UNIVERSITY OF BIRMINGHAM

FEMALE SEX HORMONE FLUCTUATIONS ACROSS THE FEMALE LIFE CYCLE AND THEIR INFLUENCE ON VASCULAR FUNCTION TESTS

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ABSTRACT

Vascular dysfunction in the cerebral and peripheral vasculature has implications for cardiovascular and cerebrovascular diseases. Fluctuations in the levels of female sex hormones across the menstrual cycle, and over the course of the menopause appear to alter several vascular responses. These changes may have implications for vascular related diseases, however the extent of this in the peripheral and cerebral circulations is not fully understood. Therefore, this thesis aims to compare cerebral and peripheral vascular function over the course of the menstrual cycle in pre-menopausal females, in comparison with the responses to post-menopausal females. **Methods:** Six pre-menopausal females at two phases of their menstrual cycle and six post-menopausal females underwent a CO_2 inhalation cerebrovascular reactivity protocol and a flow-mediated dilation protocol to assess vascular function. Comparisons were made between the phases of the menstrual cycle in pre-menopausal females and these results were compared to post-menopausal females. **Results:** CVR_{MCAv} and CVR_{PCAv} values were comparable over the course of the menstrual cycle in pre-menopausal females. FMD% in pre-menopausal females was higher in the ML phase than the EF phase of the menstrual cycle (p = .046). CVR to hypercapnia was higher in pre-menopausal females when compared to post-menopausal females (p = .014) , yet FMD% was only higher in pre-menopausal females when compared to post-menopausal, when the former were in the ML phase of their menstrual cycle (p= .006). **Conclusion:** In pre-menopausal females, CVR responses were similar between the EF and ML phases of their menstrual cycles. As expected, CVR to hypercapnia and FMD measures were higher in pre-menopausal females when compared to post-menopausal females and these were dependent on menstrual cycle phase. More research is required to look into the cardioprotective mechanisms of female sex hormones on different cohorts (including those on HRT) and how these have implications for health and disease.

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ACRONYMS/ABBREVIATIONS

ACA – Anterior Cerebral Artery

ACTZ – Acetazolamide

ATP – Adenosine Triphosphate

BFV – Blood Flow Velocity

BHF – British Heart Foundation

BHI – Breath-hold Index

BMI – Body Mass Index

CA – Cerebral Autoregulation

CBF – Cerebral Blood Flow

CBFV – Cerebral Blood Flow Velocity

CBVD – Cerebrovascular Disease

CO_2 – Carbon Dioxide

CPP – Cerebral Perfusion Pressure

CT – Computed Tomography

CV – Coefficient of Variation

CVC – Cerebrovascular Conductance

CVD – Cardiovascular Disease

CVR – Cerebrovascular Reactivity

ECA – External Carotid Artery

FMD – Flow Mediated Dilation

HGEX – Handgrip-exercise

HPF – Hyperpolarisation Factor

HRT – Hormone Replacement Therapy

ICA – Internal Carotid Artery

ICP – Intracranial Pressure

LDL – Low Density Lipoprotein

MAP – Mean Arterial Pressure

MCA – Middle Cerebral Artery

MRI – Magnetic Resonance Imaging

NO – Nitric Oxide

NVC – Neurovascular Coupling

OC – Oral Contraceptives

$PaCO_2$ – Arterial Carbon Dioxide

PCA – Posterior Cerebral Artery

$PETCO_2$ – End Tidal Carbon Dioxide

PG -Prostaglandins

PI – Pulsatility Index

RI – Resistance Index

SNA – Sympathetic Nerve Activity

TCD – Transcranial Doppler

VE – Minute Ventilation

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1.1. Introduction

Vascular dysfunction in the cerebral and peripheral circulation has implications for various conditions that have become the most prominent causes of death within the last 15 years. Cardiovascular disease (CVD) (including coronary heart disease, cerebrovascular diseases, stroke and peripheral artery disease) is the leading cause of death worldwide and accounted for the deaths of 17.9 million people in 2016 (31% of all deaths worldwide) (WHO, 2017). It is estimated that, by 2030, there will be 23.6 million annual fatalities from CVD (WHO, 2017). In the UK, cardiovascular diseases have become a huge cost for national healthcare, costing the UK economy an estimated £9 billion each year (BHF, 2020). Ageing is a major risk factor for CVD, as vascular function declines with ageing due to vessel structure alterations and the imbalance of vasoactive substances, which can accelerate the development of cardiovascular-related diseases (Nyberg et al., 2015). With the increase in the ageing population in the UK, age-related disease prevention is becoming even more crucial. There is a difference in the rate of cardiovascular decline between the sexes. For example, men (aged <50 years) have a higher incidence of atherosclerotic vascular disease (Barrett-Connors, 1997) and a 33% increase in stroke risk (Appleros et al., 2009), when compared to age-matched females (<50 years old; pre-menopausal). Furthermore, cerebrovascular disease (CBVD) in general is higher in males compared to females (Bain et al., 2005). This sex disparity diminishes in the 6th decade of life, when female risk equals or exceeds that of males (Roger et al., 2012). Female menopause is highly likely to be the key event that changes cardiovascular disease rates between men and females. For example, a number of studies have shown that post-menopausal females have a higher risk of developing CBVD when compared to pre-menopausal females (Pérez-López et al., 2009; Bain et al., 2005; Pellegrino et al., 2001). Furthermore, there is an increase in CVD in females after the age of 50 (Lloyd-Jones et al., 2010) and it is interesting that the average

age of menopause is 50 years old (Gold 2011). In amongst the several physiological changes that occur after menopause, the dramatic reduction in oestrogen levels has been discussed as a main factor that influences the vascular-related disease rates. Prognostic markers of cerebral and peripheral vascular function can be used to provide a link between vascular health and female sex hormone levels. This could be done by comparing post-menopausal females (with or without hormone replacement therapy) to pre-menopausal females over varying levels of females sex hormone levels (across the menstrual cycle), to see the effect on the vasculature.

1.2 Peripheral Vascular Function

Endothelial Function

The peripheral vasculature is responsible for the regulation of blood flow around the body and is essential for oxygen and nutrient transport. It does this by regulating vascular smooth muscle tone as well as inhibiting leukocyte and platelet cell adhesion, amongst others factors (Celermajer et al., 1997). Blood flow in the periphery can be manipulated by changing the resistance of the arteries to meet the metabolic demand. The endothelium is a single-cell thick layer that responds to hormonal and haemodynamic stimuli (Schechter and Gladwin, 2003) and can influence the smooth vascular muscle cells to change their vascular tone and increase or decrease blood flow. The endothelium responds to shear stress stimuli (e.g. reactive hyperaemia) by releasing vasoactive substances. This results in vasodilation of the artery via the smooth vascular muscle cells which increases the vessel diameter (Ando et al., 2009). These vasoactive substances include nitric oxide (NO), prostaglandins (PG) and hyperpolarisation factor (HPF).

Endothelial dysfunction is a key step in the formation of atherosclerosis (Laketta et al., 2003) and is a result of imbalances in vasoactive substances and an increase in inflammation

(Rodriguez-Miguelez et al., 2016). Endothelial dysfunction is a maker of CVD (Deanfield et al., 2007). Flow-Mediated Dilation (FMD) is widely used as a measure of endothelial function, with a decrease in FMD strongly predictive of cardiovascular disease/event risk (Green et al., 2011). Indeed, FMD has been shown to be an independent predictor and marker of CVD, with an 8-13% decrease in CVD risk per 1% increase in FMD% (Matsuzawa et al., 2015). There is also an association between the Framingham risk score and FMD, with a higher FMD% associated with a lower Framingham risk score (a measure of CVD risk; Maruhashi et al., 2013; Figure 1.1). FMD is also lower in individuals with diabetes, hypertension or other cardiovascular diseases (Maruhashi et al., 2013). Thus, it is important for FMD protocols to be standardised and therefore aid CVD diagnostics.

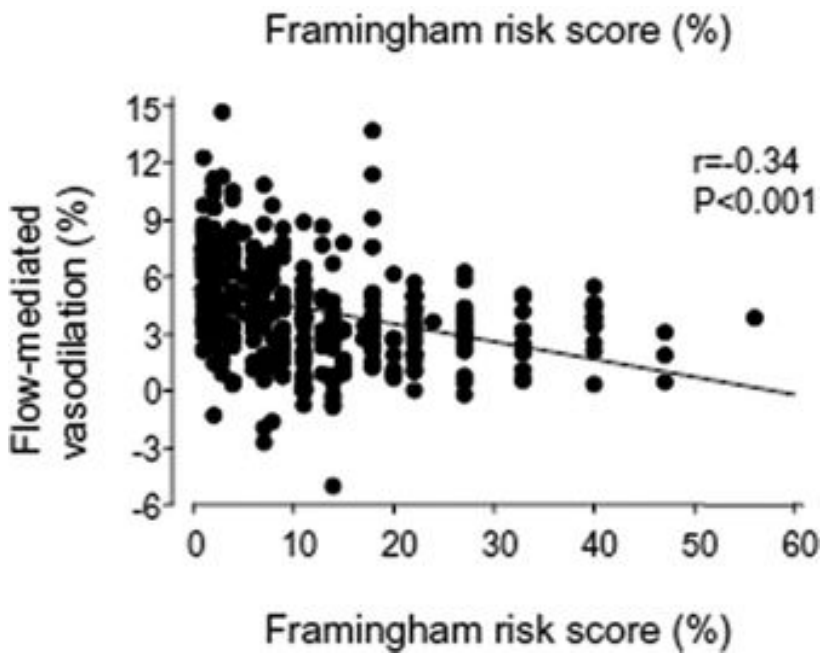


Figure 1.1 graph showing Framingham risk score (%)(used to calculate 10-year cardiovascular risk. A higher score = higher risk of CVD) and FMD. The higher the FMD, the lower the Framingham risk score. (Maruhashi et al., 2013).

Flow-Mediated Dilation

Flow-mediated dilation (FMD) is a method used to quantify endothelial function and measure reactivity of a conduit vessel, commonly the radial, brachial or femoral artery. During FMD, a stimulus (e.g. a sudden increase in blood flow from the release of pneumatic cuff compression) is used to evoke shear stress (reactive hyperaemia) on the artery, resulting in a dose-dependent increase in diameter of the vessel (Pyke et al., 2008; Figure 1.2). This shear stress stimulus causes endothelial cells to release vasoactive substances, which promote vasodilation (NO, PG HPF) (Ando et al., 2009). For example, an increase in shear stress stimulus increases endothelial nitric oxide synthase (eNOS) activity, which in turn converts L-arginine to NO, causing relaxation of the smooth vascular muscle cells and vasodilation (increase in vessel diameter) (Sessa 2004). A meta-analysis comparing studies that measured NO involvement in FMD revealed that FMD is largely mediated by NO (Green et al., 2014). Furthermore, studies that used the common and accepted FMD method (Thijssen et al., 2011)have shown that NO inhibition (using either saline or NO synthase blocker) resulted in a lower or blocked shear stress response in the conduit arteries (Green et al., 2014). Thus, FMD measures NO-dependent endothelial function.

To measure the increase in diameter and velocity caused by FMD's shear stress response, B-mode duplex Doppler ultrasound is used as a non-invasive, high resolution method (Thijssen et al., 2011). User variability is high; therefore, sufficient training is essential. The main stimuli used to evoke a shear-stress response is pneumatic cuff inflation and subsequent deflation. Other stimuli have been used in the literature, such as handgrip-exercise (HGEX) to examine the shear-stress response (D'Urzo et al., 2018). However, HGEX is intensity-dependent and may evoke a different shear stress response through NO-independent mechanisms (Findlay et al., 2013).

The FMD method using cuff inflation also varies throughout the literature, with duration of cuff inflation (Harris et al., 2010) and position of the cuff (Betik et al., 2004) leading to large changes in the FMD response. Commonly accepted guidelines by Thijssen et al., (2019) state that the cuff should be distal to the ultrasound transducer, to reflect a more NO-dependent mechanism (Betik et al., 2004) and that cuff inflation duration should be 5 minutes (for comfort and consistency) (Harris et al., 2010). FMD can be analysed by edge-detection and wall tracking software, that continuously measures diameter and velocity, to calculate FMD% (% change in diameter from baseline to post-deflation) and shear rate. By using this literature-wide accepted method of FMD (Thijssen et al., 2011) endothelial function can be accurately assessed, with a higher FMD% reflecting a greater endothelial function (Celermajer et al., 1994).

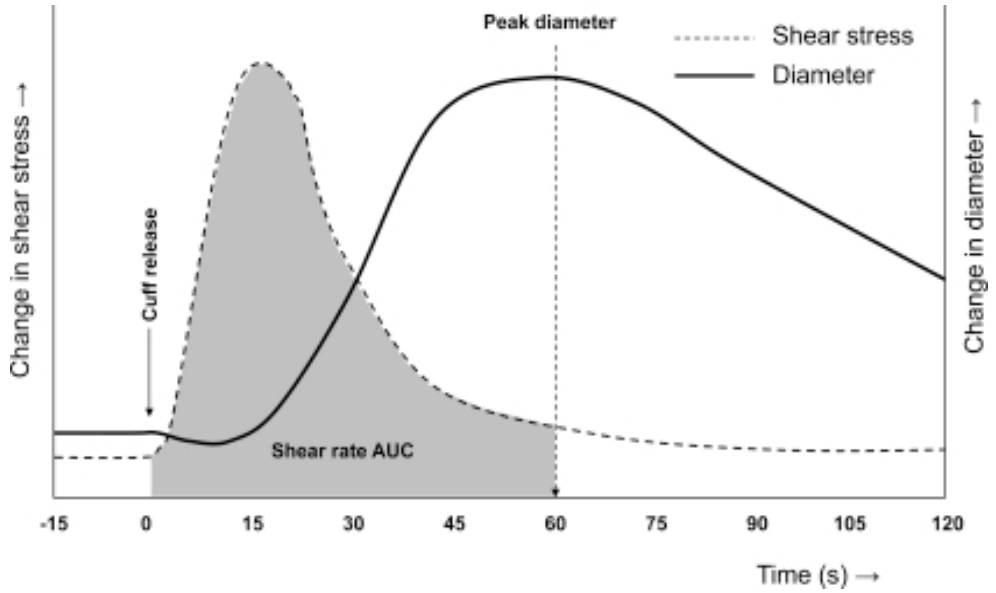


Figure.1.2. The typical response expected with FMD. The cuff is released (following 5 minutes of occlusion) which results in an increase in shear stress. Secondary to the increase in shear stress (measured by shear rate), there is an increase in vessel diameter as a result of vasodilation. The peak diameter is then compared to baseline and a % is given. (Thijssen et al., 2011).

1.3 The Cerebral Circulation

The Circle of Willis is the main blood distribution hub of the brain, feeding into several conduit arteries. In particular, it feeds into the middle cerebral artery (MCA), anterior cerebral artery (ACA) and posterior cerebral artery (PCA), which each supply different regions of the brain and therefore, match different metabolic demands (Figure 2.1). These arteries act as a conduit for blood distribution and exhibit little change in diameter, yet are crucial in blood flow regulation due to their large influence on downstream smaller arterioles (Olufsen et al., 2002). In the anterior circulation, the MCA runs laterally and anteriorly to the internal carotid artery (ICA) and takes up 21% of all cerebral perfusion (Willie et al., 2014). It has a high baseline velocity, is easy to locate with measures such as transcranial Doppler ultrasound (TCD) and is the most common site of pathology within the brain (Navarro-Orozco et al., 2018). This makes it a common site of study within the literature. It is split into four segments that extend from the ICA to the cerebral cortex, with its primary aim to supply this area of the brain with oxygenated blood (Willie et al., 2014). Further within the anterior circulation, the ACA supplies oxygenated blood to the middle of the cerebral cortex. The ACA arises from the ICA and splits into 5 segments (Chandra et al., 2017). The posterior circulation supplies only 30% of the total brain blood flow yet is important for various functions (Chandra et al., 2017). The PCA forms part of the posterior circulation. The PCA starts at the basilar artery and extends to the posterior regions of the brain, including the temporal and parietal lobe (Willie et al., 2014). The PCA (and posterior circulation) may differ in cerebral blood flow (CBF) regulation to the anterior circulation (Zarrinkoob et al., 2016), and therefore both circulations need to be studied to get a wider picture on global CBF regulation.

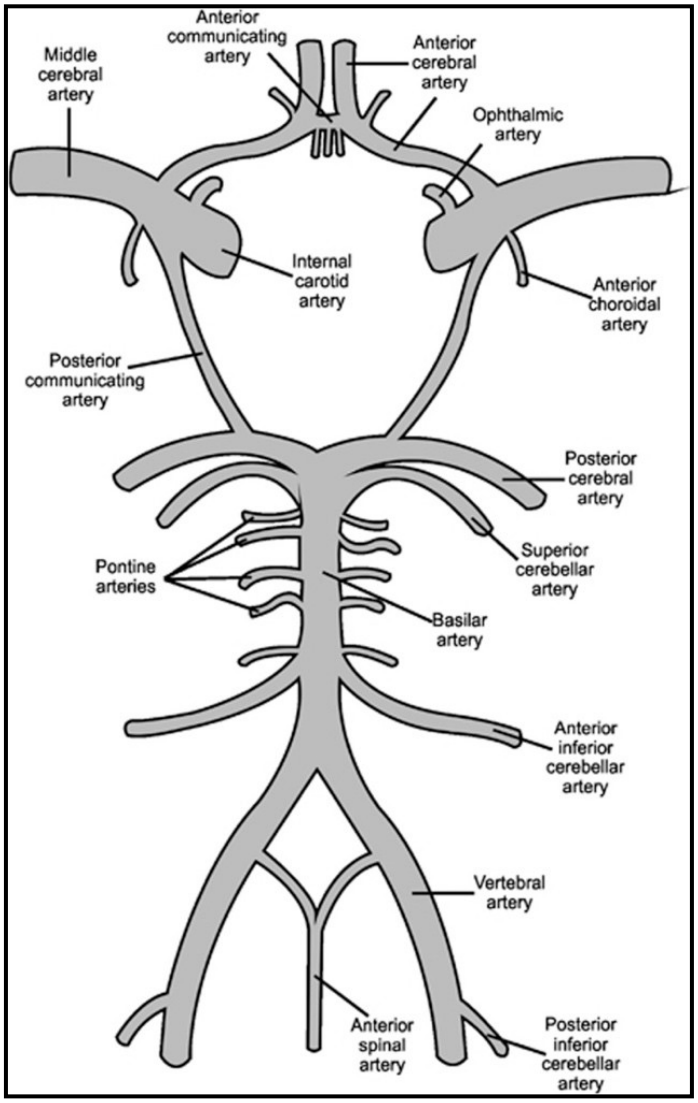


Figure 2.1 The cerebral circulation (the Circle of Willis). (Vrselja et al., 2014)

Cerebral Blood Flow Regulation

Despite only making up 2% of total body weight, the brain takes up 15-20% of all cardiac output (Williams et al., 1989), highlighting the importance of understanding how CBF is controlled and regulated. In normal healthy adults, CBF is regulated to match the metabolic demands of the brain, allowing oxygen, glucose and other metabolites to cross the brain blood barrier and reach the cerebral tissues (Jones-Muhammed and Warrington, 2019). Cerebral perfusion pressure (CPP) (the pressure gradient between the mean arterial pressure (MAP) and

the intracranial pressure (ICP) (Ainslie and Duffin, 2009)) is the driving force that allows metabolites to reach cerebral tissue. Keeping CPP constant is important to avoid hyper-perfusion (seizure) or hypo-perfusion (ischaemia). CBF regulation is also dependent upon the resistance of the blood vessels (Aaslid et al., 1992). This resistance can be localised, allowing areas of the brain to respond differently and flow to be distributed to meet demand (Mandell et al., 2008). The increase or decrease in vessel radius, and subsequent change in resistance, impacts CBF and is thought to occur in the capillary and arteriolar bed (Edvinsson et al., 2002). The relationship between CPP, CBF and resistance is explained by Poiseuille's Law (see equation 1), where resistance in inversely proportional to blood flow and pressure difference.

$$Cerebral\ Blood\ Flow = \frac{\pi \Delta P r^4}{82\lambda}$$

Equation 1: Poiseuille's law (McDonald et al., 1974) ΔP: Pressure difference, r: radius, η: viscosity of the blood, λ: length of the vessel. It explains the relationship between CBF, CPP (ΔP) and cerebral resistance (8ηλ).

In order to regulate CPP and vessel resistance, hence CBF, several factors need to be tightly controlled (Figure 2.2). These factors include cardiac output, blood pressure, sympathetic nerve activity (SNA), arterial gases, and nerve cell activity. The extent to which CBF responds to these different factors is commonly tested in three ways:

1. Cerebrovascular reactivity (CVR) in response to inhalation of different gas concentrations

2. Cerebral Autoregulation (CA) in response to blood pressure (BP) changes

3. Neurovascular Coupling (NVC) responses to metabolic demands

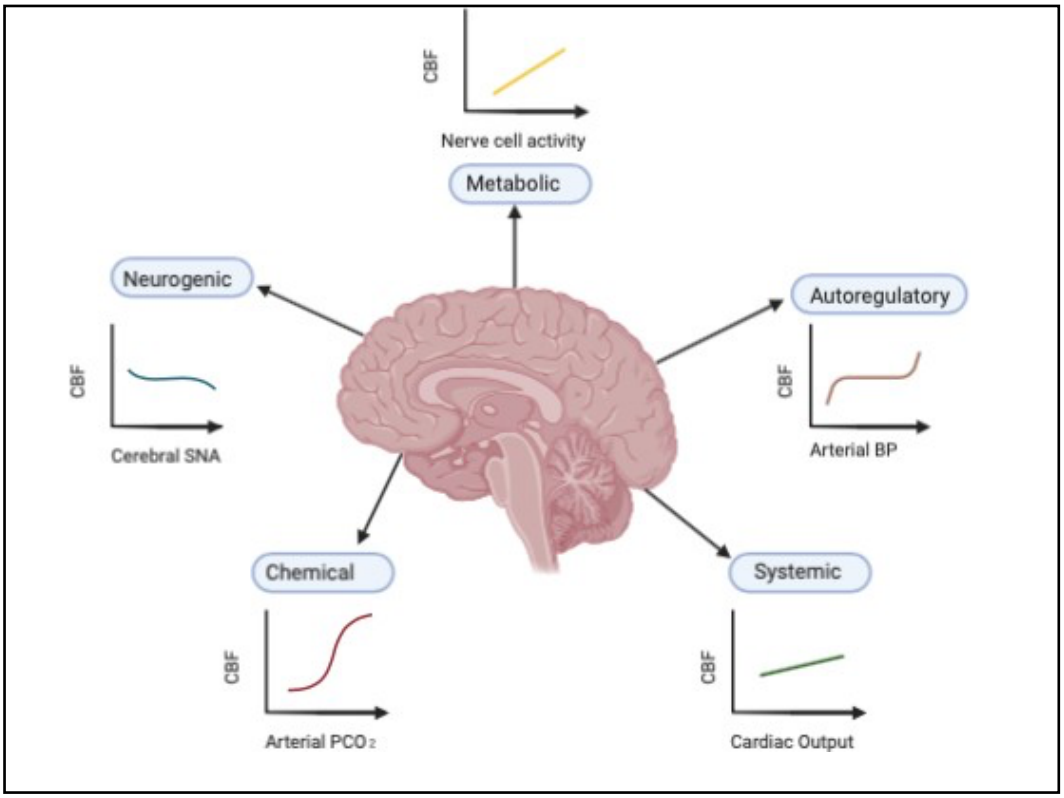


Figure 2.2. A summary of the main factors that affect CBF (Adapted from Smith and Ainslie, 2017).

