 2012). In adults, vascular adaptation to

exercise adapts rapidly to physical inactivity (Euser and Cipolla, 2007). Specifically, 8-weeks of bed rest has previously shown a 20% increase in carotid artery wall thickness and increased IMT-to-lumen ratio. It is possible that any exercise adaptation that may have taken place in the offspring had been reduced during the time frame to when the measurement was conducted (12 weeks PP). To demonstrate further, a follow up study at 3, 5 and 9 months in exercised swine exposed to the same exercise stimulus as in Newcomer et al. (2012), observed no difference in femoral artery cell functioning between offspring of exercised and sedentary swine; in fact the authors observed a reduction in vascular smooth muscle functioning (Kanaley et al., 2001). The authors reported that the differential expression of genes and proteins associated with vascular tone observed between the cohorts might explain the differing functional phenotyping and not via the effect of maternal exercise on NO production in utero. Taken together, is it possible that maternal exercise induces long-term vascular programming in adult offspring, which is not evident at the endothelium.

There is some evidence that offspring vascular responses to exercise differ depending on the duration and intensity of the exercise (Newcomer et al., 2012, Blaize et al., 2015). In contrast to the enhanced endothelial function observed following a high dose of exercise investigated by Newcomer et al. (2012), voluntary maternal exercise in rodents has shown no effect on offspring endothelial cell function at 4- and 8-months of age (Blaize et al., 2015). It is plausible that the exercise dose in this study was not enough to have a positive impact on cIMT. In Chapter 4, this exercise dose was also insufficient to enhance maternal vascular function. Although previously inactive women are encouraged to become progressively active during pregnancy, the upper limit to warrant CV benefits has not yet been established (Khatun et al., 2013). The evidence presented here implies that the exercise stimulus may need to be greater to program CV benefits from mother to offspring. Further research is required to explore maternal exercise frequency and intensity to develop a better understanding of the

vascular benefits to offspring exposed to maternal exercise.

A birth weight of between 2500g and 4000g is considered normal, with deviation beyond these boundaries associated with increased risk of CVD and T2DM in adult life (Nyberg et al., 2014). The findings of this study illustrate no effect of maternal exercise on offspring birth weight in agreement with previous authors (Kramer and McDonald, 2006, Sturek et al., 2007). That said, prospective cohort studies have demonstrated previously active women, who maintain a high volume of exercise into the third trimester, tend to deliver infants weighing 200–400 g less than non-exercise controls (Greenland et al., 2000, Natoli et al., 2005). The discrepancy between studies has been explained by Greenland et al. (2000), who suggested that the prescription of exercise (type, duration, frequency, and intensity) and the stage of pregnancy at which it occurs, have varying effects on weight and body composition at birth. For instance, a low volume of exercise in mid and late pregnancy appears to stimulate foetal growth, regardless of exercise performance in early pregnancy (Clapp et al., 2000, Greenland et al., 2000), while a high volume of weight-bearing exercise in later gestation restricts foetal fat deposition (Greenland et al., 2000). In this study, a reduction in exercise compliance in T3 was observed compared to T2. Moreover, women only engaged in aerobic exercise which was not always weight bearing exercise (cycling and swimming), which may explain the lack of difference in offspring weight between the EX and CONT group. It also noteworthy that the women recruited to this study were of normal weight and gained weight within normal limits during pregnancy. It appears that birth weight is not related to exercise and may be more valuable in the future to monitor dietary intake surplus to an exercise intervention during pregnancy (Hunter et al., 1996).

There were no apparent differences between groups for overall and domains of QOL assessed in the PP period. QOL questionnaires used in the PP period are often

specific to the research topic such as urinary incontinence, sleep, oral health, psychometric health and breastfeeding as reviewed by (Taddei et al., 2000). To avoid specificity, the questionnaire used in this study is a valid, generic tool that has been used to asses QOL during pregnancy (Smith et al., 1997), 4 weeks (Evans et al., 2017) and 6 weeks PP (Webster et al., 2010) with no specific recall time frame suggested. The rationale for administering the questionnaire in this study within 12 weeks following birth was for logistical reasons such that it was completed during the visit where offspring IMT data was collected. Due to the time at which this visit took place to accommodate the mothers, it is possible that any exercise effect on PP QOL that may have taken place, had been overlooked during the time frame to when the data was collected. It is plausible that these women had a high perception of their QOL on entering the study based on their voluntary contribution to participating.