Changes in the outcome of these three tests can have implications for health and disease. The cerebrovasculature is very susceptible to changes in CBF regulation, making it vulnerable to damage. Impaired CBF regulation can result in reduced cerebral oxygen consumption, glucose delivery and reduced ATP production (Chen et al., 2015). CBF regulation is impaired in individuals with dementia, small cerebral vessel disease, carotid artery stenosis or those at increased risk of stroke (Silvestrini et al., 2000; Zarrinkoob et al., 2015; Ritz et al., 2014). Damage to the cerebrovasculature can limit the ability of the vessels to respond to various physiological factors which help regulate CBF. For example, atherosclerosis is a common

disease that results in cerebral endothelium damage and a reduction in cerebrovascular compliance, which can impair CBF regulation. Measuring CBF can aid in the diagnostics and prognostics of certain diseases and can be used as a marker of overall cerebral health.

Measuring CBF – Transcranial Doppler Ultrasound (TCD)

CBF was originally measured by nitric oxide (N_2O) (Kety and Schmidt, 1944) and Xenon-133 inhalation methods, however, there was a shift towards more non-invasive, dynamic measuring techniques such as Doppler ultrasonography in the 1980s. Doppler ultrasonography uses the Doppler Effect, where the sound waves emitted by an ultrasound transducer are reflected back by red blood cells. The speed the red blood cells (the difference in the frequency of the transducer waves and reflected waves) are moving is proportional to the velocity of the blood moving through the isonated vessel (Aaslid et al., 1986). In peripheral vessels, Duplex Doppler is used, which can measure both blood velocity and diameter (due to spatial resolution) and subsequently, absolute flow can be calculated. In the cerebral arteries, TCD is used (Aaslid 1982) which has high temporal resolution but poor spatial resolution, meaning it is unable to measure absolute blood flow. Therefore, although actual CBF cannot be measured, blood flow velocity (CBFV) of the cerebral vessels is used as a substitute for blood flow. If the diameter of the vessel remains unchanged, CBFV can be assumed to reflect blood flow in that absolute CBF is proportional to velocity. However, this is controversial in the MCA and PCA, as some studies have shown that vessel diameter of these large cerebral arteries remains unchanged while others have shown the diameter does change (Wilson et al., 2011; Skow et al., 2013). Regardless, TCD remains a reliable method to measure the cerebral blood flow velocity (CBFV) of the large cerebral vessels when knowledge of the potential diameter changes in certain conditions is understood. Compared to alternative methods (MRI, CT; Brunser et al., 2009), TCD is used in clinical settings and during exercise at a relatively little expense and can

also give beat-to-beat recordings (figure 2.3; Willie et al., 2014). It is commonly used in responsiveness tests such as cerebrovascular reactivity (CVR), effective cerebral autoregulation assessment (CA) and in neurovascular coupling (NVC). (See methods section for more information on the technique and protocol).

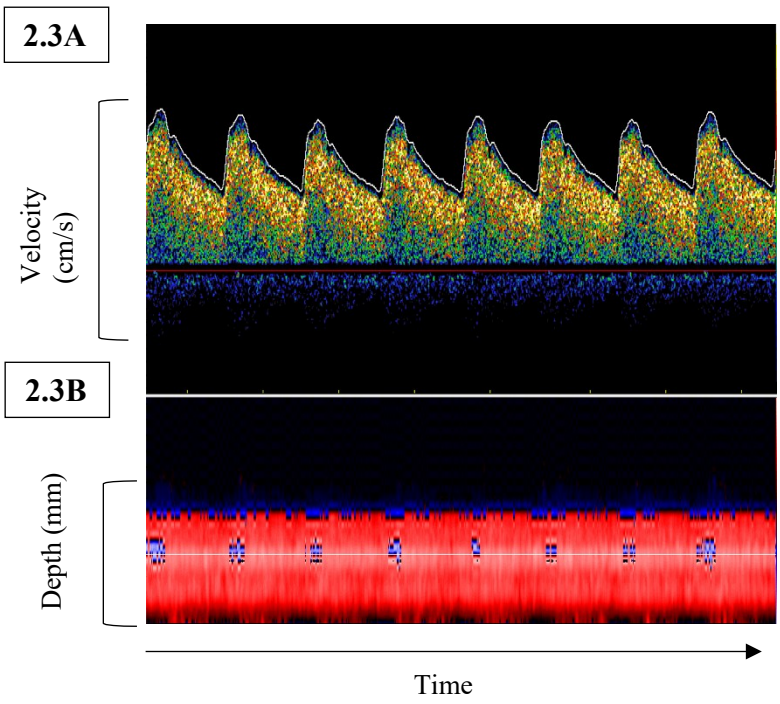


Figure 2.3. A typical trace of the MCA found using TCD in M mode. 2.3A shows the velocity trace(cm/s) and 2.3B shows the depth of the vessel (mm).

Cerebrovascular Reactivity

The cerebrovasculature is extremely sensitive to changes in arterial blood gases such as carbon dioxide (CO_2). At a cellular level (see figure 2.4), it has been proposed that an increase in P_aCO_2 (arterial CO_2) leads a decrease in pH and an increase in extracellular H+, which fires the central chemoreceptors (Willie et al., 2014). The lower pH activates the K+ channels in the smooth muscle cells, resulting in a K+ influx which results in the vasodilation of the vascular smooth muscle walls by opening the Ca^{2+} channels (Kisler et al., 2017). This change in vasomotor tone decreases the resistance to flow and subsequently local CBF increases, increasing the washout of CO_2. (Figure 2.4; Ainslie and Duffin, 2009). The increase in vasodilator substances is another mechanism responsible for the reduction of resistance in the cerebral vessels during hypercapnia (Figure 2.5). There is also evidence for the release of nitric oxide in cerebral vessels in response to increased $PaCO_2$ (hypercapnia). Although the extent of this action is unclear, it has been postulated that this increase in NO reduces resistance in the cerebral vessels, increasing CBF (Iadecola and Zhang, 1996). The extent of how reactive the cerebral vessels are depends upon the level of $PaCO_2$. A 1mmHg increase (above baseline) in $PaCO_2$ has been found to raise CBF by 3% (Fortune et al., 1992) and a 5% CO_2 stimulus (≈5mmHg increase in $PaCO_2$ from baseline in healthy individuals) can increase CBF by 50% (Kety and Schmidt, 1948). A decrease in CO_2, although less potent, has the opposite effect, it causes vasoconstriction and a decrease in CBF, implying that the CBF-CO_2 relationship is non-linear (Peebles et al., 2007). Subsequently, cerebrovascular reactivity to CO_2 has been described as a sigmoidal relationship (Battisti-Charbonney et al., 2011; Bohgal et al., 2014). Differences in CBF changes during hypercapnia vs. hypocapnia is thought to be due to vasodilators (in hypercapnia) having greater potency than vasoconstrictors (in hypocapnia) (Peebles et al., 2007). A plateau is found when maximum vasodilation (around 50mmHg

$P_{ET}CO_2$; Battisti-Charbonney et al., 2011) and vasoconstriction (around 10-15mmHg $P_{ET}CO_2$ below baseline values; Brugniaux et al., 2007) have occurred. These plateaus indicate the cerebral vasculature's capacity to respond to extreme high and low levels of CO_2.

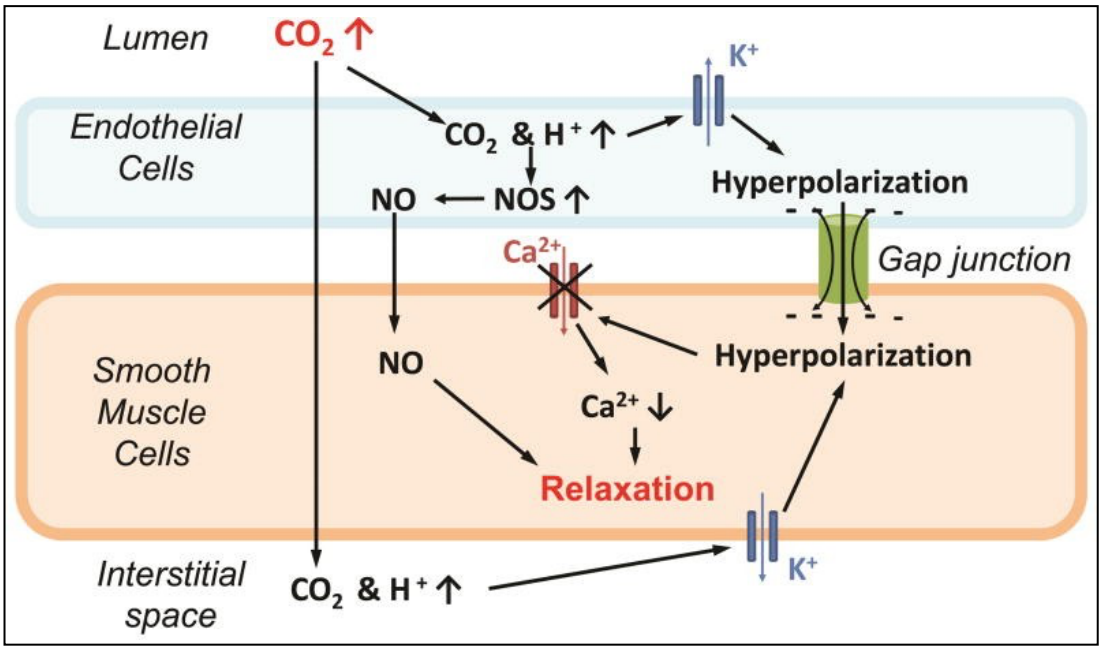


Figure 2.4. Summary of the cellular events that happen in the vascular smooth muscle and endothelial cells during hypercapnia that results in vasodilation. (Liu et al., 2019).

CVR is defined as the sensitivity and ability of the cerebrovasculature to vasodilate/constrict in response to a stimulus (most commonly CO_2) (Fierstra et al., 2013). Measuring it has been shown to be an important indicator of overall cerebrovascular health. Studies have shown CVR is decreased in those with sleep apnoea, hypertension, heart failure, carotid artery stenosis, cerebral ischaemic events, stroke, Alzheimer's Disease and Parkinson's Disease (Burgess et al., 2010; Serrador et al., 2000; Widder et al., 1994; Xie et al., 2005; Silvestrini et al., 2000; Meel van den Abeelen et al., 2014). Furthermore, it has been used as a prediction of CVD,

stroke and overall mortality in those with certain risk factors (Markus et al., 2001; Dahl et al., 1992; Portegies et al., 2014).

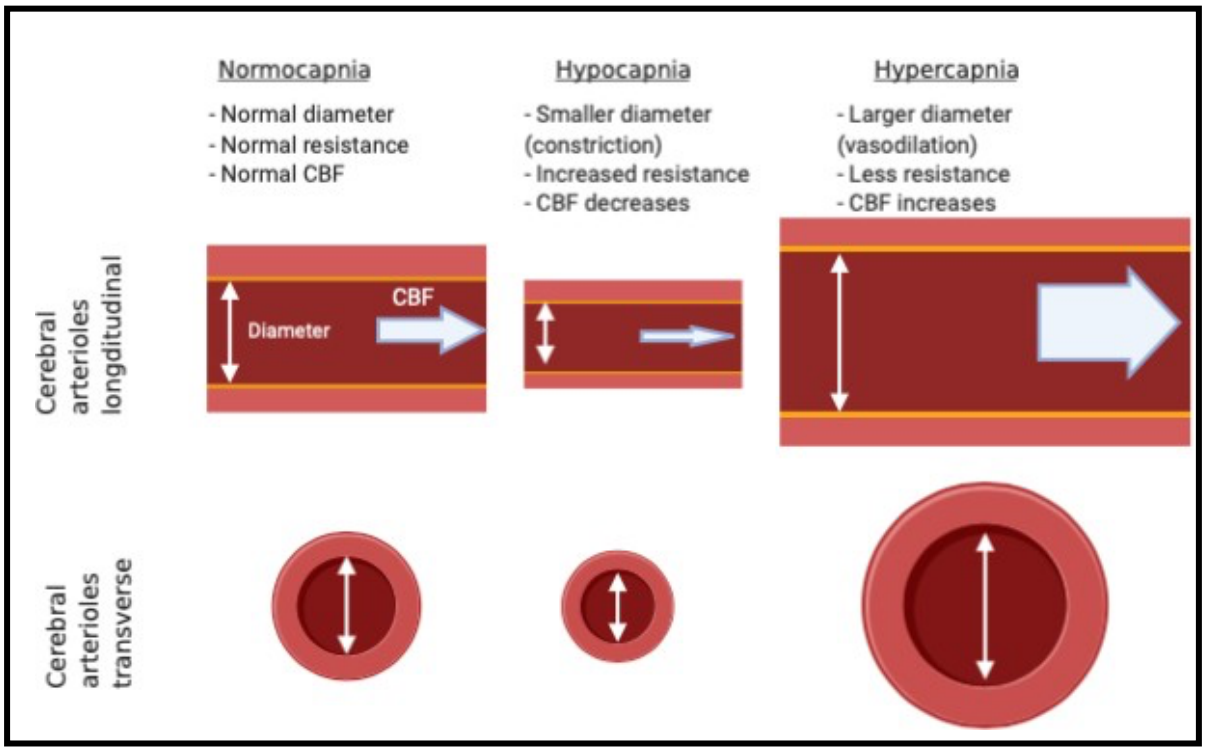


Figure 2.5. The change in the diameter of the cerebral arterioles during different levels of CO_2 (normocapnia, hypocapnia and hypercapnia; adapted from MacKay et al. 2016)

There are several methods and stimuli used to measure CVR. For example, acetazolamide (ACTZ) causes maximum vasodilation of the cerebral vessels due to its inhibition of carbonic anhydrase and the extent of the vasodilation can be used to measure CVR (Vorstrup et al., 1986). However, cerebrovascular response to ACTZ is highly variable between individuals and can lead to overestimation/underestimation of CVR (Dahl et al., 1992). Carbon dioxide is the most common non-invasive stimulus to measure CVR. There are numerous ways to measure CO_2-CVR, each with strengths and limitations (summarised in Table 1). Steady state inspired CO_2 is the method used in the current study, due to its small interindividual variability in a healthy cohort, the inexpensive equipment needed and its ability to be used in clinical settings

(Portegies et al., 2014). This steady state method of CO_2 inhalation to measure CVR has been shown to predict risk of death from stroke occurrence (Portegies et al. 2014).

Table 1. The strengths and limitations of several CO_2-CVR breathing methods.

	CO_2-CVR METHOD	STRENGTHS	LIMITATIONS
BREATH HOLD INDEX (BHI)	Participants hold their breath causing an increase in $PaCO_2$ with time. (Ratnatunga and Adhiseshiah, 1990)	It can show strength of stimulus and can use 'breath hold index' (BHI)to compare different CVR values (Silvestrini et al., 1999)	Affected by metabolic rate, size of lungs, ventilation history, prior inspiration/ expiration. Highly variable between subjects (Fierstra et al., 2013)
REBREATHING PROTOCOL	Participants inhale and exhale into either: 1. Empty Douglas bag (small amount of O_2 in it) 2. A douglas bag filled with CO_2 balanced with 100% O_2 (Read, 1967)	Dynamic relationship. No external CO_2 is required (Fierstra et al., 2013) It can be used to measure central respiratory chemo-sensitivity.	Rate of rise of PCO_2 is difficult to control Dependent upon individual rate of CO_2 production. Inspired O_2 not kept constant. Can't be used with MRI. (Fiersta et al., 2013).
STEADY STATE FIXED INSPIRED CO_2 INHALATION	Fixed concentration of CO_2 inspired such as 5% or 7% CO_2. 21% O_2 and N_2 is balanced (Ringlestein et al., 1988)	Standardised stimulus. It can look at steady state response rather than dynamic. Constant O_2 levels. $PETCO_2$ can be used instead of $PaCO_2$. It can be used in MRI (Ringlestein et al., 1992)	Varies with ventilation and metabolic rate – chemo sensitivity differs in individuals (Duffin et al., 2011)

The MCA is the most common TCD measurement site for CVR-CO_2. However, it has been suggested that there are inter-hemispheric differences between the anterior and posterior circulation (Sato et al., 2012; Skow et al., 2013). MCA reactivity to hypercapnia has been found to be higher than the PCA reactivity, in absolute terms (Skow et al., 2013; Sorond et al., 2005). The reasons for this difference in CVR between the MCA and PCA have been suggested to be due to the larger downstream tissue bed that is perfused by the MCA, hence allowing a larger increase in diameter (Skow et al., 2013.) Consequently, measuring and comparing the response to CO_2 in different circulations creates a better picture of global cerebral reactivity.

1.4 Cerebral and Peripheral Vascular Function

Cerebral and peripheral vascular function are both impaired in several cardiovascular and cerebrovascular diseases. For example, CVR and FMD are both reduced in carotid artery stenosis (Oz et al., 2012; Markus et al., 2001) and can both predict future CVD risk (Deanfield et al., 2007; Dahl et al., 1992). An impaired FMD response has been shown to be predictive of white matter lesions in those with a history of cerebrovascular disease (CBVD) (Chen et al., 2006) and has also shown to be reduced in several cerebrovasculature related diseases such as vascular dementia and Alzheimer's disease (Techibana et al., 2016). This leads to the question of whether vascular impairment in the peripheral is indicative of cerebrovascular impairments. In a study that looked at reductions in vascular function in the early morning, they found that a reduction in FMD response was related to a reduced CVR (measured via 5% CO_2 inhalation) in males (Ainslie et al., 2007). Furthermore, a study looking at older adults (aged 72-75 years) found a possible relationship between FMD and CVR in the hypocapnic range (but not the hypercapnic range) (Brar et al., 2013). In another study looking at older adults, a relationship was found between CVR (via BHI) and FMD, with both markers decreasing in patients with

white matter lesions (Staszewski et al., 2018). This may suggest an age effect. Contrary to the aforementioned studies, others have found no correlation between FMD and CVR. Palazzo et al. (2013) found no correlation between FMD and CVR (via BHI) in young healthy adults. They attributed this to differences in stimulus (hyperaemia vs. hypercapnia) between the two and the difference in location (MCA vs. brachial artery). Furthermore, in a study that looks at FMD and CVR (via 4% and 7% CO_2 inhalation), no correlation was found in young healthy male subjects (Junujo et al., 2020). FMD has been suggested to be solely NO-mediated (Green et al., 2014) whereas it is unclear as to the involvement of NO in CVR in the cerebral vessels. When NO synthase is inhibited, CVR has been shown to be unaffected (White et al., 1998). Moreover, studies that have looked at CVR to L-arginine (a substance that synthesises NO) have found no correlation between this and FMD response (Pretnar-Oblak et al., 2007; Perko et al., 2011). However, NO has been reported to contribute to changes in CBF (Iadecola and Zhang, 1996), though other mechanisms (e.g. other neurogenic and metabolic factors) appear to have a stronger influence (Lavi et al., 2003). Therefore, the contribution of NO to FMD, as compared to CVR, may be the reason for the lack of correlation found between FMD and CVR measures. Furthermore, studies that have looked at younger healthier populations have found no correlation between FMD and CVR, thus it appears that ageing is the primary factor that links corresponding declines in FMD and CVR.

1.5 The Role of Female Sex Hormones on the Peripheral and Cerebral Vasculature

Circulating female sex hormones, in particular, oestrogen and progesterone are secreted by ovarian follicles and can have an important impact on vascular function and health. These sex hormone levels increase and decrease throughout the female life cycle, for example, over the course of the menstrual cycle, during pregnancy and at the onset of menopause (as previously described). In post-menopausal females, the amount of ovarian follicles is only 1% of those that are in pre-menopausal females (Dhandapani et al., 2006). This leads to a drop in circulating oestrogen levels from up to 2.8nM at ovulation in the menstrual cycle to 0.15-0.2nM post-menopause, which is equal to circulating levels in men (Yen et al., 1991). In comparison, when female sex hormones are high during pregnancy, oestrogen levels can go up to 70nM (Yen et al., 1991). The reduction in female sex hormone levels, particular oestrogen, has been linked to the increased risk of several pathologies that occur at menopause. Oestrogen has been shown to have protective actions on the vasculature through a large number of mechanisms/pathways (Mendelsohn et al., 2000). The effect of oestrogen can be rapid (non-genomic) or longer term (genomic), can be endothelial dependent or independent and can have varying effects on different vasculatures e.g. peripheral and cerebral. In the peripheral vasculature, immediately following oestrogen exposure (non-genomic), there is an increased response to shear stress (Huang et al., 1998) due to the increased NO levels, resulting in vasodilation (Figure 3). Mendelsohn et al., 2000). 17B estradiol levels have been shown to positively correlate with nitrate levels (Rossielli et al., 1994), with post-menopausal females (lower estradiol levels) showing lower NO activity (Majmudar et al., 2000). Furthermore, in the ovulatory phase of the menstrual cycle (when oestrogen is at its highest), females have been shown to exhale twice as much NO when compared to the EF phase (Kharitonov et al., 1994). Longer term genomic

effects of having elevated oestrogen levels determines that females can have increased gene expression of eNOS and prostacyclin synthase, which increase NO bioavailability (Chang et al., 1980). The increase in vascular function markers (e.g. FMD and CVR) when oestrogen levels are higher (e.g. pre-menopausal, pregnancy etc) may be influenced by the higher levels of NO.

Oestrogen has been shown to effect other properties of the vasculature. For example, oestrogen levels are negatively associated with CRP (a biomarker of inflammation) (Wander et al., 2008). This female sex hormone also reduces free radical species (increasing NO synthase) (Stirone et al., 2005) and subsequently provides vascular protection by reducing atherosclerosis. Furthermore, oestrogen lowers serum low-density lipoprotein (LDL) levels (Guetta et al., 1996 and decreases vasoconstrictor (endothelin-1) levels (Sudhir et al., 1997). All of these are increase in post-menopausal females when oestrogen levels lower (Figure 4.3) (Doshi and Agarwal et al., 2013; Guetta et al., 1996).

Oestrogen receptors are found in vascular smooth muscle cells and endothelial cells of several types of tissue in humans and animals. Oestrogen receptors are activated by oestrogen (Khalil 2014) and are responsible for several cellular effects (Figure 3). Oestrogen receptors (in particular ER-α and ER-β) correlate with higher levels of oestrogen in animal studies (Stirone et al., 2003). In human studies, ER-α expression in the peripheral vasculature has been shown to be 30% higher in the high hormone ovulatory phase of the menstrual cycle when compared to the low hormone phase (early-follicular; Gavin et al., 2009). Moreover, in post-menopausal females, ER-α expression has been shown to be 30% lower than pre-menopausal females

during the ovulatory phase of the menstrual cycle (Gavin et al., 2009). Further results in this study found a positive correlation with ER-α expression and eNOS (r = .54).

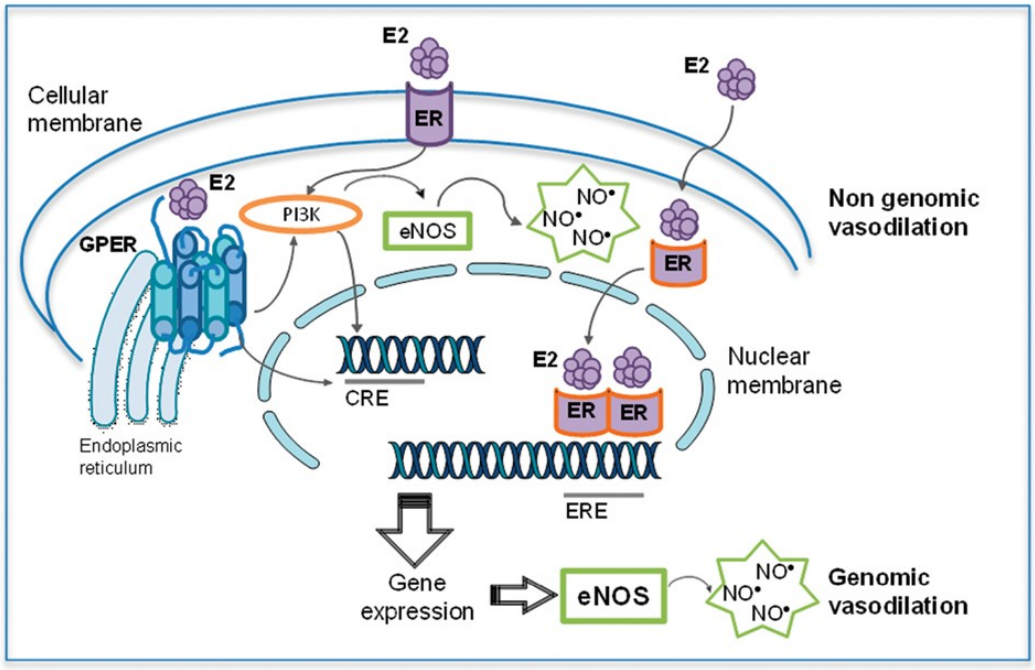


Figure 3. A summary of the genomic and non-genomic effects of oestrogen on the peripheral vasculature. (Novella et al., 2012) (E2 = oestrogen; eNOS = endothelial nitric oxide synthase; NO = nitric oxide; ER = oestrogen receptor)

Animal studies looking at the cerebral vessels have shown that ER-α and ER-β are both present in the cerebral vascular smooth muscle cells (Dan et al., 2003). The increase in vasodilation after oestrogen exposure has been shown to reduce cerebrovascular tone and therefore increase CBF in the cerebral arteries of rats (Krause et al., 2006). In ovariectomised pregnant ewes, oestrogen administration increased CBF by 21-29% (Magness et al., 2005). In cerebral tissue, an increase in ER-α receptors has been shown to modulate an increase in NO-dependent vasodilation after chronic treatment with oestrogen (Geary et al., 2000). A study that looked at ovariectomised females (producing no oestrogen) found that there was less NO-dependent

vasodilation when compared to normal females (producing normal levels of oestrogen) (Pellegrino et al., 2001). Although oestrogen has been shown to increase NO-dependent vasodilation in the cerebral vessels, measurements such as CVR have been found to be unaffected by NO synthase inhibition (White et al., 1998), implying that NO-independent mechanisms (such as other vasoactive substances) are responsible for the increase in vasodilation. It is clear that oestrogen has a profound effect on the peripheral and cerebral vasculature, yet the relationship between the exact mechanisms that result in vasodilation remain unclear.

Progesterone is another female sex hormone secreted by the ovarian follicles. There are increased levels of progesterone in pre-menopausal females during the luteal phase of the menstrual cycle compared to other menstrual cycle phases and post-menopausal females. Progesterone receptors, similar to oestrogen, have been found in the vascular smooth muscle and the endothelium of arterial walls (Guo et al., 2004). In human endothelial cells and rat aortic cells, progesterone has been shown to act through genomic and non-genomic pathways, increasing NO synthesis (Simoncini et al., 2004)) and protecting against atherosclerosis (Ma et al., 2009). In animals, progesterone has been shown to increase peripheral blood flow (Molinari et al., 2001) and increase endothelium-independent relaxation of arteries (Jiang et al.,1992). Contrary to this, in animals, progesterone has also been shown to induce vasoconstriction (Sarrel et al., 1999) and reduce the response of oestrogen endothelium-dependent vasodilation (Williams et al., 1994). Furthermore, progesterone is positively associated with a biomarker of inflammation (C-reactive protein), which restricts vasodilation (Wander et al., 2008).

In humans, natural progesterone acts on progesterone receptor A (progesterone in other forms may not act on this receptor therefore may not produce the same response) (Toth et al., 2009). During the menstrual cycle, when progesterone levels increase during the luteal phase, oestrogen receptors are down-regulated and the vasodilator response is reduced (Kawano et al., 1996). Progesterone has been shown to reduce the endothelium-dependent vasodilator response of oestrogen in FMD, yet progesterone alone does not attenuate endothelial function (Miner et al., 2011). Different, stronger forms of progesterone (such as medroxyprogesterone acetate) are commonly found in HRT in post-menopausal females and these may have differing responses on the vasculature (see section HRT). For example, medroxyprogesterone acetate was shown to correlate with reduced endothelial function (as measured by FMD%) (Meendering et al., 2008). There is very little information in the literature as to the effect of progesterone on the cerebrovasculature in humans and although probable, there are no reports of progesterone receptors in cerebral vessels (Krause et al., 2006). In contrast to oestrogen, progesterone does not appear to affect the eNOS pathway in cerebral vessels (McNeill et al., 2002).

1.6 Factors Affecting Vascular Function

1.6.1 Ageing

Vascular ageing describes the degeneration of the vessels due to increased age, and is one of the main risk factors for cardiovascular diseases such as stroke, hypertension and atherosclerosis (Quinn et al., 2012; Franklin, 1999). Vascular ageing is characterised by endothelial dysfunction and arterial wall stiffness (AWS; van Bussel et al., 2011) and other known risk factors (e.g. blood pressure, smoking, obesity etc). Endothelial dysfunction (characterised by a reduced FMD response) leads to an overall reduction in vascular

vasodilation through age-related mechanisms, such as increased inflammation (increased TNF-α), and increased oxidative stress (Moreau and Hildreth, 2015). For example, by the 5th decade FMD% decreases and pulse wave velocity (PWV; an indicator of arterial wall stiffness) increases (Benjamin 2004; Celermajer et al., 1994; Herrington et al., 2001). Furthermore, FMD% is 50% less in older adults (66 ±7 years) when compared to young adults (24 ±4 years; Coverdale et al., 2016). The reduction of NO and the subsequent reduction in FMD% in the peripheral vasculature has been shown to be a predictor of cardiovascular events (Laketta et al., 2003), with the incidence of CVD increasing from the 5th decade in both males and females (Figure 4.1; Kannel et al., 1976).

Endothelial dysfunction and altered vascular resistance are highly important in other vasculatures, such as the cerebrovasculature. MCAv has been shown to decrease with increasing age (Bakker et al., 2004), with CBF velocity decreasing by 60 cm/s from the 6th to the 7th decade of life (Aaslid et al., 1982). This is the result of increased arterial stiffness and endothelial dysfunction (Yang et al., 2017; Scicchitano et al., 2019). These structural changes with ageing can lead to changes in cerebrovascular function and the development of cerebrovascular diseases (Iadecola et al., 2004). Related to this, CVR has been shown to decrease with age (Peng et al., 2018) . Ageing has been found to be negatively associated with CVR across MRI and TCD studies using steady state CO_2-CVR challenges (Miller et al., 2019; Chen et al., 2018; Sorond et al., 2005; Peng et al., 2018). However, many studies that look at the impact of ageing on CVR do not take into consideration sex and the influence of female sex hormones and it has been suggested that CVR changes with ageing are oestrogen/female specific (Miller et al., 2013).

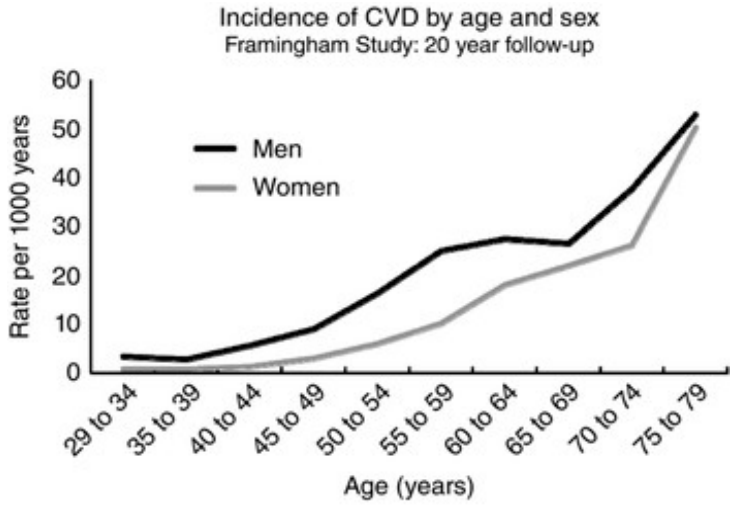
1.6.2 Sex

The incidence of cardiovascular disease differs between the sexes, with males having a higher incidence of CVD across their lifetime (Figure 4.1). Markers of cerebrovascular and cardiovascular function that impact CVD and cerebrovascular disease risk are different between the sexes. For example, using FMD as a measurement of endothelial function, studies have shown that females exhibit a higher FMD response when compared to men (Celermajer et al., 1994; Juonala et al., 2008; Yao et al., 2014; Green et al., 2016). However, all of these studies failed to control for menstrual cycle, which have shown to influence FMD response due to changes in female sex hormone concentration (Brandao et al., 2014; Williams et al., 2011). In studies that controlled for menstrual cycle, Hashimoto et al., (1995) found that FMD values in males and females were comparable when females were measured in the low hormone phase of the menstrual cycle (early follicular phase). Furthermore, Harris et al., (2012) found that FMD response was significantly higher in the high-hormone phase in female when compared to men.

There are differences in the cerebral circulation between the sexes. There is an increased mean baseline CBFV in females when compared to age-matched males (aged 20-60 years) (Vriens et al., 1989; Olah et al., 2000; Krejza et al., 2005). This higher global CBF in females during their reproductive years (Liu et al., 2016) has been linked to the impact of female sex hormones. However, many of these studies did not control for menstrual cycle phase. Furthermore, females have been shown to have a smaller cerebral vessel diameter (Shatri et al., 2017). The small diameter in the MCA and PCA of females may be the reason for the higher CBFV when compared to males. Studies examining sex-related differences in CVR are less conclusive. In healthy young adults (aged 20-40), some studies have found a higher CVR in females

compared to age-matched males (Olah et al., 2000; Kastrup et al., 1997), while others have found no sex-related differences (Peltonen et al., 2015; Madureira et al., 2017). Varying methodology and small sample sizes likely influence these results and may explain the conflicting literature. For example, in Kastrup et al., 1997 a 5% CO_2 stimulus was used, with 95% O_2. Hyperoxia has been found to influence the vasculature by increasing CBF and enhancing brain metabolism (Ainslie and Duffin, 2009), therefore this hyperoxic-hypercapnic gas mixture used by Kastrup et al., (1997) may have influenced their results, making it incomparable to other studies. The two studies that found no difference in CVR between the sexes (Peltonen et al., 2015; Madureira et al., 2017) measured females in the early-follicular (low hormone) phase of the menstrual cycle, whereas the other studies (Olah et al., 2000; Kastrup et al., 1997) did not control for menstrual cycle phase. Thus, the influence of female sex hormone on the cerebrovascular responses remains inconclusive.

A



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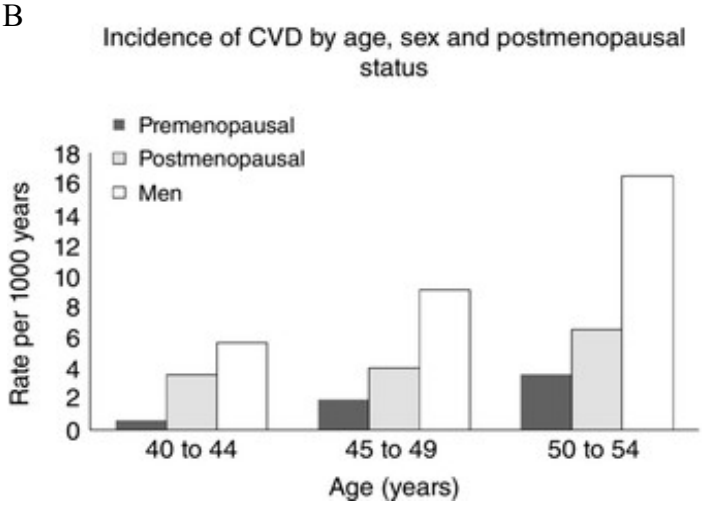

Figure 4.1. The Framingham Study showing incidence of CVD across age, sex and menopausal status. (A) A higher incidence in males when compared to females. (B) Showing that irrespective of age, males have the highest incidence, followed by post-menopausal females (Kannel et al., 1976)

1.6.3 The Menstrual Cycle

The menstrual cycle is a monthly pattern in pre-menopausal females that involves changes in the ovaries and uterus lining to prepare for pregnancy. Natural cyclic oscillations in female sex hormones occur over the course of menstrual cycle (Figure 4.2) depending on the cycle phase (follicular, ovulation, luteal). The average secretion rates of estradiol, progesterone and testosterone for each menstrual cycle phase are shown in Table 2. The changing levels of oestrogen and progesterone have been found to have an influence of cardiovascular events, for example, the incidence of acute coronary effects are higher during menses (when oestrogen levels are low) (Hamelin et al., 2003). In order to understand the mechanisms of this, it is important to look at the influence of female sex hormone levels on cerebral and cardiovascular haemodynamics. Furthermore, many previous studies have failed to account for the potential confounder of menstrual cycle when measuring pre-menopausal females. It is important to establish the effects of changing female sex hormone levels on cerebral and cardiovascular haemodynamics to establish how findings from previous studies are affected.

Table 2. Average ovarian secretion levels of female sex hormones over the course of the menstrual cycle (Adapted from Baird et al., 1974).

Female sex hormone	**Early-Follicular (days 1-3)**	**Late-Follicular (days 13-16)**	**Mid/Late-Luteal (days 18-28)**
Estradiol (μg)	36	380	250
Progesterone (mg)	1	4	25
Testosterone (μg)	144	171	126

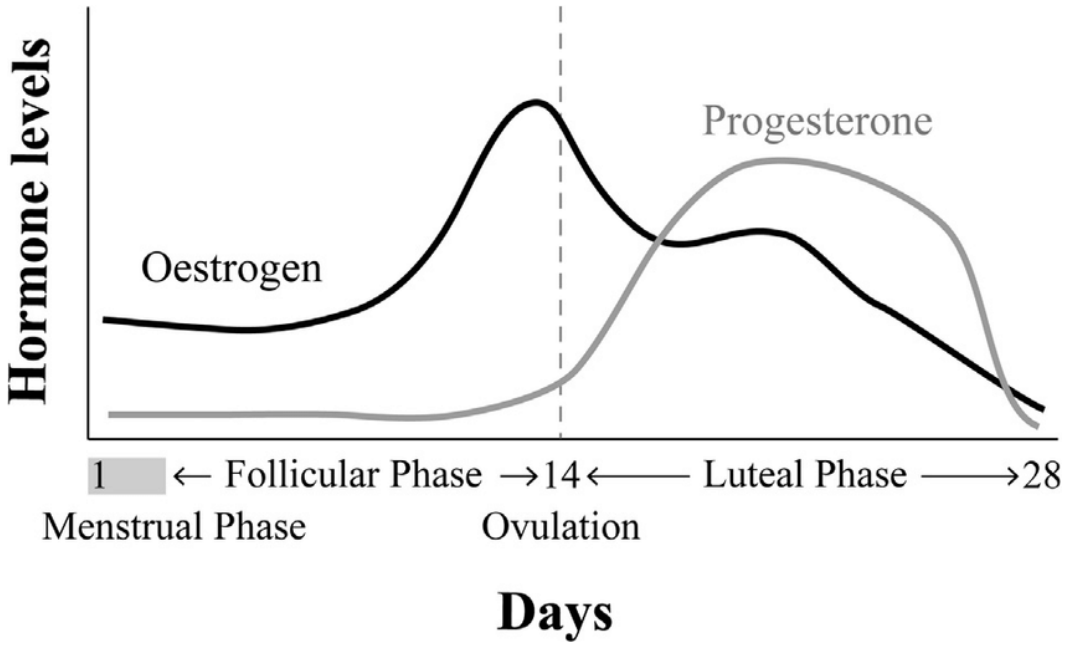

Figure 4.2. A graph showing oestrogen and progesterone levels over the course of the menstrual cycle (28 days) (Vink et al., 2017)

Flow Mediated Dilation

The effects of the menstrual cycle have been established in the peripheral vasculature due to several studies using the same uniform study design, making the results comparable and clear. Furthermore the peripheral arteries are easily accessible by ultrasound equipment and therefore, FMD is a common non-invasive, accessible mode to assess vascular function. Despite baseline diameters being similar across the menstrual cycle, several studies have shown that FMD% is significantly different across the different phases of the menstrual cycle, with FMD% being the highest in the late-follicular phase (days 12-14) and the lowest in the early-luteal phase (days 16-18) (Brandao et al., 2014; Williams et al., 2001). Another study using the same protocol found a similar result, with FMD% 53% higher in the high hormone

late-follicular phase when compared with all other phases, and FMD% at its lowest levels in the early-luteal phase (Adkisson et al., 2010). All of these studies identified menstrual cycle phase through timing and/or temperature and confirmed them with serum female sex hormone level concentrations. In slight contrast to these findings, two other studies found that FMD% was comparable between late-follicular and luteal phases of the menstrual cycle, however there was still a significant decrease in FMD% from the early-follicular phase (days 1-3) to the late-follicular and luteal phases (days 16-27) (Hashimoto et al., 1995; Harris et al., 2012). However, in these latter studies, female sex hormone levels between the late-follicular and luteal phases were comparable, with large individual variation in hormone levels reported (ranging from 149 to 594 pg/ml in the late-follicular phase). This may be the reason for this non-significance between the FMD% during the late-follicular and luteal phases. When looking at specific hormone concentrations, Schnabel et al., 2013 found that increased FMD (defined as vascular reactivity) correlated with increased oestrogen levels. Furthermore, increased progesterone levels were inversely correlated with brachial artery diameter (Schnabel et al., 2013) which may explain lower FMD results in the luteal phase of the menstrual cycle (when progesterone is high).

In contrast, a study that compared the mid-follicular (days 9-13) to the late-luteal phase (day 26-30) found no difference in FMD%, despite there being a significant increase in estradiol levels (increasing by 230 ± 207 p/mol) in the mid-follicular phase when compared to the late-luteal phase (Shenshouda et al., 2018). In this study, there was no significant relationship found between oestrogen levels, progesterone levels and brachial artery FMD (Shenshouda et al., 2018). Furthermore, another study showed that FMD% did not change between either the early-follicular and early-luteal phases (D'Urzo et al., 2018) or across the whole menstrual cycle

(Jochmann et al., 2009). All of these studies used the same universally accepted FMD protocol, however, potential individual confounders (such as physical exercise or age) or molecular differences (variation in oestrogen receptors and eNOS activity (Gavin et al., 2009)) were not controlled for in these studies and may have influenced the results. As the literature is conflicting, it is important to further assess endothelial function across the menstrual cycle in pre-menopausal females to assess the influence of high levels of oestrogen/progesterone. These female sex hormone levels and corresponding FMD% in pre-menopausal females could then be compared to values in post-menopausal females.

Cerebral Blood Flow

Several studies in humans have looked into the effect of the menstrual cycle on the basal cerebral blood flow velocity. Observational studies on the carotid arteries have shown a decrease in pulsatility index (PI) and resistance index (RI) during the menstrual cycle in the internal carotid artery (ICA) during the late-follicular phase (days 13-14, nearing ovulation) (Krejza et al., 2004; Brackley et al., 1999) when compared to the early-follicular and luteal phases. Conversely, in the external carotid artery (ECA), resistance and pulsatility index have been shown to increase in the late-follicular phase (nearing ovulation) (Krejza et al., 2003). In the MCA, the literature varies, with some studies seeing no change in mean flow velocity over the course of the menstrual cycle (Diomedi et al., 2001; Krejza et al., 2013) and others saw a significant increase in MCAv in the late-follicular phase (Brackley et al., 1999; Peltonen et al., 2016). Variance in results may be due to small sample size and lack of significant sex hormone change in each phase.

Cerebrovascular Reactivity

The literature surrounding CVR and menstrual cycle is also conflicting, stemming from the difference in methods and the stimulus used (Table 1). When using ACTZ as a stimulus for maximum vasodilation, CVR has been shown to increase blood flow through the ICA and ECA during the mid-luteal phase when compared to the late-luteal phase (Krejza et al., 2013). Yet in this study, female sex hormone levels didn't match to the correct phase (average values shown in table 2). Studies that have used the breath-hold index as a stimulus for CVR have shown varying results depending on the phase of the menstrual cycle assessed. One study has found CVR in the MCA to be increased in the early-follicular phase (low hormone) when compared to the ovulatory phase (high oestrogen), despite a significant 150 pg/ml increase in oestrogen levels during the ovulatory phase (Diomedi et al., 2001). Another study has found no difference in CVR in the MCA when comparing the late-follicular phase (high oestrogen) to the mid-luteal phase (high oestrogen and high progesterone) (Matteis et al., 1998). This indicates that when comparing low hormone levels to high hormone levels, there is a difference in CVR. A study that looked at dynamic end-tidal CO_2 forcing showed a greater CVR to hypercapnia in the luteal phase (high oestrogen and progesterone) when compared to the early-follicular phase (low hormone) and mid-cycle (oestrogen high, progesterone low) phases (Debert et al., 2012). Using a graded hypoxia and hypercapnic protocol, another study found that values for CVR_{MCAv} were higher in the late-follicular phase when compared to the early-follicular phase, despite a significant 78 pg/ml rise in oestrogen levels in the late-follicular phase (Peltonen et al., 2016). Contradicting this, another study has found that during 5% CO_2 inhalation and guided hyperventilation (10mmHg below baseline $PETCO_2$ levels), there was

no difference in CO_2 reactivity between the early-follicular, late-follicular and mid-luteal phases of the menstrual cycle (Favre et al., 2019). In the latter study, there was no significant difference found in estradiol levels, potentially due to the unreliable nature of salivary estradiol measurements. However, this may also be the reason for the insignificant results. Different phases of the menstrual cycle correspond to different levels of progesterone and oestrogen, both of which may affect CVR differently. Currently no studies assess menstrual cycle phase and CVR to the PCA. To allow comparison between studies, a universal protocol needs to be adopted in which the CVR stimulus used is the same, and phases of menstrual cycle phases correspond to clear differences in correct female sex hormone levels.