Limitations

This study is limited by a small sample size, which may reduce the ability to determine differences between groups and reach statistical significance. The data will allow us to calculate an appropriate sample size for a larger study. Interpretation of the findings might be limited by the time lapse between birth and when the IMT measurement was taken. PP measurements were taken within 12 weeks of delivery and given the rapid vascular responses to training and detraining described earlier, this time-frame may have prevented detection of any adaptive response to maternal exercise, although the time course for vascular adaptation in neonates is currently unknown. Although we aimed to recruit inactive women, the data presented in Chapter 4 outlines these women to have an active lifestyle. The tool used to assess QOL is generic and may not have been the most sensitive tool for the detection of changes to QOL following maternal exercise participation. While there were alternatives to assessing QOL in the PP, many tools are specific to various dimensions of health in the PP period as mentioned above. There is a gap in the

literature for the development of a specific questionnaire relevant to activity participation in pregnancy and further studies to be conducted with larger sample sizes, differential maternal exercise frequency, duration and intensity.

5.5 Conclusion

In summary, engaging in moderate intensity aerobic exercise from the first trimester of pregnancy until birth appears to non-significantly influence cIMT and artery lumen size in offspring of exercising mothers, however does not affect birth weight or maternal quality of life.

Chapter 6: Synthesis of Findings

6.1 Aims and Objectives

The research presented within this thesis aimed to investigate cerebrovascular function, and peripheral vascular function and structural adaptations during menopause and pregnancy, with simultaneous assessment of cardiorespiratory fitness and PA. We also aimed to investigate the effect of moderate intensity exercise during pregnancy on these cerebral, peripheral vascular parameters. Lastly, we aimed to identify the effect of maternal exercise on offspring vascular structure, delivery outcomes and maternal quality of life PP.

6.2 Major findings

Chapter 3 demonstrated that menopausal women have lower MCAv, central and peripheral vascular function and higher artery wall thickness in comparison to PRE-M women; all of which occur alongside reduced cardiorespiratory fitness and vigorous PA. Interestingly, not all decrements occurred within the first five years of menopause with Early-POST-M women presenting with higher carotid artery IMT and lower femoral FMD compared with Late-PRE-M women. Taken together, this suggests that while some parameters of vascular function and structure are impacted by the onset of menopause, the duration of time spent in menopause alongside increased age, reductions in cardiorespiratory fitness and vigorous PA may have a greater influence on the systemic vasculature than oestrogen reduction *per se.*

Chapter 4 demonstrated a decline in MCAv during pregnancy and reduced vascular resistance distal to the cerebrovasculature. Pregnancy resulted in an increase in conduit artery size evident at the carotid, brachial and femoral arteries and a reduction in fFMD. These pregnancy related changes were not impacted by moderate aerobic exercise recommend by the RCOG.

Chapter 5 was a pilot trial which investigated the effect of the gestational exercise intervention described in Chapter 4, on offspring carotid artery vascular structure. This is the first study to examine the impact of gestational exercise on intrauterine vascular programming in humans. Although non-significant, we reported a smaller cIMT, cIMT:lumen and a larger carotid diameter in offspring of exercising mothers compared to controls. This study suggests that gestational exercise programs the vascular profile of progeny, however a full trial is warranted with larger sample sizes and varying doses of exercise to confirm this.