1.6.4 The Menopausal Transition

Menopause is defined as the decline in ovarian function due to the loss of ovarian follicles and the decline of female sex hormones, it can occur naturally or surgically (Davis, 2000). Several physiological changes take place during menopause including augmented lipid profile, fat distribution, insulin sensitivity and increase in blood pressure (Celermajer et al., 1994). These exacerbate vascular ageing in females, so much so that cardiovascular disease rates significantly increase in post-menopausal females (Taddei et al., 1996). For example, Figure 4.1 shows that from the ages 40-54, incidence of CVD is higher in post-menopausal females compared to pre-menopausal females of the same age (Kannel et al., 1976). This has been attributed to the lack of female sex hormones (in particular oestrogen and progesterone; Figure 4.3). By comparing measures of vascular function between pre-menopausal and post-menopausal females, the wider role of menopause and female sex hormone levels on the vasculature can be found.

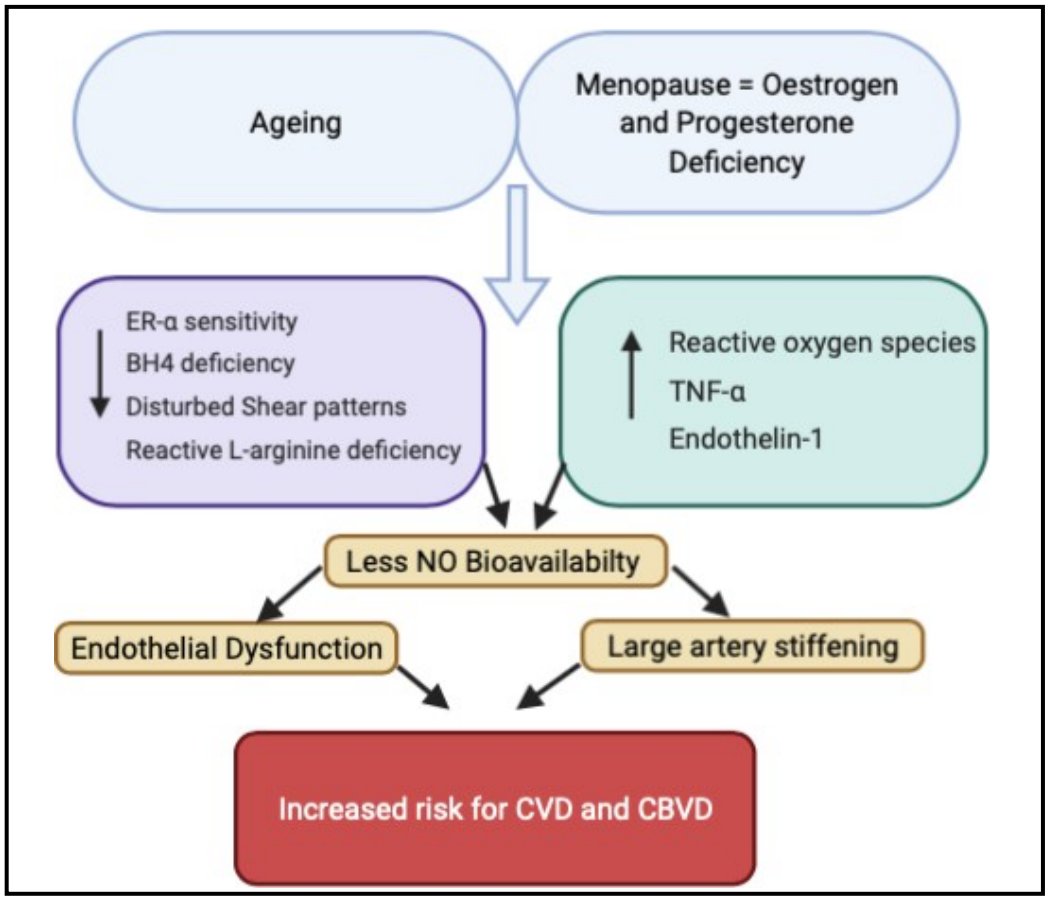

Figure 4.3. A summary of the effects of ageing and menopause (oestrogen and progesterone deficiency) on endothelial function and the increased risk of CVD and CBVD. (Adapted from Moreau and Hildreth, 2014).

Flow Mediated Dilation

Endothelial-dependent vasodilation and the effect of menopause has been extensively studied in the literature. The onset of menopause has been shown to lower lipid metabolism, reduce endothelial function and reduce vascular reactivity (Mendelsohn et al., 2000). The FMD response is lower in post-menopausal females, as compared to pre-menopausal females (Brislane et al., 2019; Green et al., 2016; Celermajer et al., 1994; McCrohen et al., 1996; Holder et al., 2019; Sorensen et al., 1998). This reduction in FMD% has also been shown to decrease across the menopausal stages, with females in early peri-menopause exhibiting a greater

FMD%, corresponding to higher female sex hormone levels when compared to late peri-menopause stage, corresponding to higher female sex hormone levels at the former stage (Moreau et al., 2012). Despite other functional and structural changes occurring at the onset of menopause, reduced oestrogen concentration correlated with this reduced FMD response at the late-perimenopausal stage independent of other CVD risk factors (Moreau et al., 2012). However, increased shear stress (oscillatory and retrograde) in the brachial artery with advancing menopausal age has also been shown, which is also associated with endothelial dysfunction (Somani et al., 2019). In contrast to other studies, Brislane et al., 2019 found no difference in brachial artery FMD% when comparing late pre-menopausal females and early post-menopausal females of a similar age. A larger age gap between pre-menopausal and post-menopausal females in other studies may be a stronger factor when comparing females. Another study found that pre-menopausal females had a greater FMD% when compared to sedentary older females, yet when compared to older fit females, there was no difference in FMD% found (Black et al., 2009). These data indicate that fitness level could be a confounder in several studies and should be considered. It is clear that the menopause in females significantly impairs endothelial function in the peripheral vasculature. There is less literature surrounding the influence of menopause on the cerebrovascular and how these correspond to peripheral vascular changes.

Cerebral Blood Flow

Vascular ageing has a prominent effect on the cerebrovasculature. In females, changes in the hormonal environment at the onset of menopause can influence cerebral regulation and therefore incidence of cerebrovascular diseases (Rosano et al., 2007). Basal CBF has been shown to be lower in post-menopausal compared to pre-menopausal females, when measuring

MCAv using TCD (Brislane et al., 2019; Bakker et al.,2004). In all of these studies, the pre-menopausal and post-menopausal cohort were not age-matched. Of note, one study has shown that MCAv was not different between age-matched pre- and post-menopausal females (Matteis et al., 1998). Furthermore, although Brislane et al., (2019) found a significant difference in MCAv when comparing pre- to post-menopausal females, however when comparing age-matched late pre-menopausal to early post-menopausal females (age-matched), there was no significant difference in MCAv. This indicates that ageing may be a confounder when looking at changes in cerebral blood flow velocity. Despite this, when controlling for mean arterial blood pressure (MAP) and comparing cerebrovascular conductance, there was a trend (p = 0.07) for an increase in cerebrovascular conductance in late pre-menopausal females when compared to early post-menopausal females (Brislane et al., 2019). The small sample size used in this particular cohort (n=10 for each group) may increase the likelihood of type II errors, decreasing the power of the effect. In summary, it appears that ageing as oppose to menopause is largely responsible for the decrease in CBFv described in menopausal females.

Cerebrovascular Reactivity

Due to the structural and functional changes that occur in the vasculature with ageing and the onset of menopause, it would be expected that CVR would change also. Despite this, the literature is contradictory. This may be due to a wide range of factors such as small sample size, the type of method used, the age of participants and other risk factors that can accelerate at menopause in different individuals. All the following studies mentioned measure CVR in the MCA and no studies have currently looked at CVR to PCA across the menopausal transition. A study that used ACTZ as the stimulus showed that post-menopausal women exhibit a lower CVR compared to pre-menopausal, and the results match that of men (Olah et

al., 2000). In agreement with this, CVR induced via BHI has been shown to be higher in pre-menopausal women as compared to post-menopausal women (Matteis et al., 1998). Using CO_2 inhalation, Kastrup et al., 1998 found that there was a decline in CVR after the 5th decade of life in women and that this was significantly lower than age-matched women taking HRT. In this study as previously mentioned, the gas mixture used may mean that hyperoxia influenced these results. Furthermore, the lack of control for menopause stage or sex hormone concentration levels means that it is difficult to determine whether Kastrup et al., (1998) findings are due to age or menopause. Another study using CO_2 inhalation, found no significant difference in CVR between post-menopausal and pre-menopausal females (Brislane et al., 2019). This study controlled for several factors and had a large sample size, linking the non-significance of CVR to the fact that the population studied were healthy, had no diseases and were not on medication. Furthermore, mean arterial pressure (MAP) and cerebrovascular conductance (CVC) (a measure of CBFV that includes MCAv and MAP) were shown to be significantly different between post-menopausal and pre-menopausal females, yet CVR was not significantly different. Further research is needed looking at the difference in CVR and the mechanisms behind it, when comparing women across the menopause.

1.6.5 Hormone Replacement Therapy

The drop in female sex hormone levels at the onset of menopause not only causes vasculature changes (as discussed previously) but also cause other symptoms. Examples of these include hot flushes, sleep problems, mood swings and weight gain (Santoro et al., 2015). To relieve these symptoms, hormone replacement therapy (HRT) has become increasingly popular. The impact of HRT on cardio- and cerebrovascular diseases and symptoms is contradictory, with

vascular responses appearing to be affected by type of HRT used, timing of HRT since menopause, length of HRT, ageing as well as the prescense or absence of other CV risk factors. Studies that have shown benefits show a 30-35% decrease risk of coronary heart disease (Barret-Connnor et al., 1998) and decreased BP and low density lipoprotein (LDL) levels (Canderelli et al., 2007). Stroke risk and HRT is contradictory with some studies showing reduced risk (Carrasquilla et al., 2017), no effect (Lokkegaard et al., 2017; Qureshi et al., 2016) or an increased risk of stroke whilst using HRT (Anderson et al., 2004). The effects of HRT on CVD has also appears to be inconsistent, either showing little effect on risk of CVD in a 2015 review (Boardman et al., 2015) or no effect on risk (Manson et al., 2017). Due to the influence of HRT on several cardiovascular diseases, it is important to look at how HRT impacts cerebrovascular and cardiovascular responses.

Flow Mediated Dilation

Short and long term effects of HRT on FMD in the literature are in general agreement. In post-menopausal females, short term studies (ranging 4-9 weeks) looking at females taking oestrogen-only HRT, have shown an increase in FMD% compared to baseline and a placebo group (Hurtado et al., 2016; Koh et al., 1999; Leiberman et al., 1994). One of these studies controlled for age, BMI and time since menopause, and still showed an increase in FMD (Hurtado et al., 2016). In longer studies (ranging from 3 months – 6 years), those taking oestrogen-only HRT also showed a increased FMD compared to placebo (Bush et al., 1998). Under a similar time frame, the literature surrounding oestrogen + progesterone HRT varies, with some studies showing an improved FMD response (Koh et al., 2001; Gerhard et al., 1998) compared to baseline and placebo, while other studies show no difference (Sorensen et al., 1998; Herrington et al., 2001). This has been attributed to the varying effect of progesterone

on the vasculature. In all of these studies highlighted above, participants were not on HRT prior to taking part. A retrospective study that looks at differences in FMD response in those taking two different types of HRT compared with those not on HRT, have shown an increase response in FMD% in HRT users as compared to non-users (McCrohen et al., 1996). Furthermore, in this study there was no difference in FMD% between the two types of HRT, despite one containing progesterone (McCrohen et al., 1996). Another long term observational study looked at FMD responses in HRT users vs. non-users, and showed no difference in relative or absolute diameter change in the brachial artery after FMD (Herrington et al., 2001). However, all women in this study were >65 years and therefore these results may have been influence by age and time since menopause as increased time since menopause has been found to be correlated with decreased FMD% (Vitale et al. 2007). Surgically menopausal women have also shown an increased FMD% after 6 weeks oestrogen-only HRT (Zegura et al., 2003). It must be noted that studies looking at HRT in post-menopausal females are hard to compare due to contrast in several factors such as administration route, age and type, timing and dose of HRT.

CBF and Cerebrovascular Reactivity

Cerebrovascular studies comparing post-menopausal women with or without HRT typically focus on measuring CBF and/or CVR at the carotid arteries. ICA pulsatility index (PI) (a measure of cerebrovascular impendence) has been shown to decrease across a 12-month period in post-menopausal females taking varying levels of oestrogen/progesterone HRT when compared to placebo (De Leo et al., 2003; Gangar et al., 1991; Caccitore et al., 1998; Penotti et al., 1993). MCA PI has shown a similar trend, with a 24% decrease in PI in the first 6 weeks of taking transdermal combined oestrogen/progesterone HRT (Penotti et al., 1993). Furthermore, an MRI study found that cerebrovascular resistance decreases following 12

weeks of HRT as compared to a placebo (Sorensen et al., 2001). Limited studies have looked at the effect of HRT on CVR in the MCA. An observational study looking at the long term effects of HRT showed CVR increased in women on HRT compared with those not on HRT and pre-menopausal women (Kastrup et al., 1998). Despite this, they had a small sample size, did not measure the dosage or length of time on HRT and the method used to measure CVR was influence by hyperoxia.

Another observational study looked at changes in CVR after women stop taking different types of HRT (Barnes et al., 2018). In this study, participants were 3-year post-HRT. Despite participants being 3-years post-HRT (previously taking oral oestrogen), there was an increase in MCAv and CVCi to steady state open circuit 4% and 6% CO_2 inhalation (Barnes et al., 2018) when compared to a placebo group, despite no differences found between the two in baseline values. In contrast to this, an interventional study where post-menopausal females took 2 different types of HRT (oestrogen only or oestrogen/progesterone) or placebo for 3 months, showed that CVR to ACTZ did not change across the three groups (Bain et al., 2004). However, MCA PI decreased and MCAv increased in participants taking the oestrogen/progesterone HRT. Of note, this study used a relatively short time frame and had a large age-range in participants, which may have affected the results. The potential reasons and mechanisms for the change in the cerebrovasculature due to oestrogen and progesterone supplementation are discussed in the below section (other hormone states). From the literature, it appears that HRT has an effect on the vasculature. However, differing methods between HRT studies make results hard to compare and more research is needed to be more clearly establish how HRT influences disease rates.

1.6.6 Other hormonal states

1. Pregnancy

Pregnancy is a natural event that is common during a women's lifetime. During pregnancy there is a dramatic increase in progesterone and oestrogen, which can have a large effect on the vasculature. During pregnancy, plasma volume increases by 40% and cardiac output also increases (Cipolla et al., 2013). This causes the cerebral circulation to adapt to avoid hyper-perfusion, and it does so by changing CBF. Furthermore, CVR to CO_2 has been shown to increase from pre-pregnancy to 36 weeks of pregnancy (Steinback et al., 2015). Interestingly, a lower CVR-CO_2 in the first trimester of pregnancy has been linked to the development of a pregnancy-related hypertensive disorder, preeclampsia (Riskin-Mashiah et al., 2002). This is more common in the posterior circulation (Cipolla et al., 2013) leading to questions about the differences in regional CBF regulation when hormone levels are high. Systemic vasodilation has been shown to increase during pregnancy, reducing the resistance and therefore increase blood flow (Phippard et al., 1986). FMD (a marker of NO-dependent vasodilation) has been shown to increase by up to 47% when comparing non-pregnant women to women in the 3rd trimester of pregnancy in a cross-sectional and longitudinal study (Dorup et al., 1999). Similar to the cerebrovasculature, in those with preeclampsia, FMD is reduced in women with preeclampsia (Weissgerber et al., 2016). High hormone levels have therefore been shown to affect markers of vascular health and can perhaps be used as a clinical tool to diagnose patients with hypertensive disorders.

2. Ovarian hyper/hypo stimulation

Ovarian hyper-stimulation can occur in women undergoing in vitro fertilization or ovulation induction when the ovaries and the pituitary glands are stimulated to increase circulating oestrogen levels. No studies as of yet have looked at the influence of ovarian hyperstimulation on either cerebrovascular reactivity or FMD. Ovarian hypo-stimulation (pituitary gland suppression) is used in laboratory settings to lower the levels of naturally circulating oestrogen to be used as a comparison to compare to normal or high levels of oestrogen. One study that compared ovarian-hypo-stimulation to normal levels, found that there was a reduction in MCAv that was associated with the reduction in oestrogen levels (Shamma et al., 1992). Furthermore, ICA flow has been shown to be decrease following ovarian hypo-stimulation as compared to normal levels (Nevo et al., 2007). These studies show that manipulating oestrogen levels experimentally can influence cerebral haemodynamics. No studies as of yet have looked at the influence of flow-mediated dilation and ovarian hypo/hyper-stimulation.

3. Oral Contraceptives

Oral contraceptives (OC) are a form of birth control than contain synthetic oestrogen and/or progesterone. Several studies have looked at the effect of hormonal contraceptives on the vasculature, but are affected by several caveats, such as the type of OC and the timing and duration of taking OC. One study that looked at transdermal oestrogen supplementation for 10-12 days after suppression of natural female sex hormone levels (using gonadotropin-releasing hormone antagonist) showed that there was significant increase in FMD% after oestrogen increase. (Miner et al., 2011). Interestingly, this increase in FMD% was removed after progesterone was added. Furthermore, progesterone administration (200mg orally) only

showed a trend towards decreased FMD%. Other studies looking at FMD and OC use have shown differing results, with either a higher FMD% in OC users when compared to controls (Friedman et al.,2011), no change (Shenshouda et al., 2018) or a lower FMD% in OC users compared to controls (Lizarelli et al., 2009). The length of OC use has been shown to significantly affect FMD % (Shenouda et al., 2018), with females taking OC for 12 years having lower FMD% as compared to females taking OC for up to 1 year and this may result in small atherosclerotic changes that promote endothelial dysfunction (Lizarelli et al., 2009). Currently there is no evidence looking into the influence of oral contraceptives on CVR-CO_2. Given that there is evidence that use of oral contraceptives for example, has been associated with risk of stroke in females with migraines (Sacco et al.,2017), it may be of interest to look at the impact of CVR and more research is needed.

2. Aims

The main aim of this study was to compare cerebral and peripheral vascular function over the course of the menstrual cycle in pre-menopausal females, and compare these response to post-menopausal females. The secondary aim was to assess how HRT may influence peripheral and cerebrovascular function in age-matched post-menopausal females.

2.1 Hypotheses

It was hypothesised that within pre-menopausal females, peripheral and cerebrovascular function measures (FMD and CVR) will be higher in the mid-luteal phase of the menstrual cycle (when oestrogen and progesterone levels are high) when compared to the early-follicular phase (when progesterone and oestrogen levels are low). It was further hypothesised that FMD and CVR measures will be higher in pre-menopausal when compared to post-menopausal

females. In summary, the cohorts that have higher female sex hormone levels will have higher FMD and CVR values, indicating a lower risk of CVD. It is hypothesised that the anterior circulation (MCA) will have a higher CVR when compared to the posterior circulation (PCA).

3. Methods

3.1 Repeatability Study

Measurements taken during duplex ultrasonography of the brachial artery have large variability and are highly operator dependent and therefore adequate training beforehand is necessary. To ensure results were reliable and repeatable, a Flow-Mediated Dilation (FMD) repeatability study was conducted by R Davies prior to testing.

3.1.1 Flow-Mediated Dilation

Duplex ultrasonography is a non-invasive technique used to measure brachial artery flow velocity and diameter (uSmart 3300, Terason) using a flow-mediated dilation technique (FMD) as described in previous guidelines (Thijssen et al., 2011). A 7.5MHz frequency linear array ultrasound transducer (Sonos 7500, Philips, The Netherlands) connected to a portable ultrasound system (Terason T300, USA) was placed 5-7cm from the elbow crease over the right biceps brachii muscle (Figure 5.1). Ultrasound gel was used to help transmit the signal from the transducer to the skin. The Doppler angle was maintained at 60 degrees to minimise the error in calculating blood velocity and was held in place by a mechanical probe holder. This was standardised throughout all participants (Figure 5.1). A suprasystolic pressure cuff was placed on the right forearm, 1-2cm distal to the anticubital fossa. After one minute of baseline measurements, the cuff was pumped up to 220mmHg for 5 minutes to occlude blood flow to the forearm. Measurements were also recorded 5 minutes' post-deflation. Blood

velocity was measured using pulse wave mode. Doppler gain, scale, contrast and angle correction were adjusted accordingly. Longitudinal 2D B-mode images were collected, measuring blood velocity and vessel walls and images were stored for offline use on FMD studio in Cardiovascular Suite (v.2.81, Quipu, Italy). Automatic edge detection software calculated baseline and peak diameter, FMD% change and all shear rate calculations.

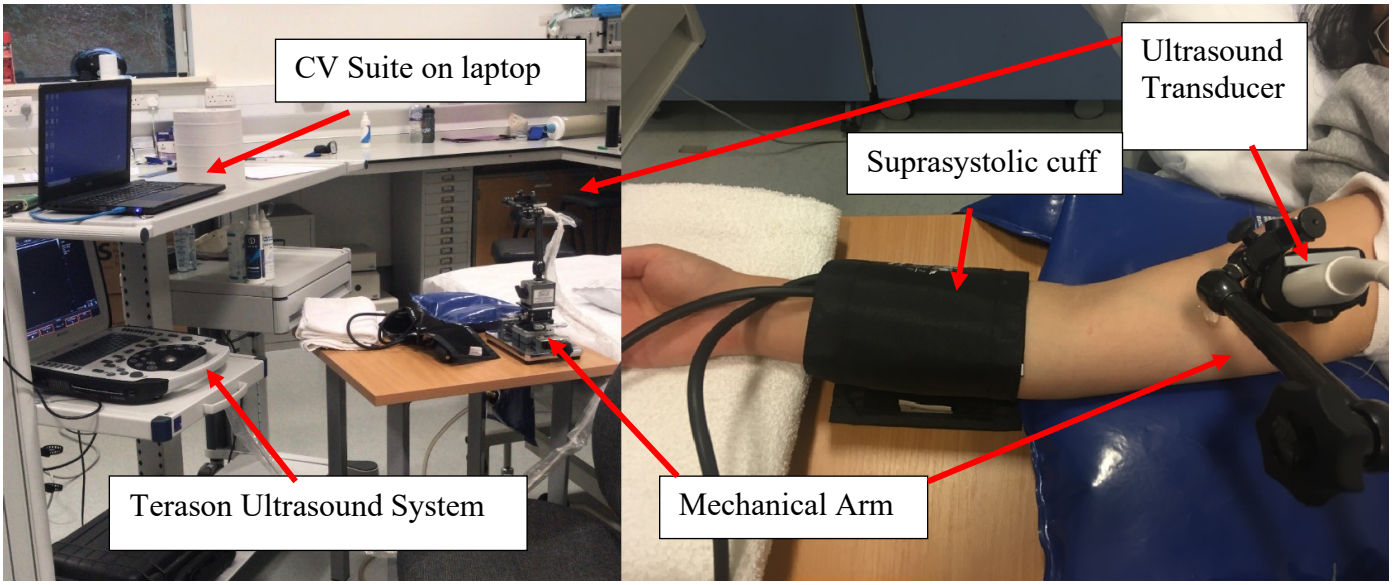

Figure 5.1. Ultrasound setup for FMD protocol.

3.1.2 Repeatability Protocol

The Flow-Mediated Dilation (FMD) protocol was conducted on the right brachial artery in healthy individuals (age range 18-35 years, n =10) to assess inter-day and intra-day measurements. Participants came into the laboratory on three separate occasions (at least 24hrs apart) and underwent the FMD protocol twice during each session (1hr apart). They were asked to refrain from caffeine 12 hours prior and alcohol and vigorous physical activity for 24 hours

prior to testing. For each testing session, participants lay supine at room temperature twenty minutes prior to measurements taken.

3.1.3 Analyses

Inter- and intra-day FMD measurements were analysed using Bland and Altman plots, coefficient of variation as well as Pearson correlation coefficient analyses. Results are presented as means (x) and standard deviations (SD). Coefficient of variation (CV) was calculated using the formula:

$$\text{Equation 2 : Coefficient of variation (CV)} = SD/x$$

Ten participants' arteries were assessed, with 60 brachial artery measurements taken in total. Baseline arterial diameter and FMD % were variables assessed.

Intra-day measurements were compared between two different times points (0 hour (a) and + 1 hour (b)) at each of the three visits the participants came to the lab. The Pearsons correlation coefficient between intra-day FMD% measurements was very strong for all three visits, with an overall r value of 0.896. The mean intra-day coefficient of variation for FMD% across all 3 visits was 2.66% ±0.51. Bland Altman graph analysis (Figure 6.1) showed that mean overall intra-day difference between FMD% values was 0.0923%. Limits of agreement ranged from -1.15% to 1.8%.

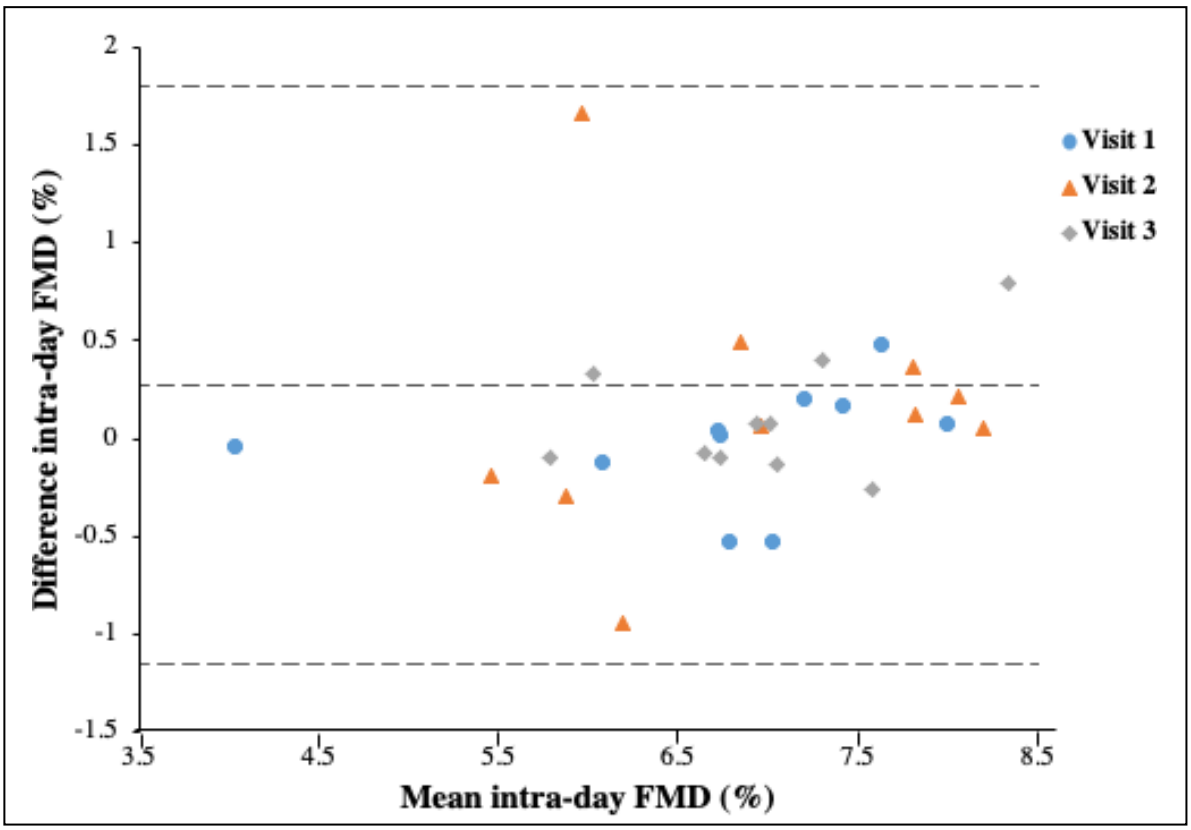

Figure 6.1. Bland-Altman graph of mean intra-day FMD% vs. differences in intra-day FMD% across the 3 visits.

The Pearson correlation coefficient for intra-day baseline diameter was very strong, with an overall r value of 0.990. The intra-day coefficient of variation for baseline arterial diameter was and 0.97% ±0.39. Bland Altman graph analysis (Figure 6.2) showed that the mean intra-day difference between baseline diameter values was 0.028mm. Limits of agreement ranged from -0.14mm to 0.18mm.

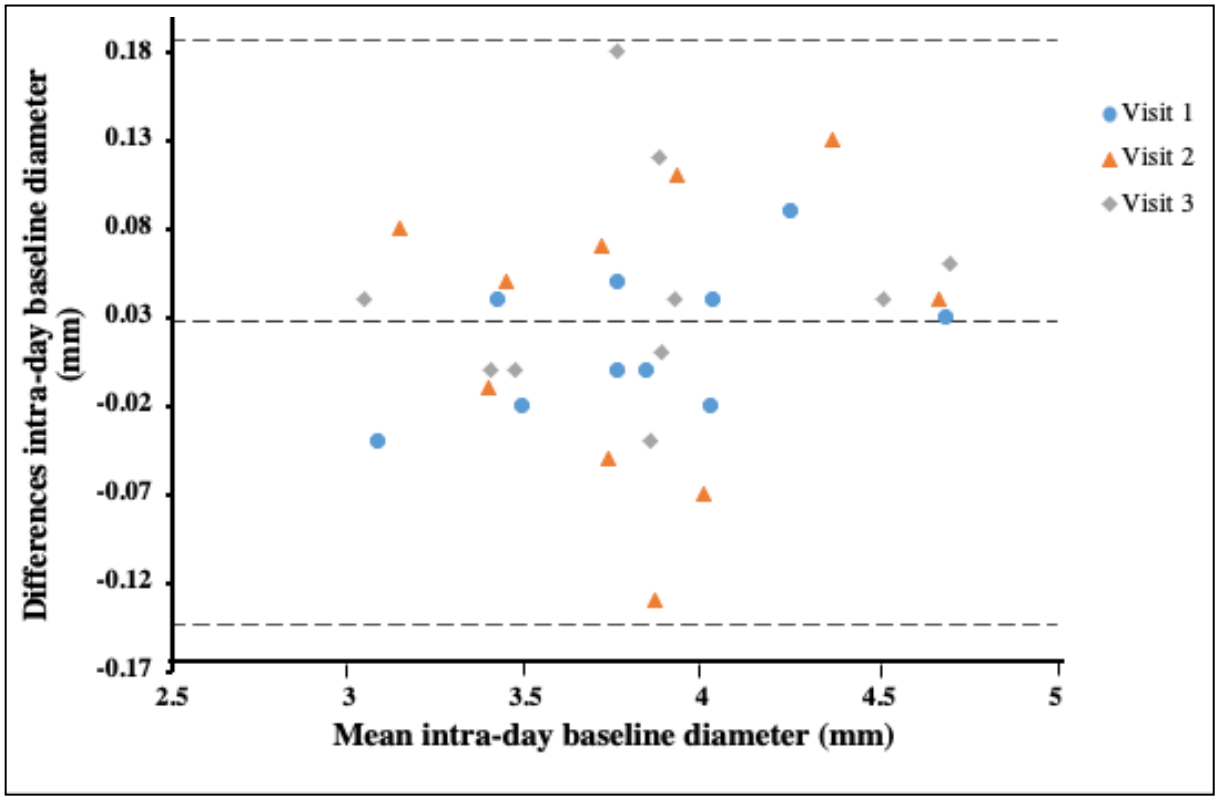

Figure 6.2. Bland-Altman graph of mean intra-day baseline diameter vs. differences in intra-day baseline diameter across the 3 visits.

The Pearson correlation coefficient for inter-day FMD% was strong, with an overall r value of 0.763.The mean inter-day coefficient of variation for FMD% was 6.17% ±0.84. Bland Altman Graph (Figure 6.3A) analysis showed that the mean inter-day difference between FMD% values was -0.163%. Limits of agreement ranged from -1.72% to 1.31%. The Pearson correlation coefficient for inter-day baseline diameter was strong, with an overall r value of 0.978. The mean inter-day correlation coefficient for baseline diameter was 1.55% ± 0.04. Bland Altman graph (Figure 6.3B) analysis showed that mean inter-day differences was -0.046mm. Limits of agreement ranged from -0.23mm -to 0.22mm. Recommended levels of good repeatability are <15% CV for FMD% and <2% CV for artery diameter (Thijssen et al., 2019). This was achieved in the current repeatability study, indicating that ultrasonographer (R Davies) measurements are sufficient enough to produce repeatable and reliable results.

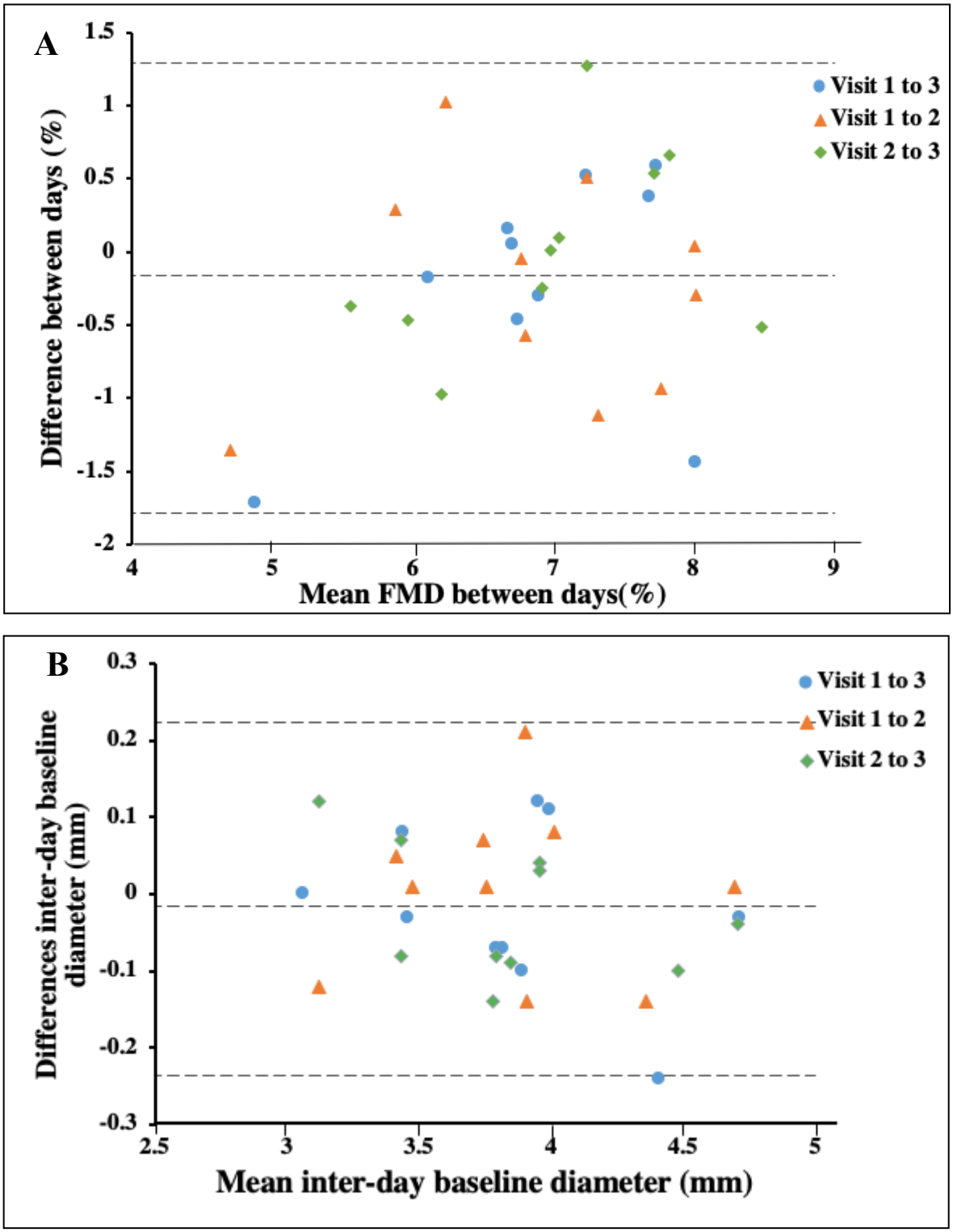

Figure 6.3. Bland-Altman graphs of the inter-day repeatability of FMD% measurements (A) and baseline diameter measurements (B). Dashed lines show the mean of differences and limits of agreement analysis.

3.2 Main Study

3.2.1 Participants

Nine pre-menopausal women and six post-menopausal women from the University of Birmingham and the local community volunteered to participate in this study. All pre-menopausal women had self-reported regular menstrual cycles, were on no form of hormonal contraception and were not pregnant. Data sets for both phases of the menstrual cycle were completed for six participants and these were used to compare results between the two phases. Post-menopausal women were recruited if aged 45-60 years and were at least >2years post-menopausal (defined as the absence of a menstrual cycle). Three of the post-menopausal women were using HRT (1mg oestradiol/0.5mg norethisterone acetate). Exclusion criteria included history of cardiovascular or respiratory diseases and those with history of migraines or prior preeclampsia during pregnancy. Written and informed consent was obtained from all participants and participants were made aware of their right to withdraw at any time. The study was approved by the University of Birmingham Ethics Committee and conformed to the Declaration of Helsinki. All testing took place in the School of Sport and Exercise and Rehabilitation Sciences at the University of Birmingham.

Participant Preparation

Pre-menopausal women were tested during two phases of the menstrual cycle and therefore were required to attend the laboratory on two occasions. The early follicular phase (EF) (days 1-3) and mid-luteal phase (ML) (days 22-24) were initially determined through self-report and an oral thermometer. These phases were then confirmed by blood levels of oestrogen and progesterone. The two phases chosen corresponded to low and high levels of circulating sex hormone levels. This wasn't completed at the time of writing this thesis due to time constraints.

Post-menopausal women were required to attend the laboratory on one occasion. Participants were instructed to consume no food 2 hours prior, no alcohol or vigorous exercise 24 hours prior and no caffeine 12 hours prior to testing.

3.2.2 Study Design

Each laboratory visit lasted approximately 2.5 hours and was divided into four parts to measure (familiarisation session, flow-mediated dilation, hypercapnic and hypocapnic challenges and a squat-to-stand protocol; figure 7.1). Pre-menopausal participants were familiarised only during their first visit. A squat-to-stand protocol was included to assess cerebral autoregulation. These data were not the focus of this thesis and therefore not included in the results. Pre-menopausal experimental sessions took place at the same time of the day to reduce the effect of circadian rhythm (Thosar et al., 2019; Strohm et al., 2014). The laboratory conditions were kept constant throughout all experimental visits.

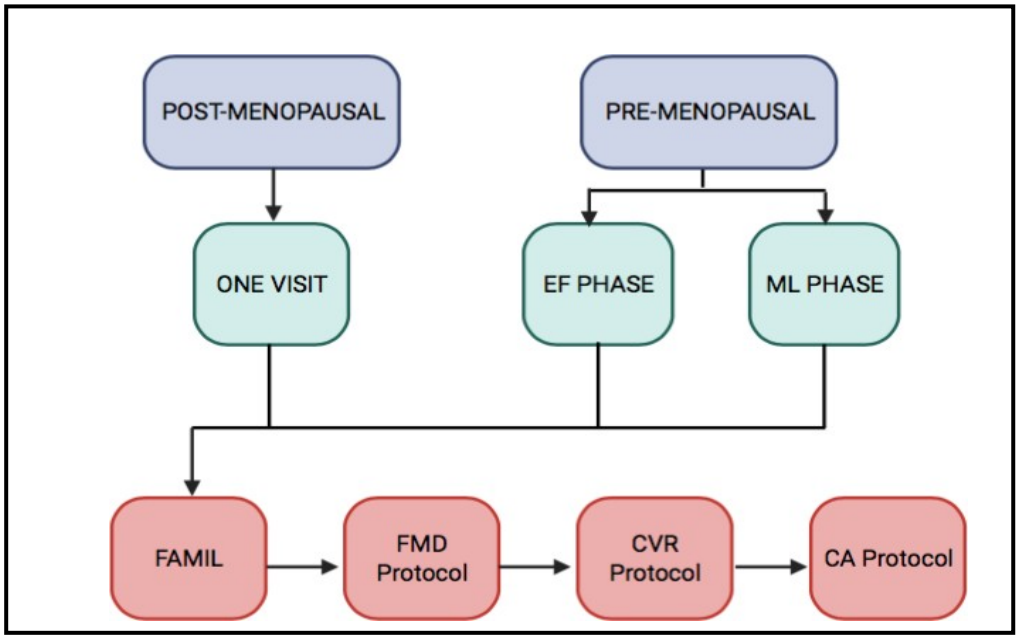

Figure 7.1. Schematic diagram of visits for post- and pre-menopausal females. EF: Early-Follicular, ML: Mid-Luteal, FMD: Flow-mediated Dilation, CVR: Cerebrovascular Reactivity, CA: Cerebral Autoregulation (analyses not included in this thesis).

Familiarisation Session

For pre-menopausal females, a familiarisation session took place prior to the actual testing sessions to familiarise participants to the equipment and ensure the participant was comfortable. Post-menopausal females were familiarised at the start of the actual testing session. Participants lay supine for 20 minutes before completing a full FMD protocol. They were then asked to breath in 2% and 5% CO_2 for 2 minutes each, a shorter time than used in the full protocol. This was followed by the hypocapnic challenges, in which participants hyperventilated for 2 minutes at two different levels of $PETCO_2$ (30mmHg and 24 mmHg).

Flow-Mediated Dilation Protocol

Measures were taken as described above (repeatability study) in a quiet room with the participant lying supine for 20minutes prior. To avoid inter-observer error, the same ultrasonographer (R Davies) took the FMD measurements for each participant.

Cerebrovascular Reactivity Protocol - Hypercapnic and Hypocapnic challenges

Following the FMD protocol, instrumentation of the Transcranial Doppler ultrasound (TCD) was set up in addition of respiratory and cardiovascular equipment was performed (see Figure 7.2). Participants wore a face mask (leading to a Douglas Bag with 2% and 5% CO_2) and a nose clip, to ensure they were only breathing through the mouth. Hypercapnic and hypocapnic challenges were performed in a similar manner to previous studies (Peebles et al., 2007). Figure 7.2 summaries the CVR protocol.

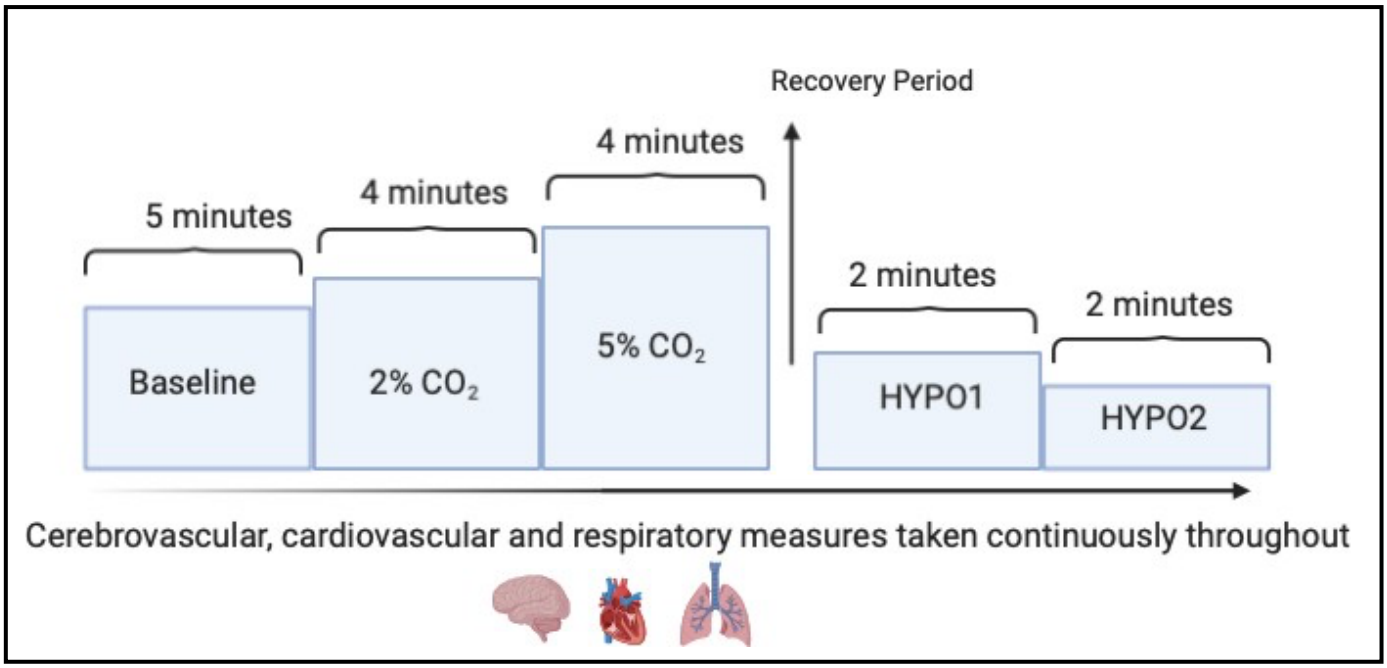

Figure 7.2. Summary of the Cerebrovascular Reactivity Protocol: the time-frame of each challenge (baseline, Hypercapnic challenge 1 1 (2%CO_2) and Hypercapnic challenge 2 (5% CO_2), Hypocapnic challenge 1 (Hypo1, 30mmHg $PETCO_2$ target) and Hypocapnic challenge 2 (Hypo2, 24mmHg $PETCO_2$ target).