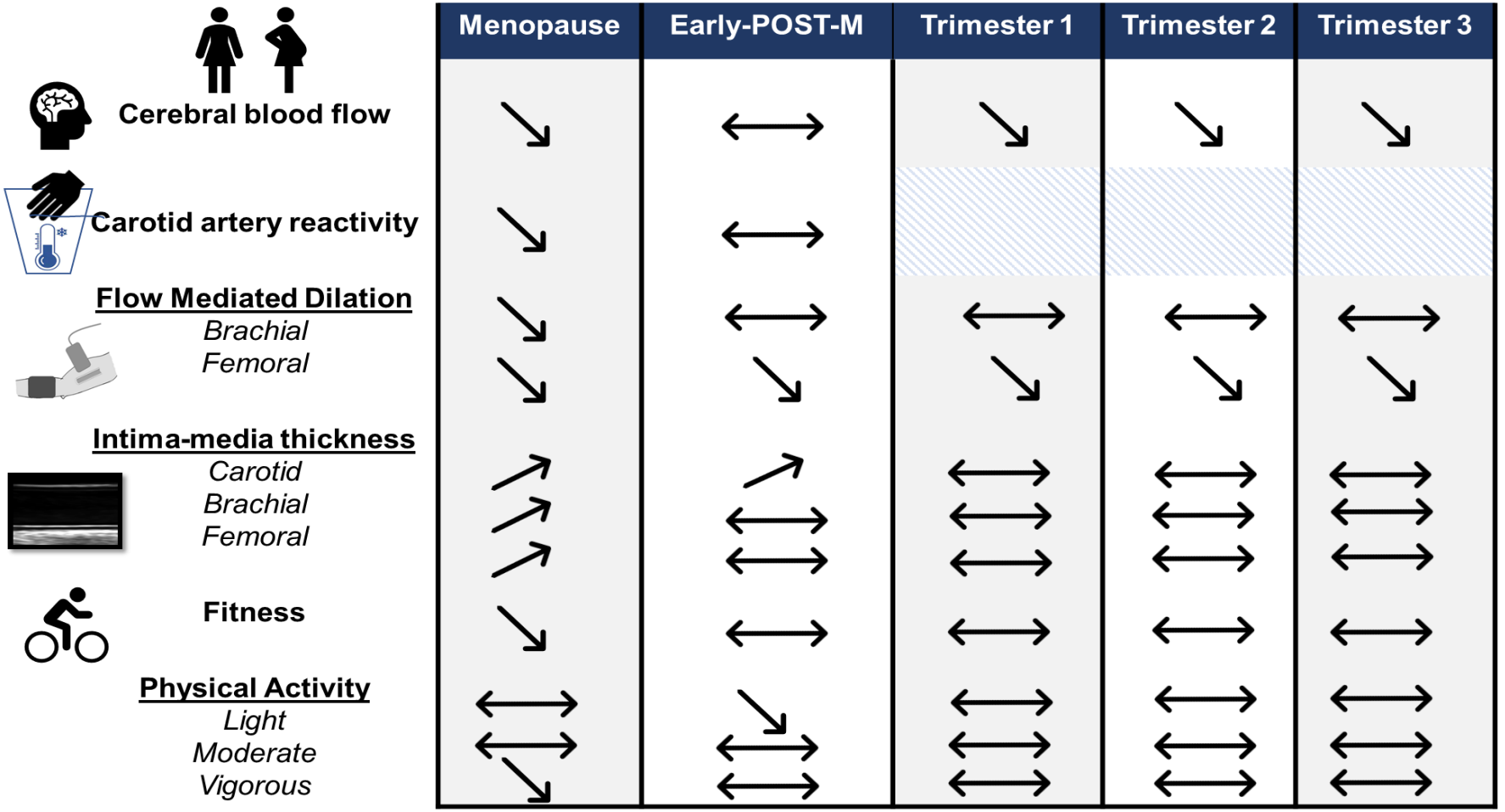


Figure 6.2 Illustration of observed differences for physiological and modifiable lifestyle factors in menopausal (compared to pre-menopausal women) and early-post-menopausal (compared with late-pre-menopausal women). The effect of each trimester of pregnancy on the same parameters are illustrated in columns *Trimester 1*, *Trimester 2* and *Trimester 3*.

6.3 General Discussion

The section that follows will discuss the major findings in this thesis under the themes of the **i)** cerebrovascular system, **ii)** peripheral and conduit vascular system, **iii)** role of maternal exercise on offspring vascular structure and **v)** confounders to CVD risk.