The cerebrovascular reactivity protocol is summarised in figure 7.2. With the participant remaining in a supine position, baseline data was recorded for 5 minutes. This was followed by the hypercapnic challenge, in which participants breathed in 2% carbon dioxide (CO_2) for 4 minutes (21% oxygen (O_2), nitrogen (N_2) balanced) from an open-circuit Douglas bag, connected via a tube to a three-way valve (Figure 7.3). Immediately after this, participants breathed in a gas mixture of 5% CO_2. After a brief recovery period to allow $P_{ET}CO_2$ to return to baseline values, hypocapnic challenges were performed. Here participants were asked to voluntarily hyperventilate until a $P_{ET}CO_2$ target of 30mmHg and 24mmHg was reached. Each stage of $P_{ET}CO_2$ level was maintained for 2 minutes each. Verbal guidance by the

experimenter allowed the participant to reach these desired $P_{ET}CO_2$ levels. The order of the hypercapnic and hypocapnic challenges in this study were performed to eliminate the blunting effect that prior hypocapnia can have on the cerebral responses in hypercapnic conditions (Peebles et al., 2007).

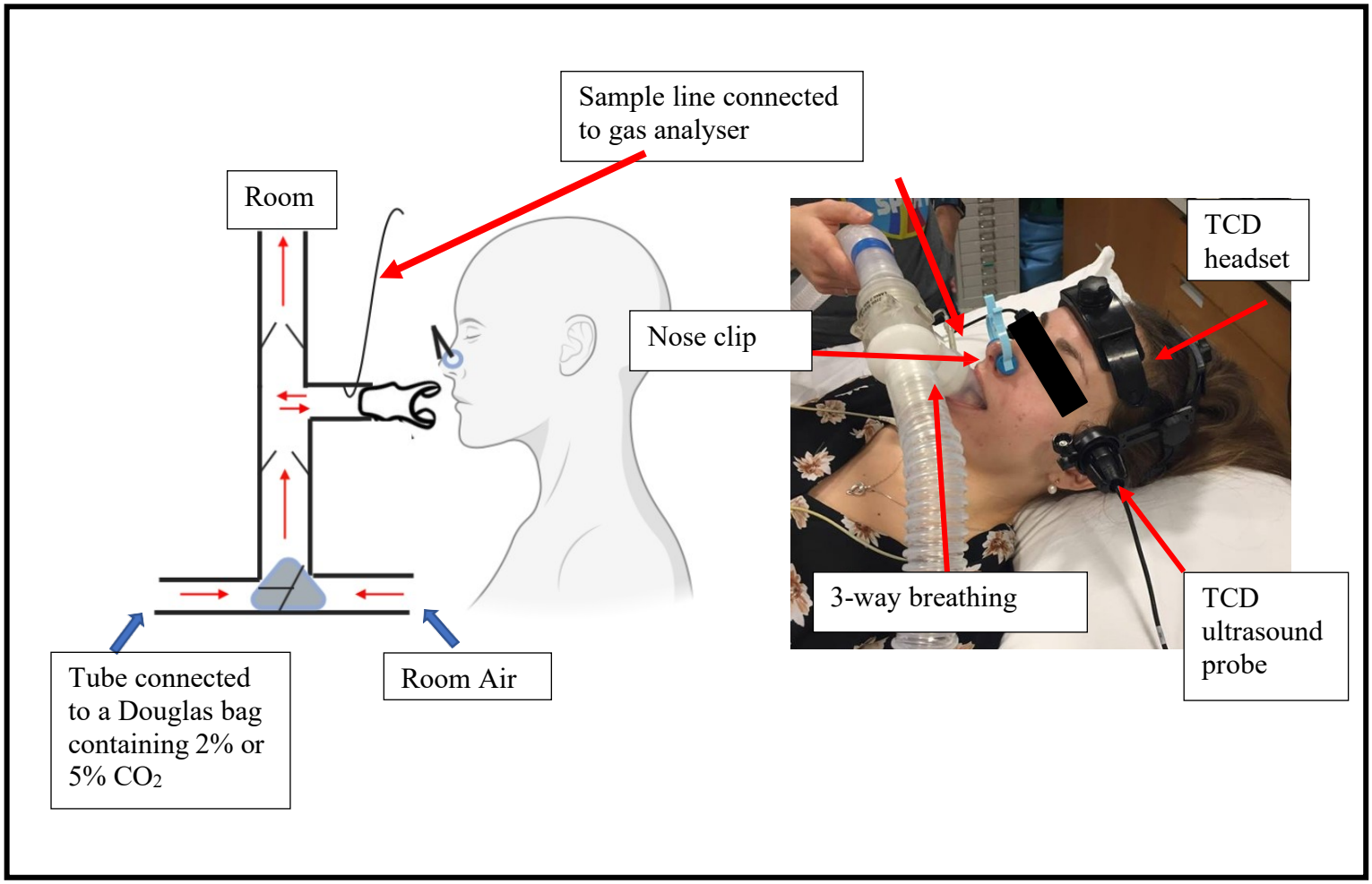

Figure 7.3. Breathing tubes and valves set up for CO_2 inhalation. Adapted from Liu et al., 2018. Red arrow represent direction of air flow and the real life setup of TCD and breathing equipment for cerebrovascular reactivity protocol.

2.2.3 Measures

Transcranial Doppler Ultrasound

Two 2-MHz frequency ultrasound probes with ultrasound gel were used to examine the posterior and middle cerebral artery (Multi Dop X, DWL, Compumedics Ltd, Germany) and secured in position using a headband (DWL, Compumedics Ltd, Germany) to allow correct position and angle to be maintained throughout the entire protocol. The right middle cerebral artery velocity (MCA) was located through the transtemporal acoustic window, superior to the zygomatic arch, running anteriorly and laterally to the ICA (Willie et al., 2011) at depths of 25 to 55mm. The left posterior cerebral artery (PCA) was located also through the transtemporal window running posterior to the ICA bifurcation in the same plane as the Circle of Willis, at depths of 60-70mm. The PCA exhibits a lower blood flow velocity when compared to the MCA (Prukayastha and Sorond, 2012). The direction of blood flow towards the Doppler probe (appearing red on the screen) as well as blood flow velocity and depth were used as confirmation that the correct vessel was isonated. The PCA was confirmed by using a simple eye movement task (closing and opening eyes). If there was a substantial change in flow velocity between closing the eyes to opening them and moving them left to right, it was confirmed that the PCA was found. Gain was adjusted to allow an optimal velocity signal with minimal interference. Beat to beat measurements of MCAv and PCAv were taken throughout the protocol. Data was recorded and stored on LabChart (v7.2.g, ADInstruments,USA).

Cardiovascular Measures

Heart Rate was measured continuously throughout the TCD protocol using a 3-lead ECG (BioAmp, ADInstruments, New Zealand). Mean arterial blood pressure (MAP) was measured throughout the course of the experiment using finger photoplethysmography (Finometer,

Finapress Medical Systems BV, Netherlands) and was placed on the middle finger of the left hand. Manual blood pressure measurements were also taken using a brachial bicep cuff (705-IT, Omron, UK) on the right arm at various time points throughout the protocol to verify finger photoplethysmography measures. Systolic and diastolic recordings were taken from this measurement and MAP was calculated the same way as LabChart (MAP = 1/3 systolic BP + 2/3 diastolic BP). These blood pressure measurements were used to measure cerebrovascular conductance (CVC).

Respiratory Measures

Participants wore a mouthpiece and a nose clip (see diagram 7.3). Changes in inspired and expired O_2 and CO_2 were measured from a sample line connecting to a breathing tube and a gas analyser (ML206 ADInstruments). Respiratory flow was measured using a pneumotachometer (Hans-Rudolph 3813, USA). This was then used to calculate minute ventilation (VE) using LabChart. Calibration was performed prior to testing using a 3-L syringe for volume and known concentrations of CO_2 and O_2 (~15% O_2, 5% CO_2) for calibration of the gas analyser.

Blood measures

Venous blood samples were taken at the end of each session to measure female sex hormones (oestrogen and progesterone levels). Due to the nature of this project, female sex hormone levels for the participant data reported in this thesis have yet to be analysed and therefore are not reported.

3.2.4 Data Analysis

Diameter of the brachial artery during the FMD protocol was measured using continuous edge detection and wall tracking software. A one minute mean was taken during the first minute (baseline), 2-minutes immediately post-deflation and the last minute of the protocol. Brachial blood velocity was measured throughout the protocol. Brachial diameter and blood velocity were both used for the calculation of shear rate. All calculations were automated using specific software (Cardiovascular Suite, v.2.81, Quipu, Italy). The FMD response is presented in absolute (mm) and relative (%) change.

Equations 3:

$$FMD\ (mm) = p*ak\ diam*t*r - r*stikg\ diam*t*r$$

$$FMD\% = \frac{P*ak\ diam*t*r}{R*stikg\ Diam*t*r}\ x\ 100$$

For the cerebrovascular reactivity protocol, all variables were recorded at 1k Hz via an analogue-to-digital converter (Powerlab, ADInstruments, USA) and stored on LabChart (v7.2, ADInstruments, USA). Data were extracted from the last minute of baseline, the last 30-seconds of the hypercapnic challenges (2%,5% CO_2) and the last 10-seconds of the hypocapnic challenges (30mmHg and 24mmHg)(Figure 7.4).These values were used for other cerebrovascular calculations.

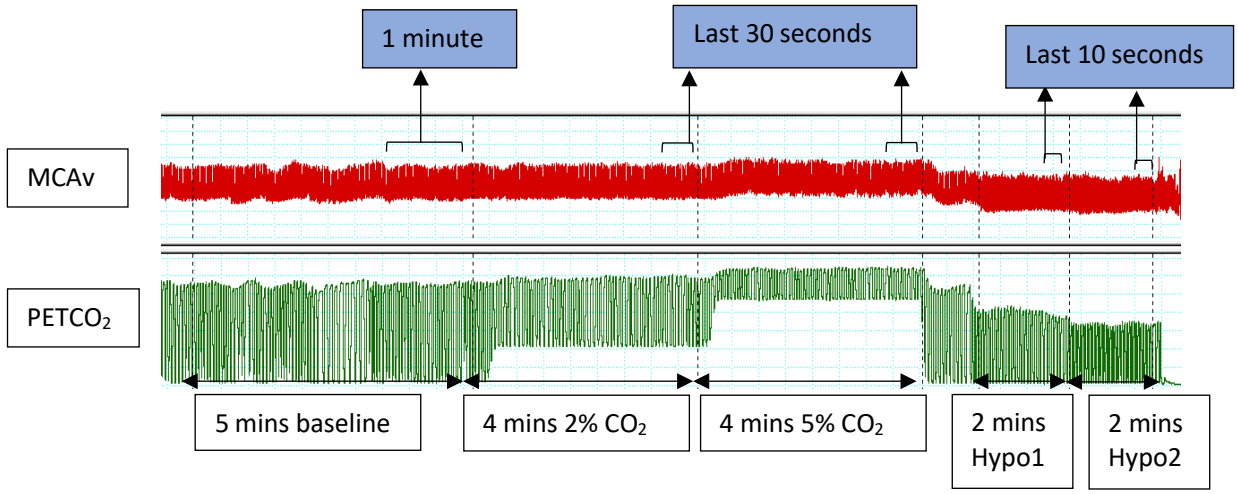

Figure 7.4: Showing a typical MCAv and $PETCO_2$ trace during baseline, 2% CO_2, 5% CO_2, hypocapnic challenge 1 (30mmHg) and hypocapnic challenge 2 (24mmHg). The blue boxes at the top represent where the data was extracted from.

Cerebrovascular Conductance (CVCi) was calculated by dividing MCAv (or PCAv) by MAP. Cerebrovascular reactivity (CVR) to CO_2 was calculated using MCAv and PCAv, using both relative (%) and absolute (cm/s) data. Furthermore, cerebrovascular reactivity was also presented as cerebrovascular conductance (CVCi) to determine CVR independent of BP changes. MCAv in the equations below are interchangeable with PCAv.

Equations 4:

Absolute Cerebrovascular Reactivity (cm/s/mmHg):

$$\frac{MCAv\ (hyp*r/hypA) - MCAv\ (bas*lik*)}{PETCO2(hyp*r/hypA) - PETCO2\ (bas*lik*)}$$

Relative Cerebrovascular Reactivity (%):

$$\frac{((MCAv\ (hyp*r/hypA) - MCAv\ (bas*lik*)/bas*lik*\ MCAv)\ x\ 100}{PETCO2(hyp*r/hypA) - PETCO2\ (bas*lik*)}$$

Absolute Cerebrovascular Reactivity (conductance):

$$\frac{\frac{MCAv}{MAP}\ (hyp*r/hypA) - \frac{MCAv}{MAP}\ (bas*lik*)}{PETCO2(hyp*r/hypA) - PETCO2\ (bas*lik*)}$$

Relative Cerebrovascular Reactivity % (conductance):

$$\frac{\left[\frac{MCAv}{MAP}\ (hyp*r/hypA) - \frac{MCAv}{MAP}\ (bas*lik*)\right] x\ 100}{PETCO2(hyp*r/hypA) - PETCO2\ (bas*lik*)}$$

3.2.5 Statistical Analysis

All statistical analysis was carried out on SPSS (IBM SPSS v.22.0, Chicago, IL,USA). A dependent sample t-test compared baseline cerebrovascular measures and FMD measures between the two phases of the menstrual cycle (EF x ML). Repeated measures within-subject analysis of variance (ANOVA) were used to compared MCAv, PCAv and $P_{ET}CO_2$ responses between menstrual cycle phases (2 levels: EF and ML) across all of the CO2 challenges (5 levels: hypo1, hypo2, baseline, 2%, 5% CO_2). Separate repeated measures ANOVAS were

conducted to compare cerebrovascular reactivity responses between menstrual cycle phases (2 levels: EF and ML) during hypercapnia (2 levels: 2% and 5% CO_2) and then again for hypocapnia (2 levels: hypo1 and hypo2). An independent sample t-test was used to compare cerebrovascular baseline measures and FMD measures between PRE and POST females. Repeated measures between-subject ANOVAs were used to compare MCAv and $P_{ET}CO_2$ responses between PRE and POST females (2 levels) across all of the CO_2 challenges (5 levels: hypo1, hypo2, baseline, 2%, 5% CO_2). Separate repeated measures ANOVAs were conducted to compare cerebrovascular reactivity responses between PRE and POST females (2 levels) during hypercapnia (2 levels: 2% and 5% CO_2) and then again for hypocapnia (2 levels: hypo1 and hypo2). An independent sample t-test was used to compare FMD measures between pre- and post-menopausal females and compare between post-menopausal females on HRT vs. those not on HRT. Dependent t-tests were used to compare between the PCA and MCA. Data are presented as means and standard deviations (SD) and statistical significance was set at $p < 0.05$. Results were tested for a normal distribution by Shapiro W Wilk tests, in which >0.05 indicated a normal distribution.

4. Results

4.1 Participant Characteristics

Pre-menopausal (PRE) women (20 ± 3yrs; 61.7±8 kg) completed two testing sessions, in the early-follicular (EF) (n = 9) and the mid-luteal phase (ML) (n =6) of their menstrual cycle. Six PRE participants completed both testing sessions and these were used to compare results between the two phases of the menstrual cycle. Post-menopausal women (POST (all); n=6) (57 ± 2yrs; 64.2 ± 5 kg) completed one testing session. Of these, three POST participants were taking hormone replacement therapy (HRT).

4.2 The menstrual cycle: the influence of short term natural cyclic oscillations in female sex hormones in pre-menopausal females

4.2.1 Cerebrovascular Reactivity Protocol

Baseline Measures

A dependent sample t-test compared baseline measures in pre-menopausal females (Table 3). There was no significant difference between PRE-EF and PRE-ML in baseline MCAv ($t(5) = .723$, $p = .510$) or baseline PCAv ($t(5) = .673$, $p = .549$). When these values were adjusted to account for MAP, there was no significant difference between the two menstrual cycle phases for baseline $CVCi_{MCAv}$ ($t(5) = .343$, $p = .749$) or $CVCi_{PCAv}$ ($t(5) = 3.17$, $p = .051$; Table 3). There was also no significant difference in baseline values between PRE-EF and PRE-ML for MAP ($t(5) = .944$, $p = .389$), HR ($t(5) = .634$, $p = .561$), or VE ($t(5) = .1.034$, $p = .360$). There was a significant main effect of menstrual cycle phase when comparing baseline $PETCO_2$ values

between PRE-EF and PRE-ML (t(5) = .2.956, p = .032), with $PETCO_2$ 2.7±. 0.6 mmHg higher in PRE-ML when compared to PRE-EF.

Table 3. Absolute cerebrovascular and cardiorespiratory baseline measures in pre-menopausal females (PRE; n = 6) at the early-follicular (EF) and mid-luteal (ML) phases of their menstrual cycle. Data presented as means and SD (±). * p = <0.05 when compared to PRE-EF.

	Baseline	
	EF	**ML**
MCAv (cm/s)	70±9	75±12
PCAv (cm/s)	48±2	45±10
$CVCi_{MCAv}$ (cm/s/mmHg)	0.81±0.11	0.79±0.11
$CVCi_{PCAv}$ (cm/s/mmHg)	0.57±0.06	0.48±0.07
MAP (mmHg)	88±12	82±11
$P_{ET}CO_2$ (mmHg)	37±1	40±1*
HR (bpm)	63±8	64±8
VE (l/min)	4.1±0.9	3.6±0.9

Abbreviations: EF = Early Follicular, ML = Mid-Luteal; MCAv = Middle Cerebral Artery Velocity; PCAv = Posterior Cerebral Artery Velocity; CVCi = Cerebrovascular Conductance Index; MAP = Mean Arterial Pressure; $P_{ET}CO_2$ = End-Tidal Carbon Dioxide; HR = Heart Rate; VE = Minute Ventilation.

Across the CO_2 challenges

A repeated measures ANOVA compared MCAv, PCAv and $P_{ET}CO_2$ values across all the CO_2 challenges in PRE females in the EF and ML phases of the menstrual cycle (Figure 8.1). There was no main effect of menstrual phase on MCAv across all of the CO_2 challenges ($F(1,5)$ = .022 , p = .890 , $\eta 2$ = .005), though there was a significant main effect of CO_2 challenge on MCAv ($F(4,20)$ = 64.96, p = <0.01, $\eta 2$ = .942; Figure 8.1B). Post-hoc analyses revealed that PRE female's MCAv at the 5% CO_2 challenge was 22.6±2.5 cm/s and 15.5±2.6 cm/s higher than baseline (p = 0.009) and the 2% CO_2 challenge (p = .04), respectively. When compared to baseline, MCAv was 22.1±2.5 cm/s and 27.4±4.2 cm/s lower at the hypo1 (p = 0.038) and hypo2 (p = .003) challenges, respectively. There was no main effect of menstrual phase on PCAv across all of the CO_2 challenge on PCAv ($F(1,5)$ = .3.879 , p = .120 , $\eta 2$ = .492; Figure 8.1A), though there was a significant main effect of CO_2 challenge on PCAv ($F(4,20)$ = 33.37 , p = <0.01, $\eta 2$ = .893). Post-hoc analyses revealed that PRE female's PCAv at the 5% CO_2 challenge was 19.5 ±3.1 cm/s and 12.9±2.1 cm/s higher than baseline (p= .035) and the 2% CO_2 challenge (p =.036) , respectively. When compared to baseline, PCAv was 16.3 ±2.6 cm/s and 20.3±3.7 cm/s lower at the hypo1 (p = .034) and hypo2 challenges (p = 0.05), respectively. There was no main effect of menstrual phase on $P_{ET}CO_2$ across all of the CO_2 challenges ($F(1,5)$ = .5.111 , p = .073, $\eta 2$ = .505), though there was a significant main effect of CO_2 challenge on $P_{ET}CO_2$ ($F(4,20)$ = 379.001 , p = <0.01, $\eta 2$ = .987). Post-hoc analyses revealed that PRE female's $P_{ET}CO_2$ at the 2% CO_2 challenge was 1.7±.0.4 mmHg higher than baseline (p = .033). $P_{ET}CO_2$ at the 5% CO_2 challenge was 5.2±0.6 mmHg and 3.4±0.5 mmHg higher than baseline (p = .005) and the 2% CO_2 challenge (p = .008), respectively. Furthermore, $P_{ET}CO_2$ at the hypo1 and hypo2 CO_2 challenges was 8.7±0.7 mmHg and 13.6± 0.6 mmHg higher than baseline, respectively (both p = .01).

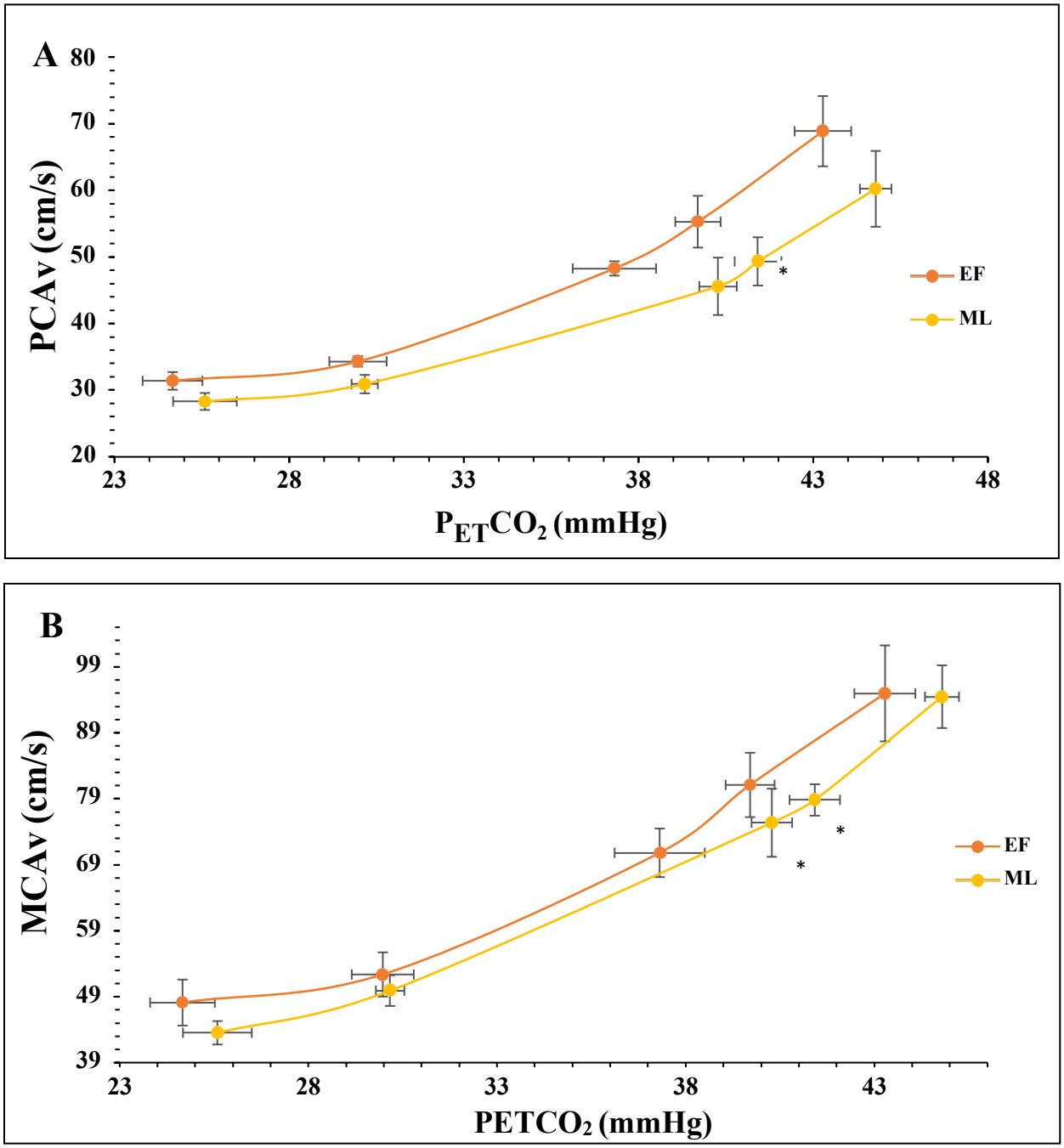

Figure 8.1. The PCAv (A) and MCAv (B) response to changing levels of $P_{ET}CO_2$ between pre-menopausal (PRE) females in the early-follicular (EF) and mid-luteal (ML) phases of the menstrual cycle. Data presented as means and SE bars. * = $p < 0.05$ compared to PRE-EF $PETCO_2$.