6.3.1 Cerebrovascular system

The incidence of cerebrovascular disease including Alzheimer's, dementia (Beam et al, 2018) and stroke (Reeves et al, 2008) is greater in women compared to men. This is hypothesised to relate to women's longer life expectancy (Townsend et al, 2015; Mazure and Swendsen, 2016) and to differences in sex hormones (Reeves et al, 2008). Accordingly, menopause-related oestrogen reduction has been associated with reduced cognition, increased risk of stroke and Alzheimer's disease (Wong et al., 2016, Davey, 2017, Evans et al., 2017), while pregnancy is also associated with increased risk of stroke compared to the non-pregnant state (Cipolla et al., 2011, Sloan and Stern, 2003). Furthermore, pregnancies complicated by pre-eclampsia have a greater risk of cerebrovascular disease in later life (Leslie and Briggs, 2016). Despite these associations, there is a paucity of evidence characterising the normal cerebrovascular adaptation that arises during menopause and pregnancy.

In Chapter 3, MCAv was lower in POST-M compared with PRE-M women to POST-M and was accompanied by a simultaneous reduction in cardiorespiratory fitness and vigorous PA. These reductions were not apparent in early menopause, implying that duration of menopause, increased age (Ainslie et al., 2008) and reductions in cardiorespiratory fitness and vigorous PA may influence MCAv decline to a greater extent than or in conjunction with oestrogen reduction *per se*. Elevations in cardiorespiratory fitness have elsewhere shown to improve basal cerebral blood flow

velocity in healthy ageing (Ainslie et al., 2008) and in particular, during menopause (Bailey et al., 2016). It is understood that PA influences cerebral blood flow velocity via enhanced endothelium-dependent vasodilation (Taddei et al., 2000) and NO bioavailability (Ainslie et al., 2007). Despite reductions in MCAv, the PA level achieved by the women in this study (PRE-M, 55±2min/d; POST-M, 49±24 min/d) may have been sufficient to maintain parameters of cerebrovascular function (CBV-R and cerebral autoregulation. The reduction in vigorous PA may be in part responsible for reduced cardiorespiratory fitness and cerebral blood flow velocity. The extent to which vigorous PA and improvements in cardiorespiratory fitness could be used as a strategy to prevent MCAv decline in menopausal women is unknown but worthy of further investigation.

During pregnancy, MCAv declined in line with previous findings (Serra-Serra et al., 1997, Ikeda and Mori, 1990, Williams and Wilson, 1994). This decline has been attributed to both reduced SBP (Williams and Wilson, 1994); although this did not change in our study, and distal vasodilation of resistance vessels to accommodate increased blood volume (Belfort et al., 2001). It is plausible that reduced MCAv is related to an increased carotid diameter and reduced vascular resistance which in turn reduces blood flow velocity (Baltgaile, 2012, Kerber and Heilman, 1992). Furthermore, the structural adaptation of the carotid artery may have also downregulated any possible MCAv and/or PCAv response to the CBV-R and NVC tests.

Another possible explanation for reduced MCAv may be due to altered cerebral autoregulation. In this study, there was a trend towards increased parameters of cerebral autoregulation including normalised gain and phase which have been explained in Chapter 4 and supported by previous research (van Beek et al., 2008, Kodama et al., 2009). Our findings imply that despite increased BP oscillations (increased normalised gain), MCAv response time is improved to maintain

synchronised wave forms (increased phase) and ensure intact cerebral autoregulation during pregnancy. Interestingly, it has been speculated that the upper and lower limits of cerebral autoregulation that typically lie between pressures of ~60-150mmHg in non-pregnant adults (Cipolla et al., 2011) are extended during pregnancy according to an animal model (Chapman et al., 2013). This extension is thought to arise to protect the maternal brain against possible acute and drastic fluctuations in blood pressure that arise during pregnancy and labour (Cipolla et al., 2011). It is plausible that the observed increase in phase and normalised gain in this study reflect a protective mechanism of the cerebrovasculature, however more research is warranted to confirm this. Taken together, the evidence presented in this thesis demonstrates a reduction in MCAv after menopause, although this is may be related to increased age, time from menopause and a decline in vigorous PA and cardiorespiratory fitness rather than oestrogen reduction *per se*. MCAv is also reduced in pregnancy; likely a result of reduced vascular resistance and altered cerebral autoregulation in an effort to maintain homeostasis of the cerebrovascular system.

6.3.2 The peripheral and conduit vascular system

Menopause. A key observation from Chapter 3 was the heterogenous response of peripheral and conduit arteries to menopause. In general, peripheral vascular endothelial function, artery wall thickness and stiffness were impaired from PRE- to POST-M however, these decrements were only apparent for fFMD and cIMT within the first five years of menopause. This suggests that the duration of time in menopause combined with increased age and possibly, a reduction in vigorous PA and cardiorespiratory fitness may have a greater impact on the peripheral vasculature than the event of menopause alone.

Femoral and brachial FMD declined from PRE- to POST-M, however, only fFMD; which has been investigated here for the first time in menopausal women, was reduced in early menopause. The femoral artery is most susceptible to atherosclerosis (Thijssen et al., 2008) and appears to be exacerbated by the early phase of menopause. Secondly small increases in femoral artery diameter were observed in Early-POST-M women which is associated with reduced shear stress (Green, 2009b); an important physiological stimulus for the release of NO (Davies, 1995). It is plausible that this structural adaptation and reduced shear may have further compounded the production of NO which, combined with reduced oestrogen could explain the decline in fFMD in early menopause. Importantly, Early-POST-M women engaged in significantly less light PA compared to Late-PRE-M women. An acute bout of inactivity has elsewhere shown to reduce femoral but not brachial FMD (Thosar et al., 2014). Considering this, a significant reduction in light PA over time may also contribute to reduced fFMD in early menopause. Taken together, the femoral artery appears to be affected in early menopause potentially due to the loss of oestrogen which knowingly compounds endothelial dysfunction further to negligible increases in artery diameter and reduced light PA.

Menopause results in significant carotid artery remodelling (Muscelli et al., 2009) which was evident in early menopause, and appears to occur more rapidly than brachial and femoral arteries. The development of IMT has shown to be comparable between central and peripheral artery beds (van den Munckhof et al., 2012), however our data illustrates a time-dependent development of IMT between arteries. The reason for higher cIMT in early menopause above other arteries is unclear, however it may be related to the elastic nature of the carotid artery (Binder et al., 1996). Oestrogen increases elastin/collagen ratio and attenuates collagen deposition in aortic smooth muscle cells in vitro (Chironi et al., 2003, Natoli et al., 2005). Oestrogen reduction may therefore have an opposing effect on the connective tissue component of the artery

wall leading to increased stiffness and altered structure (Westendorp et al., 1999). In contrast, the brachial and femoral arteries are muscular in nature. Muscular arteries have thicker medial layers and contain more smooth muscle cells compared with elastic arteries (Sturek et al., 2007). Oestrogen inhibits smooth muscle cell hypertrophy through oestrogen receptor-dependent and -independent mechanisms (Dubey et al., 2001, Dubey et al., 2000). Therefore, the heterogenous development of IMT is likely specific to the properties of the artery wall that result in different oestrogen receptor expression, or different local metabolism of oestradiol (Dubey et al., 2001). In addition, habitual exercise may exert heterogeneous influences on the vessel wall of different arterial segments depending on variations in local metabolic requirements, hydrostatic pressures, and blood flow patterns, resulting in different mechanical and shear forces applied against the vessel wall (Greenland et al., 2000) which may explain the slower progression of IMT in the periphery compared to the carotid artery.

Moreover, maintaining vigorous PA in early menopause may have influenced several putative factors known to modulate smooth muscle cells in the arterial wall including sympathetic-adrenergic activity, circulating ANG II and endothelin-1, and locally released vasoactive factors such as NO (Kingwell, 2000, Espeland et al., 1995, Majmudar et al., 2000). Interestingly, when all POST-M were included in analysis, vigorous PA was significantly reduced compare to PRE-M. Alongside this, brachial and femoral IMT were increased proposing a plausible role for vigorous PA in reducing the magnitude of IMT development. Taken together, the heterogenous progression of IMT across multiple arteries may be explained by differential artery wall properties and the influence of PA in attenuating peripheral IMT development.