Hypercapnia

When comparing cerebrovascular reactivity (CVR) between the two phases of the menstrual cycle, neither absolute CVR_{MCAv} or CVR_{PCAv} values were significantly different between PRE-EF and PRE-ML during the hypercapnic CO_2 challenges (2% and 5% CO_2) ($F(1,5) = 0.25$, $p = .883$, $\eta 2 = .006$; $F(1,5) = .317$, $p = .604$; $\eta 2 = .073$, respectively; Table 5). Differences were also non-significant between PRE-EF and PRE-ML when comparing relative CVR_{MCAv} and CVR_{PCAv} values during the hypercapnic challenges ($F(1,5) = .305$, $p = .604$, $\eta 2 = 0.058$; $F(1,5) = .386$, $p = .587$, $\eta 2 = .114$, respectively; Figure 8.2A and 8.2B,respectively). There was no significant difference in absolute $CVCi_{MCAv}$-CVR or $CVCi_{PCAv}$-CVR between PRE-EF and PRE-ML ($F(1,5) = 2.52$, $p = .173$, $\eta 2 = .335$; $F(1,5) = .875$, $p = .403$, $\eta 2 = .179$, respectively). No significant difference was found between PRE-ML and PRE-EF in MCAv ($F(1,5) = 2.83$, $p = .191$, $\eta 2 = .486$) and PCAv during the hypercapnic challenges ($F(1,5) = 3.34$, $p = .165$, $\eta 2 = .527$, respectively; Table 4). There was no significant difference in $CVCi_{MCAv}$ ($F(1,5) = .522$, $p = .510$, $\eta 2 = .115$) or $CVCi_{PCAv}$ ($F(1,5) = .479$, $p = .539$, $\eta 2 = .138$) during the hypercapnic challenges between PRE-EF and PRE-ML. There was no significant difference in MAP ($F(1,5) = ..864$, $p = .395$, $\eta 2 = .147$), VE ($F(1,5) = .967$, $p = .381$, $\eta 2 = .195$) or HR ($F(1,5) = .062$, $p = .815$, $\eta 2 = .015$) during the hypercapnic challenges between PRE-EF and PRE-ML. However, there was a significant main effect of menstrual cycle phase on $P_{ET}CO_2$ levels at the hypercapnic challenges ($F(1,5) = 4.36$, $p = .047$, $\eta 2 = .466$), with $P_{ET}CO_2$ 1.7±0.6 higher in PRE-ML than PRE-EF during the 2% CO_2 challenge.

Hypocapnia

Absolute CVR_{MCAv} and CVR_{PCAv} values were not significantly different during the hypocapnic CO_2 challenges between PRE-EF and PRE-ML ($F(1,5) = .051$, $p = .832$, $\eta 2 = 0.013$; ($F(1,5)$

= 2.009; p = .251; η2 = .401, respectively; Table 5). Relative values were also non-significant between PRE-EF and PRE-ML when comparing CVR_{MCAv} (F(1,5) = .225, p = .655, η2 = 0.043) and CVR_{PCAv} (F(1,5) = 11.1, p = .079, η2 = .848; Figure 8.2A and 8.2B). There was also no significant different in absolute $CVCi_{MCAv}$ or $CVCi_{PCAv}$-CVR to the hypocapnic challenges between PRE-EF and PRE-ML (F(1,5) = 7.103, p = .076, η2 = .703; F(1,5) = .939, p =.387, η2 = .190). No significant difference was found between PRE-ML and PRE-EF at the hypocapnic challenges in MCAv (F(1,5) = 2.84, p = .167, η2 = .415) or PCAv (F(1,5) = 4.21, p = .132, η2 = .584; Table 4). There was no significant difference in $CVCi_{MCAv}$ (F(1,5) = .019, p = .896, η2 = .005)) or $CVCi_{PCAv}$ (F(1,5) = 2.175, p = .237, η2 = .420). There was no significant difference in MAP (F(1,5) = .706, p = .439, η2 = .124), VE (F(1,5) = .865, p = .405, η2 = 1.78), $P_{ET}CO_2$ (F(1,5) = .550, p = .492, η2 = .099) , HR (F(1,5) = .009, p = .930, η2 = .002) between PRE-EF and PRE-ML females during the hypercapnic challenges.

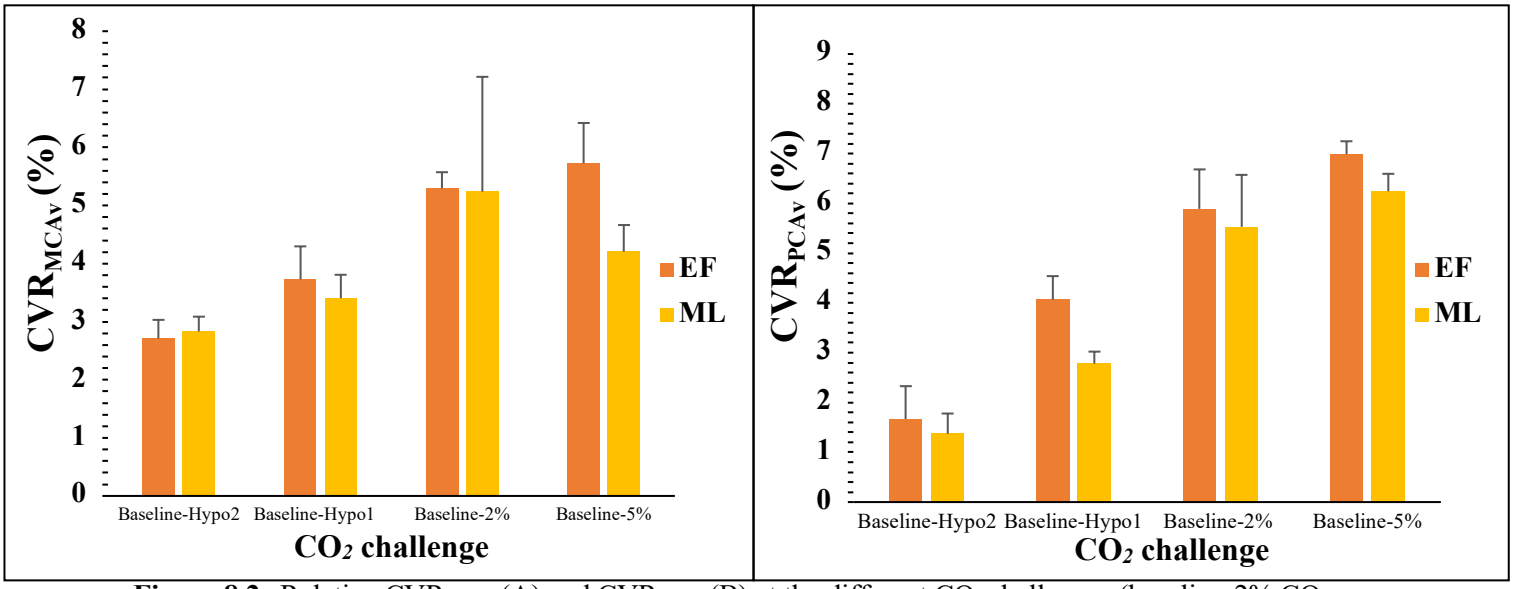

Figure 8.2. Relative CVR_{MCAv} (A) and CVR_{PCAv} (B) at the different CO_2 challenges (baseline-2% CO_2, baseline-5% CO_2, baseline-hypo1 and baseline-hypo2) between pre-menopausal females in the early-follicular (EF) and mid-luteal (ML) phases of the menstrual cycle. Data presented as means and SE bars. * = p <0.05 compared to PRE-EF.

Table 4. Absolute cerebrovascular and cardiorespiratory measures at the CO_2 challenges in pre-menopausal females at two phases of the menstrual cycle (PRE-EF and PRE-ML). * p = <0.05 when compared to PRE-EF females. Data presented as means and standard deviations (±).

	Hypo 2		Hypo 1		2% CO_2		5% CO_2	
	EF	**ML**	**EF**	**ML**	**EF**	**ML**	**EF**	**ML**
MCAv (cm/s)	48±8	44±4	52±8	49±5	81±11	78±5	94±17	94±11
PCAv (cm/s)	31±3	28±3	34±2	30±3	55±9	49±9	68±12	60±13
$CVCi_{MCAv}$ (cm/s/mmHg)	0.52±0.07	0.53±0.12	0.55±0.06	0.54±0.13	0.87±0.14	0.81±0.07	0.99±0.15	0.97±0.05
$CVCi_{PCAv}$ (cm/s/mmHg)	0.29±0.06	0.38±0.06	0.32±0.07	0.26±0.06	0.42±0.15	0.38±0.09	0.52±0.16	0.66±0.03
MAP (mmHg)	89±13	80±17	89±12	83±16	92±10	87±15	97±9	89±18
$P_{ET}CO_2$ (mmHg)	24±2	25±2	29±2	30±1	39±2	41±2*	43±2	45±1
HR (bpm)	78±11	78±9	71±8	70±10	65±6	67±5	68±7	67±8
VE (l/min)	20.7±4	21.9±5.9	13.6±2.3	16.6±6.7	5.8±1.8	6±1.1	12.2±4.7	14.1±4.9

Abbreviations: MCA = middle cerebral artery; CVCi = cerebrovascular conductance; CVR = cerebrovascular reactivity; EF = Early-Follicular Phase; ML = Mid-Luteal Phase $P_{ET}CO_2$ = end-tidal carbon dioxide; Hypo1/Hypo2 = Hypocapnic Challenge 1 or Hypocapnic Challenge 2

Table 5. Relative and absolute cerebrovascular reactivity values from baseline to hypercapnia (2% and 5% CO_2) or from baseline to hypocapnia (hypo1 and hypo2 challenges) in pre-menopausal females in the early-follicular (EF) and mid-luteal (ML) phases of the menstrual cycle. Data presented as means and standard deviations (±). * = p <0.05 when compared to PRE-EF.

	Baseline-Hypo 2		**Baseline-Hypo 1**		**Baseline-2% CO_2**		**Baseline-5% CO_2**	
	EF	**ML**	**EF**	**ML**	**EF**	**ML**	**EF**	**ML**
CVR_{MCAv} (%)	5.3±0.8	2.8±0.6	3.7±1.4	3.4±1	5.3±0.8	5.2±4	5.7±1.7	4.2±1.1
CVR_{PCAv} (%)	1.6±1.2	1.3±1	4.1 ±1.2	2.7±0.6	5.8±0.8	5.5±3	7±0.6	6.2±0.9
CVR-$CVCi_{MCAv}$ (%)	2.3±1.2	2.5±0.9	3.1±1.6	3.2±1.3	5±3.8	2.3±3	5.3±2.3	2.8±1.1
CVR-$CVCi_{PCAv}$ (%)	2.5±0.6	2.4±0.7	3.4±1.4	3.2±1.3	4.9±3.4	7.2±5.5	57±1.7	6.2±3.1
CVR_{MCAv} (cm/s/mmHg)	1.96±0.7	2.26±0.9	2.87±1.06	2.67±1.2	5.3±3.6	4.4±4	3.2±0.9	4±1.3
CVR_{PCAv} (cm/s/mmHg)	1.4±0.2	1.6±0.1	1.9±0.7	2±0.3	3.4±1.9	3.8±2.3	2.7±1.5	3.4±0.8
CVR-$CVCi_{MCAv}$ (cm/s/mmHg per mmHg $P_{ET}CO_2$)	0.02±0.01	0.02±0.01	0.02±0.01	0.02±0.01	0.04±0.03	0.03±0.02	0.04±0.01	0.02±0.01
CVR-$CVCi_{PCAv}$ (cm/s/mmHg per mmHg of $P_{ET}CO_2$)	0.01±0.01	0.04±0.07	0.02±0.01	0.02±0.01	0.03±0.02	0.08±0.1	0.03±0.01	0.03±0.01

Abbreviations: EF = Early-Follicular phase; ML = Mid-Luteal phase; CVCi = Cerebrovascular Conductance; MCA = Middle Cerebral Artery; PCA = Posterior Cerebral Artery. MC = Menstrual Cycle.

4.2.2 Cerebral circulatory differences in pre-menopausal females: Differences between the anterior and posterior circulations

A repeated measures ANOVA was used to compare CVR_{MCAv} and CVR_{PCAv} in pre-menopausal females (EF phase, n = 9). There was a significant main effect of cerebral vessel on absolute CVR ($F(1,9) = 16.73$, $p = .005$, $\eta 2 = .706$). Post-hoc analyses showed that absolute CVR in the MCA was higher by 3.5±1.2 cm/s/mmHg at the 2% challenge (p=0.029) and 1.5±0.6 cm/s/mmHg higher at the 5% challenge (p=0.035) when compared to CVR_{PCAv} (see Figure 8.3) Furthermore, CVR_{MCAv} was 0.98±0.2 cm/s/mmHg higher at the hypo1 challenge and 0.83± 0.2 cm/s/mmHg higher at the hypo2 challenge when compared to CVR_{PCAv}. When comparing relative CVR between the MCA and PCA, there was no significant main effect of cerebral vessel ($F(1,9) = .503$, $p = .496$, $\eta 2 = .053$; Figure 8.3).

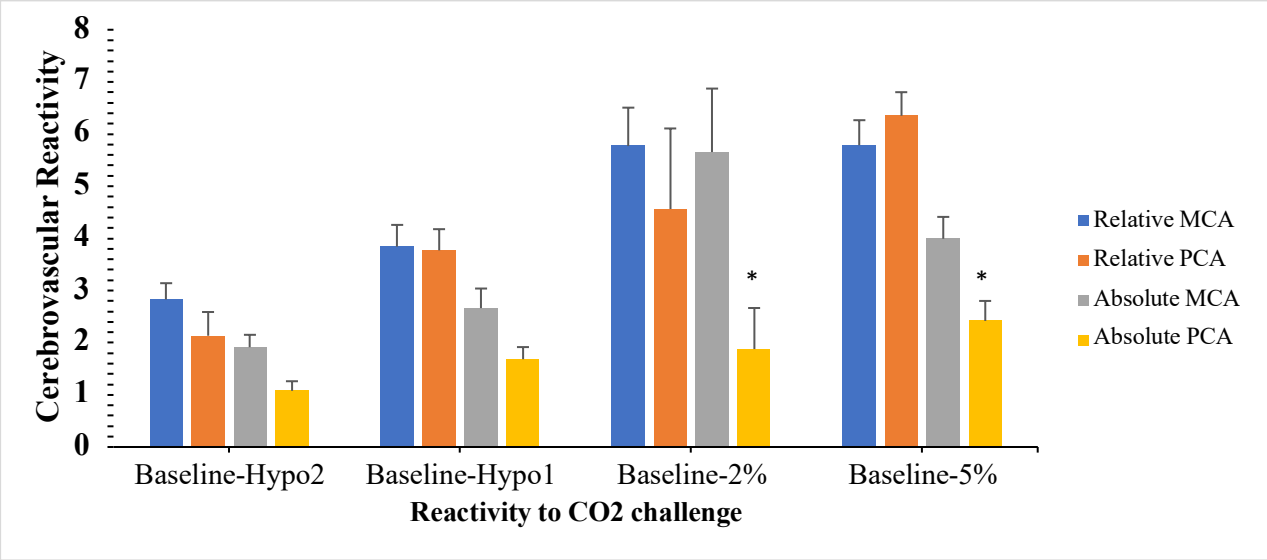

Figure 8.3. Absolute (yellow and grey bars) and relative (blue and orange bars) cerebrovascular reactivity (CVR) in the middle cerebral artery (MCA) and posterior cerebral artery (PCA) during hypercapnia (2%,5% CO_2) and hypocapnia (hypo1 and hypo2 challenges). * = $p<0.05$ when compared to absolute CVR_{MCAv}.

4.2.3 Flow-Mediated Dilation Protocol

A dependent sample t-test (two-tailed) showed there were no significant differences between PRE-EF and PRE-ML in brachial baseline diameter (t(5) = 1.77, p = .136; Figure 8.4A) and max. diameter (t (5) = .170, p =.872; Table 6). Furthermore, there were no significant differences in baseline shear rate (t (5) = .260, p = .737) and max. shear rate (t (5) = .793, p = .472). There was a significant main effect of menstrual cycle phase on FMD% (t(5) 2.61, p = 0.046), with PRE-ML having a 2.4±0.3% higher FMD (p = 0.046) when compared to PRE-EF (Figure 8.4B).

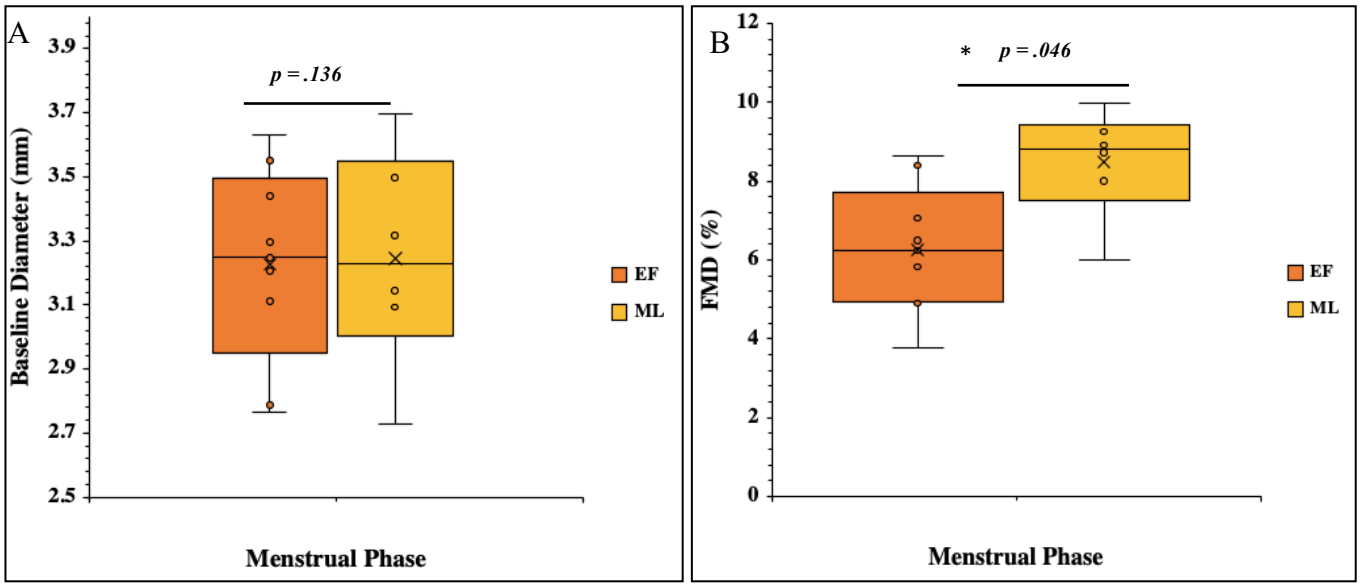

Figure 8.4A and 8.4B. A box plot showing brachial baseline diameter of the brachial artery (mm) and FMD (%) in pre-menopausal females during the early-follicular (EF) phase and mid-luteal (ML) phase of the menstrual cycle. * = p<0.05 when compared to PRE-EF females.

Table 6. Vascular function measures collected during the Flow-Mediated Dilation (FMD) protocol in pre-menopausal females (PRE) during the early-follicular (EF) and mid-luteal (ML) phases of the menstrual cycle. Data presented as means and standard deviations (±). * = p <0.05 when compared to PRE-ML. * = p <-0.05 when compared to PRE-EF AND PRE-ML.

	PRE (EF)(n=6)	PRE (ML)(n=6)
Baseline Diameter (mm)	3.3±0.3	3.25±0.3
Max. Diameter (mm)	3.51±0.3	3.51±0.3
FMD%	6.34±1.6*	8.5±1.3
Baseline Shear Rate (cm/s/mm)	466±101	524±122
Maximum Shear Rate (cm/s/mm)	1157±144	1150±147

4.3 The Effect of Menopause

Due to non-significant differences found between pre-EF and pre-ML cerebrovascular reactivity measures, females during the EF phase of the menstrual cycle (n=9) were compared to post-menopausal females (POST, the full cohort; n = 6) to examine any differences in cerebrovascular measurements. The PCA was only identified in two of the six POST participants, therefore was excluded as a measure in POST females. Three POST participants were taking hormone replacement therapy (HRT). Data for these participants as well as POST participants not on HRT (n=3) are presented but were not statistically compared due to the low n.

4.3.1 Cerebrovascular Reactivity Protocol

Baseline Measures

A dependent sample t-test showed there was no significant main effect of menopause when comparing between PRE and POST on MCAv ($F(1,13) = 2.435$, $p = .145$, $\eta 2 = .169$; Table 7). There was a significant difference in $CVCi_{MCAv}$ when compared to POST ($F(1,13) = 5.252$, $p = 0.041$, $\eta 2 = .304$). Cardiovascular measures (MAP, HR) at baseline were similar between PRE and POST females (HR = ($F(1,13) = 1.909$, $p = .19$, $\eta 2 = .128$; MAP= $F(1,13) = .148$, $p = .707$, $\eta 2 = 0.010$). There was a significant difference in ventilation between PRE and POST females ($F(1,13) = 4.699$ $p = 0.049$ $\eta 2 = .265$ but no difference in $P_{ET}CO_2$ was found ($F(1,13) = .010$ $p = .924$ $\eta 2 = .001$).

Table 7. Absolute cerebrovascular and cardiorespiratory measures at baseline in pre-menopausal females during the early-follicular phase of their menstrual cycle (EF) and post-menopausal females (POST). Data presented as means and SD (±). * p = <0.05. Mean and SD data are also presented for post-menopausal females not taking HRT (HRT) or taking HRT (no-HRT).