Pregnancy. Femoral but not brachial FMD was reduced in pregnancy highlighting the susceptibility of this vascular bed to dysfunction. These data may imply that the femoral artery is more sensitive to perturbed levels of oestrogen compared to the brachial

artery; however this remains speculative. Alternatively, pregnancy reduced fFMD may be attributed to increased body mass, which is more closely related to the femoral compared to the brachial artery (Thijssen et al., 2011b). Given that all women in Chapter 4 increased weight and BMI, this may have compounded the fFMD response. It has been proposed that, the stimulus of pregnancy alone results in arterial vasodilation which may result in maximum dilation in the first trimester of pregnancy (Skow et al., 2017). This may explain the lack of improvement in brachial FMD observed in this study given the timing of our first measurement was at the end of T1. Whilst there appears to be heterogeneity of artery function between vascular beds in the response to pregnancy, the implications and prognostic relevance of these differences are yet to be determined.

Pregnancy appears to be characterised by increased artery diameter, evident at the carotid, brachial and to a lesser extent at the femoral. These structural adaptations have been observed elsewhere (Sierra-Laguado et al., 2006) and are thought to play a role in reducing vascular resistance. To the author's knowledge, this is the first study to investigate femoral artery adaptation to a healthy and uncomplicated pregnancy. The reason for this non-significant change in femoral diameter to pregnancy is unclear however, may be a protective response. As discussed in Chapter 4, the femoral artery has the lowest mean shear rate and the highest oscillatory shear index, both of which are atherogenic (Wu et al., 2004). Given artery dilation would reduce shear stress further, (Sandoo et al., 2010, MacAllister and Vallance, 1996), it is plausible that the negligible increase in femoral diameter protects against a further decline in shear and therefore artery function. Furthermore, the femoral artery is also the largest and perhaps has a limited capacity to increase.

In summary, menopause results in reduced vascular function and increased wall thickness at each artery, although not all changes are apparent in early menopause.

Pregnancy is accompanied by increased peripheral and central artery diameters and reduced femoral artery function. The peripheral and central vascular system clearly undergoes profound upheaval during the female lifespan in response to events of menopause and pregnancy. For menopausal women, maintaining cardiorespiratory fitness and vigorous PA may prevent the vascular decline while participating in relatively low levels of MPA during pregnancy is ineffective for improving vascular function.

6.3.3 Role of maternal exercise on offspring vascular structure

In Chapter 5, we observed for the first time in humans, that offspring of exercising mothers had a lower, although non-significant, IMT and a larger lumen diameter compared to the control group. Newcomer et al. (2012) has reported improved endothelial function among porcine offspring following an intense exercise intervention comprising of treadmill running 5-days a week at 60-85% maximum heart rate. Interestingly, the same exercise intervention (Newcomer et al., 2012) demonstrated no difference in endothelial function between offspring of trained and sedentary mothers at 3, 5, and 9 months. On the contrary, vascular smooth muscle function was significantly reduced in offspring of exercise-trained pigs (Kanaley et al., 2001). This data provides novel insight that NO signalling was not programmed by maternal exercise; rather alterations to the molecular mechanisms dephosphorylating myosin light chain in vascular smooth muscle appears to be programmed by gestational exercise in- utero. Elsewhere, voluntary rodent maternal exercise has shown no effect on rodent off-spring endothelial cell function at 4- and 8-months of age (Blaize et al., 2015). The discrepancies between findings may be dependent on the time scale at which measurements were obtained, or indeed the exercise intensity imposed, as discussed in Chapter 5, which may also explain the lack of findings in our study. It must

be acknowledged that this is a pilot study and the ability to detect significant differences is limited by our small sample size. The data collected in this thesis will however allow sample size calculations to be performed in order to adequately power a full study. Nonetheless, taken with findings from Chapter 4, maternal exercise as recommended by the RCOG does not appear to infer any effect on maternal vascular function and/or structure, but may have a potential influence on offspring vascular structure.

6.3.4 Confounders to CVD

Throughout this thesis, fitness, PA and sedentary behavior were quantified due to the potential influence these variables have on parameters of CVD risk (Black et al., 2009, Colcombe et al., 2003). We observed a decline in fitness across the female lifespan, despite the majority of women in Chapter 3 (85%) achieving recommended MPA guidelines. This suggests the guidelines may be insufficient to prevent a decline in aerobic capacity, as has been previously suggested (Akbartabartoori et al., 2008). Interestingly, in the sub-analysis, we observed a reduction in light activity in the Early-POST-M women compared to Late-PRE-M. It is unclear if this reduction is related to menopause and warrants further investigation. Nonetheless, vigorous PA has shown to benefit CV risk in post-menopausal women by reducing body weight, BP, cholesterol levels alongside improvements in body composition and plasma insulin in response to glucose (Mandrup et al., 2017). Furthermore, vigorous PA in addition to MPA, is associated with a greater reduction in CV risk compared to MPA alone in older women (Manson et al., 1999). This strengthens the importance of performing vigorous PA for CV risk and may be more important than MPA with increased age, although this needs to be investigated. Certainly, improving levels of vigorous PA may be an important consideration for preserving aerobic capacity with older age and in augmenting the decline in MCAv, vascular function and increased wall thickness.

In Chapter 4, 6-months of moderate intensity exercise participation failed to improve estimated VO_{2max}. However, we have shown a greater reduction in estimated VO_{2max} in

the CONT group (-4.38±1.08ml.kg.bw) compared to the EX group (-1.7±1.30ml.kg.bw) which is clinically relevant in line with previous research whereby a reduction in aerobic capacity of 3.5ml.kg is associated with reduced longevity and increased morbidity (Kodama et al., 2009, Winett and Carpinelli, 2000). It is plausible that the exercise sessions in our study were enough to maintain, but not improve aerobic fitness during pregnancy. Moderate intensity aerobic exercise during pregnancy comprising of 45-60 minutes, performed four times/week, has shown to improve aerobic fitness (Bayliss et al., 1895). In light of this, adhering to a lower dose of exercise as per current RCOG pregnancy exercise guidelines (Bell and Dooley, 2006) may prevent a reduction in aerobic capacity which could potentially benefit long term health. Pregnancy with or without exercise, did not result in any significant change to PA time or intensity, although, the exercise group did engage in significantly more daily steps. In summary, PA is an important confounder for CVD risk and according to this thesis, altered levels of PA correspond to decrements in vascular function and structure. Based on this, PA promotion in women may take precedence around the time of menopause and pregnancy given the increased vascular vulnerability around these times.

6.4 Implications

Women are at a greater risk of cerebrovascular disease compared to men due to a longer life expectancy and possibly due to differences in sex hormones. PA should be viewed as an important mediator of cerebrovascular health and promoted by health practitioners, particularly for ageing women to protect against cerebrovascular health decline. It is important that women remain physically active for as long as possible and importantly, engage in vigorous PA in an effort to maintain cardiorespiratory fitness which may contribute to the integrity of the cerebrovascular system and prevent CV morbidity and mortality. Pregnancy is also associated with an increased risk of cerebrovascular disease compared to the non-pregnant state. The data presented in

this thesis demonstrates an adaptation of the cerebrovascular system to pregnancy, without a loss in function regardless of adhering to the RCOG guidelines or not. Importantly, this also implies that a higher dose of exercise is likely required to benefit cerebrovascular function and should be given consideration in future pregnancy exercise guidelines.

In Chapter 3 and Chapter 4, femoral artery function was reduced in early menopause and pregnancy. This implies that these groups of women should focus on lower limb exercise in an effort to increase shear and NO production and maintain or at least attenuate the loss of femoral artery function. Furthermore, menopausal women should engage in vigorous PA to maintain the integrity of peripheral and central vascular function and structure. Importantly, the PA guidelines are currently identical for adults of all ages advising that all achieve 150 minutes of moderate aerobic activity, or 75 minutes of vigorous PA across the week (WHO, 2010). Our data is suggestive that PA guidelines for older adults should potentially focus on maintaining or improving levels of moderate and vigorous PA to benefit the CV risk profile. The findings of this study may also inform future studies, particularly those investigating the impact of menopause and/or ageing on the female vascular system as our evidence highlights the importance of measuring multiple vascular beds to obtain a systemic overview of the vascular system. In addition, it is plausible that pregnant women interested in improving vascular function should engage in either longer duration or an increased frequency of exercise; both of which should be considered in future PA recommendations in pregnancy. Nonetheless, although the exercise did not enhance any vascular outcomes, it did appear to negate the decline in aerobic fitness during pregnancy which may be clinically relevant. This is encouraging for women who want to preserve fitness throughout pregnancy although any additional benefits appear to warrant a greater frequency, duration or exercise intensity. For women who have multiple pregnancies this is important as reductions in aerobic capacity aerobic

capacity is strongly related to morbidity and mortality By at least maintaining fitness, CVD risk is reduced as oppose to subsequent reductions in fitness with multiple pregnancy.

Chapter 5 provided some evidence that gestational exercise programs offspring vascular structure. The findings suggest that women who engage in MPA during pregnancy may program positive effects to their offspring vascular health in utero to potentially benefit long term CV health which highlights the potential value of pregnancy exercise in reducing the burden of CVD at a population level.

6.5 Methodological Considerations and Limitations

There are several strengths in the methodology of this thesis. The studies detailed ensured strict inclusion and exclusion criteria in addition to the control of diet and exercise prior to and during laboratory visits. Chapter 3 is the first study to our knowledge to combine measures of peripheral, conduit and cerebral outcomes in addition to considering modifiable lifestyle risk factors such as fitness, PA and sedentary behavior enabling a holistic understanding of the vascular system and some key correlates in females across the lifespan. Moreover, Chapter 4 is the first study to investigate the effect of exercise on the maternal vascular system while Chapter 5 has utilised a technically and logistically demanding approach to directly quantify the arterial structure of neonates. During laboratory assessments, methodological rigor was adhered to and optimal analysis techniques applied. As FMD assessment was undertaken according to the latest peer-reviewed consensus guidelines (Thijssen et al., 2011a), together with the use of custom-designed edge-detection and wall-tracking analysis software, the accuracy, validity and prognostic value of FMD outcomes were maximised.

Despite these methodological strengths, we must acknowledge a number of limitations within this thesis. Firstly, in Chapter 3, as with all cross-sectional study designs, we cannot make causal inference from the data. Secondly, within this study, the

recruitment of participants predominantly included highly active women limiting our ability to generalise findings to all ageing and menopausal women. Given that PA has a large impact directly on the vasculature as well as on CVD risk factors it is likely that PA levels impacted the measures in this study, and caution should be applied when relating these findings to inactive POST-M women. Similarly, inclusion criteria for the pregnancy study in Chapter 4, specified women participating in structured exercise less than twice a week. Although we were successful in screening all participants, many women were active in work and in their daily life, which again, may limit our ability to generalise findings to pregnancy exercise in previously inactive women.

A small sample size in our pilot study detailed in Chapter 4 and 5, may have limited our ability to detect differences between groups and must be interpreted with caution. Furthermore, in Chapter 4 we expected to see a change to PA levels among those exposed to an exercise intervention. Our inability to do so may be reflective of the hip accelerometer worn to quantify PA, which fails to detect all modes of exercise including cycling and swimming which were preferred modes of exercise towards the end of pregnancy. Also state that control group maintained their PA when typically PA declines in pregnancy.

Findings in Chapter 5 may be limited by the time lapse between birth and obtaining the vascular structural measurement (6.5±2.4 weeks). Additionally, based on the literature suggesting the first evidence of atherosclerosis is present in the abdominal aorta, the carotid artery may not be a true reflection of maternal exercise programming. However, we were unable to obtain sufficient quality images of the abdominal aorta, so used the carotid artery.

6.6 Future direction

Based on the evidence presented in this thesis, we have outlined some key areas to which future research can capitalise on the work presented here.

1. Further examination of the role of vigorous PA and cardiorespiratory fitness in menopausal and older women's cerebral and peripheral vascular risk would help identify changes that might arise with increased age, more years in oestrogen deficiency and altered PA levels.

2. Future research should explore differential exercise intensities and durations on peripheral and cerebrovascular outcomes during pregnancy using a larger sample size to confirm the findings of this thesis.

3. In order to provide further context to the altered cerebral autoregulation identified in Chapter 4 during pregnancy, future studies should aim to quantify cerebral autoregulation longitudinally in complicated and/or high risk pregnancy (i.e. pre-eclampsia) to perhaps enlighten the value of this measure within a clinical population at risk of cerebrovascular disease.

4. Based on the finding in Chapter 5, it is recommended that future research investigate further the impact of different types of exercise (i.e. resistance training) and intensities of maternal exercise in a fully powered study, on offspring vascular structure.

Chapter 7: References

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