	PRE (EF)(n=9)	POST (n=6)	POST (HRT) (n=3)	POST (no-HRT) (n=3)
MCAv (cm/s)	73±11	62±2	72.4 ± 2.3	53 ± 11.6
$CVCi_{MCAv}$ (cm/s/mmHg)	0.80±0.11	0.67±0.10	0.75 ± 0.14	0.59 ± 0.12
MAP (mmHg)	91±11	93±9	98±10	89±6
$P_{ET}CO_2$ (mmHg)	39±3	38±2	37.1 ± 1.1	39.2 ±2.2
HR (bpm)	63±7	58±6	55±5	60±8
VE (l/min)	4.3±1.4	2.8±1.3	3.6± 1.4	2±0.4

Abbreviations: EF = Early Follicular, ML = Mid-Luteal; MCAv = Middle Cerebral Artery Velocity; PCAv = Posterior Cerebral Artery Velocity; CVCi = Cerebrovascular Conductance Index; MAP = Mean Arterial Pressure; $P_{ET}CO_2$ = End-Tidal Carbon Dioxide; HR = Heart Rate; VE = Minute Ventilation.

Across the CO_2 challenges

A repeated measures ANOVA compared MCAv and $P_{ET}CO_2$ values across all of the CO_2 challenges in PRE and POST females. There was no significant main effect of menopause on MCAv across the CO_2 challenges between PRE and POST ($F(1,12) = 3.207$, $p = .09$, $\eta 2 = .211$; Figure 9.1). There was a significant main effect of CO_2 challenges on MCAv ($F(4,48) = 96.9$, $p = <0.01$, $\eta 2 = .890$). Post-hoc analyses revealed that in both POST and PRE females, MCAv at the 2% and 5% CO_2 challenge was 7.19±1.3 cm/s ($p = .002$) and 19.5±2.5 cm/s ($p = .012$) higher than baseline, respectively. When compared to baseline, MCAv was 17.2±2 cm/s and 22.2±2 cm/s lower at the hypo1 and hypo2 CO_2 challenges (both $p = 0.00$), respectively. There was no significant main effect of $P_{ET}CO_2$ across the CO_2 challenges between PRE and POST ($F(1,12) = 1.931$, $p = .188$, $\eta 2 = .243$). There was a significant main effect of CO_2 challenge on $P_{ET}CO_2$ ($F(4,48) = 161.7$, $p = <0.01$, $\eta 2 = .926$). Post-hoc analyses revealed that $P_{ET}CO_2$ at the 2% and 5% CO_2 challenge was 1.9±0.5 mmHg ($p = .002$) and 6.6±0.7 mmHg ($p = .000$) higher than baseline. $P_{ET}CO_2$ at the 5% CO_2 challenge was 4.6±0.3 mmHg higher than the 2% CO_2 challenge ($p= .000$). When compared to baseline, $P_{ET}CO_2$ 6.8±1 mmHg and 12.4±2 mmHg lower at the hypo1 and hypo2 CO_2 challenges (both $p = 0.01$), respectively.

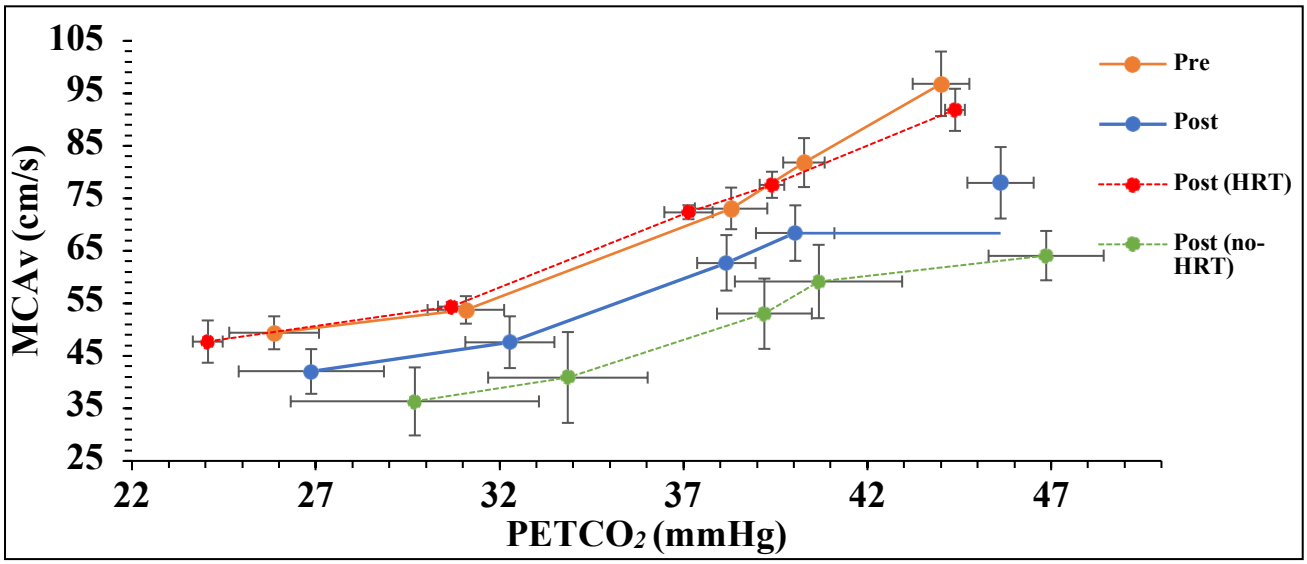

Figure 9.1. MCAv plotted against $P_{ET}CO_2$ levels throughout the CO_2 challenges comparing pre-menopausal females (PRE) and post-menopausal females (POST). Dotted lines represent sub-groups of POST females (HRT and no-HRT), where statistical analysis has not been carried out. Data presented as means and SE bars. * = $p < 0.05$ compared to PRE females.

Hypercapnia

When comparing absolute cerebrovascular reactivity (CVR) between PRE and POST females, there was a significant main effect of menopause during the hypercapnic CO_2 challenges ($F(1,13) = 8.005$, $p = .014$, $\eta 2 = .381$; Table 9). Absolute CVR_{MCAv} was 3.26±1.2 and 1.76±0.5 cm/s/mmHg higher in PRE compared to POST at the 2% (p =.041) and 5% CO2 challenges (p=.005), respectively. There was a significant main effect of menopause when comparing relative CVR_{MCAv} across the hypercapnic challenges ($F(1,13) = 4.809$, $p = .049$, $\eta 2 = .286$; Figure 9.2), with CVR_{MCAv} in PRE 1.7±0.5 cm/s/mmHg higher than POST at the 5% CO_2 challenge ($p = .034$). There was no significant difference in relative CVR between PRE and POST at the 2% CO_2 challenge (p= .106). There were no significant differences between PRE and POST in the hypercapnic challenges in absolute or relative $CVCi_{MCAv}$-CVR ($F(1,13) = 3.893$, $p = .072$, $\eta 2 = .230$; ($F(1,13) = 1.513$, $p = .242$, $\eta 2 = .112$, respectively). There was no significant main effect of menopause on MCAv between PRE and POST females ($F(1,13) = 3.795$, $p = .075$, $\eta 2 = .240$; Table 8). Despite this, there was a significant main effect of menopause on $CVCi_{MCAv}$ in the hypercapnic challenges ($F(1,13) = 4.864$, $p = .048$, $\eta 2 = .288$), with $CVCi_{MCAv}$ 0.206 cm/s/mmHg higher in PRE at the 5% CO_2 challenge, when compared to POST (p= .031). Although there was a trend for a higher $CVCi_{MCAv}$ at the 2% challenge in PRE females when compared to POST, the difference was not significant (p= .096). No significant difference was found between PRE and POST in MAP ($F(1,13) = 0.029$, $p = .868$, $\eta 2 = .002$), or $PETCO_2$ ($F(1,13) = 0.425$, $p = .526$, $\eta 2 = .032$) during the hypercapnic challenges. However, there was a significant main effect of menopause on ventilation ($F(1,13) = 4.977$, $p = .044$, $\eta 2 = .277$), with PRE having a 2.2±0.8 l/min higher VE than POST at the 2% CO_2 challenge ($p = .022$). Although there was a trend for a higher VE at the 5% CO_2 challenge in PRE females when compared the post, the difference was not significant (p= .076).

Furthermore, there was a significant main effect of HR between PRE and POST during the hypercapnic CO_2 challenges ($F(1,13) = 6.395$, $p = .025$, $\eta 2 = .330$). HR in PRE was 11±4 bpm higher than POST at the 5% CO_2 challenge ($p = .013$), yet there was no significant difference in HR between PRE and POST at the 2% CO_2 challenge ($p = .100$).

Hypocapnia

There was no significant main effect when comparing absolute and relative CVR_{MCAv} during the hypocapnic challenges between PRE and POST females ($F(1,13) = 1.125$, $p = .308$, $\eta 2 = 0.08$; $F(1,13) = .001$, $p = .974$, $\eta 2 = 0.01$, respectively; Table 9, Figure 9.2). There was also no significant differences in absolute or relative $CVCi_{MCAv}$-CVR between PRE and POST females during the hypocapnic challenges ($F(1,13) = .015$, $p = .904$, $\eta 2 = 0.001$; $F(1,13) = .236$, $p = .636$, $\eta 2 = 019$, respectively). There was no main effect of menopause when comparing MCAv or $CVCi_{MCAv}$ during the hypocapnic challenges ($F(1,13) = 1.67$, $p = .221$, $\eta 2 = 0.122$; $F(1,13) = .041$, $p = .42$, $\eta 2 = 0.03$; Table 8). There was also no significant main effect of menopause when comparing MAP ($F(1,13) = .234$, $p = .637$, $\eta 2 = .018$), VE ($F(1,13) = .1.161$, $p = .301$, $\eta 2 = 0.082$) or $P_{ET}CO_2$ ($F(1,13) = .368$, $p = .555$, $\eta 2 = .028$) between PRE and POST during the hypocapnic challenges. However, there was a significant main effect of menopause on HR during the hypocapnic challenges ($F(1,13) = 15.08$, $p = .002$, $\eta 2 = .082$), with HR 10±3 and 16±4 bpm higher in PRE at the hypo1 ($p = .009$) and hypo2 ($p = .002$) challenges, respectively.

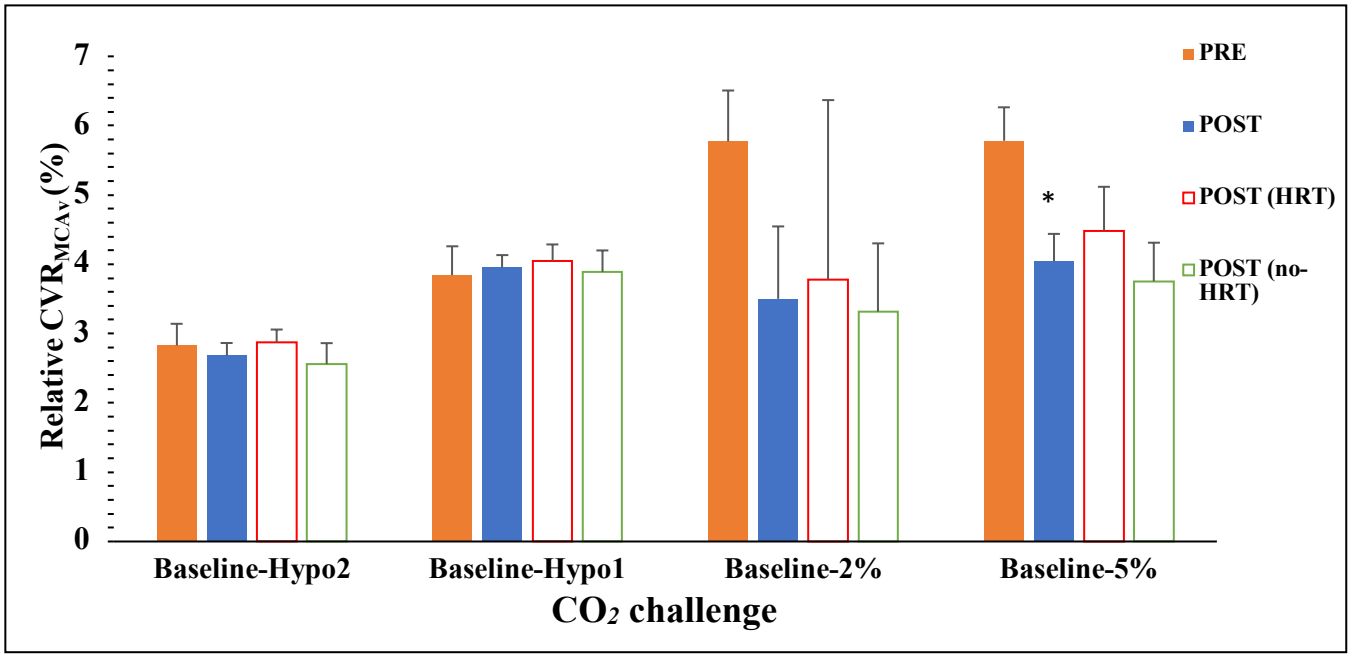

Figure 9.2. A bar graph comparing relative cerebrovascular reactivity in the middle cerebral artery (CVR_{MCAv}) between pre-menopausal females (PRE, orange filled bars) and post-menopausal (POST, blue filled bars) females during hypercapnia (2%, 5% CO_2) and hypocapnia (hypo1, hypo2). Open bars represent sub-groups of POST females (HRT, red; no-HRT, green) where statistical analyses has not been carried out. Data presented as means and SE bars. * = $p < 0.05$ compared to PRE females.

Table 8. Cerebrovascular and cardiorespiratory responses at baseline in pre-menopausal females during the early follicular phase of their menstrual cycle (PRE) and post-menopausal females (POST). Data presented as means and standard deviations (±). * p=<0.05 when compared to PRE females.

	Hypo 2		Hypo 1		2% CO_2		5% CO_2	
	PRE	POST	PRE	POST	PRE	POST	PRE	POST
MCAv (cm/s)	49±9	49±9	54±8	47±12	81±13	68±12	96±18	77±16
$CVCi_{MCAv}$ (cm/s/mmHg)	0.5±0.07	0.5±0.06	0.51±0.1	0.52±0.05	0.83±0.1	0.7±0.1	1.01±0.2	0.8±0.2*
MAP (mmHg)	89±12	92±8	90±11	92±8	93±10	92±10	96±9	96±9
$P_{ET}CO_2$ (mmHg)	25±3	26±5	31±2	32±2	40±1	40±2	44±2	45±2
HR (b/m)	80±9	63±3*	74±4	63±5*	65±7	59±6	71±7	59±4*
VE (l/min)	19.3±4.7	17.3±5	13.5±4	10.8±3	6.3±1	2.8±1.3*	12±3.6	8.6±3

Abbreviations: MCAv = middle cerebral artery velocity; CVCi = cerebrovascular conductance index; $P_{ET}CO_2$ = end-tidal carbon dioxide; VE = minute ventilation; MAP = mean arterial pressure; HR = heart rate.

Table 9. Relative and absolute cerebrovascular reactivity values from baseline to hypercapnia (2% and 5% CO_2) or from baseline to hypocapnia (hypo1 and hypo2 challenges) in pre-menopausal and post-menopausal females. Data presented as means and standard deviations (±). * = p <0.05 when compared to PRE.

	Baseline-Hypo2		Baseline-Hypo1		Baseline-2%		Baseline-5%	
	PRE	**POST**	**PRE**	**POST**	**PRE**	**POST**	**PRE**	**POST**
CVR_{MCAv} (%)	2.8 ± 1	2.7 ± 0.4	3.8 ± 1	3.9 ± 1	5.7 ± 2	3.5 ± 3	5.8 ± 1	4.04 ± 1*
$CVR\text{-}CVCi_{MCAv}$ (%)	2.6 ± 1	2.1 ± 1	4.02 ± 2	4.2 ± 1	4.5 ± 3	3.5 ± 2	4.5 ± 2	2.3 ± 2
CVR_{MCAv} (cm/s/mmHg)	1.9 ± 1	1.4 ± 1	2.7 ± 1	2.5 ± 1	5.5 ± 3	2.3 ± 2*	4.01 ± 1	2.3 ± 1*
$CVR\text{-}CVCi_{MCAv}$ (cm/s/mmHg per mmHg $P_{ET}CO_2$)	0.02 ± 0.01	0.01 ± 0.02	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.02	0.004 ± 0.02	0.04 ± 0.01	0.01 ± 0.001

Abbreviations: MCA = middle cerebral artery; CVCi = cerebrovascular conductance; CVR = cerebrovascular reactivity; EF = Early-Follicular Phase; ML = Mid-Luteal Phase $PETCO_2$ = end-tidal carbon dioxide; Hypo1/Hypo2 = Hypocapnic Challenge 1 or Hypocapnic Challenge 2.

4.3.2 Flow-Mediated Dilation Protocol

FMD values were significantly different between the phases of the menstrual cycle in PRE females therefore both phases were compared separately to POST females. Independent sample t-test was used to compare FMD values between groups. There was a significant main effect of menopause on baseline shear rate ($t(13) = 2.56$, $p = 0.025$; Table 10), with PRE-EF females having a 104±30 cm/s/mm higher baseline shear rate than POST. Furthermore, there was a significant main effect of menopause on maximum shear rate ($t(13) = 4.36$, $p = .001$; Figure 9.3B), with a 130±20 cm/s/mm higher maximum shear rate in PRE-EF compared to POST. There was no significant main effect of menopause on baseline diameter $t(13) = 1.42$, $p = .170$) or maximum diameter ($t(13) = 1.305$, $p = .215$; Figure 9.3A, Table 10). There was also no significant effect of menopause onFMD% when comparing PRE-EF and POST females ($t(13) = .753$, $p = .465$; Figure 9.4).

When comparing PRE-ML with POST, PRE-ML had a significantly higher baseline shear rate (159± 30 cm/s/mm; $t(10) = .289$, $p = 0.016$) and maximum shear rate (127±14 cm/s/mm; $t(10) = 3.86$, $p = 0.003$) compared to POST. There was no significant main effect of menopause on baseline diameter ($t(10) = 1.114$, $p = .291$) or maximum diameter ($t(10) = .669$, $p = .519$) when comparing PRE-ML and POST. There was a significant main effect of menopause on FMD% ($t(10) = 3.47$, $p = 0.006$), with PRE-ML having a 2.4%±0.6 higher FMD% when compared to POST.

Table 10. Vascular function measures collected during the Flow-Mediated Dilation (FMD) protocol in pre-menopausal females (PRE) during the early-follicular and mid-luteal phases (ML) of the menstrual cycle, as well as in post-menopausal females (POST). Data presented as means and standard deviations (±). ** = $p < 0.05$ when compared to PRE-ML. * = $p < 0.05$ when compared to PRE-EF. Mean and SD data are also presented for post-menopausal females taking (HRT) or not taking (no-HRT) hormone replacement therapy, yet no statistical analyses was carried out due to low n.

	PRE (EF)(n=9)	PRE (ML)(n=6)	POST(all)(n=6)	POST(HRT)(n=3)	POST(no-HRT)(n=3)
Baseline Diameter (mm)	3.21±0.3	3.25±0.3	3.47±0.4	3.6±0.3	3.3±0.4
Max. Diameter (mm)	3.41±0.3	3.51±0.3	3.66±0.4	3.9±0.3	3.45±0.4
FMD%	6.1±1.6	8.5±1.3	5.6±1.4**	6.8±0.5	4.4±0.8
Baseline Shear Rate (cm/s/mm)	469±86	524±122	365±54**	358.5±50	382±64
Max. Shear Rate (cm/s/mm)	1178±150	1150±147	1023±412*	1130±662	917±66

Abbreviations: ML = mid-luteal; EF = early follicular; HRT = hormone replacement therapy

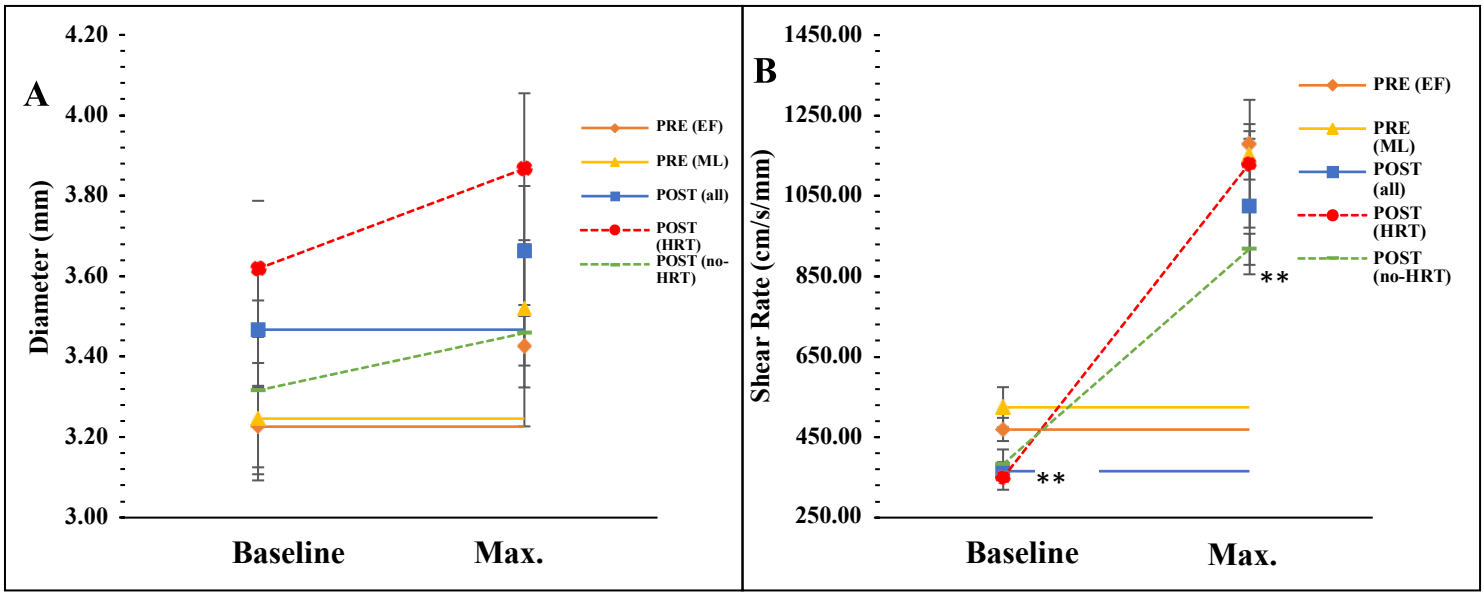

Figure 9.3A and 9.3B. Mean diameter of the brachial artery comparing baseline and maximum vasodilation as well as (B) Mean shear rate (cm/s/mm) at baseline and maximum vasodilation during the FMD protocol in post-menopausal females and pre-menopausal females ** p = <0.05 when compared to PRE-ML. Dotted lines separate out those females taking (HRT) and not taking (no-HRT) hormone replacement therapy, where statistical analysis was not carried out. See table 3 for full values and results. Values are presented as means and standard deviation bars.

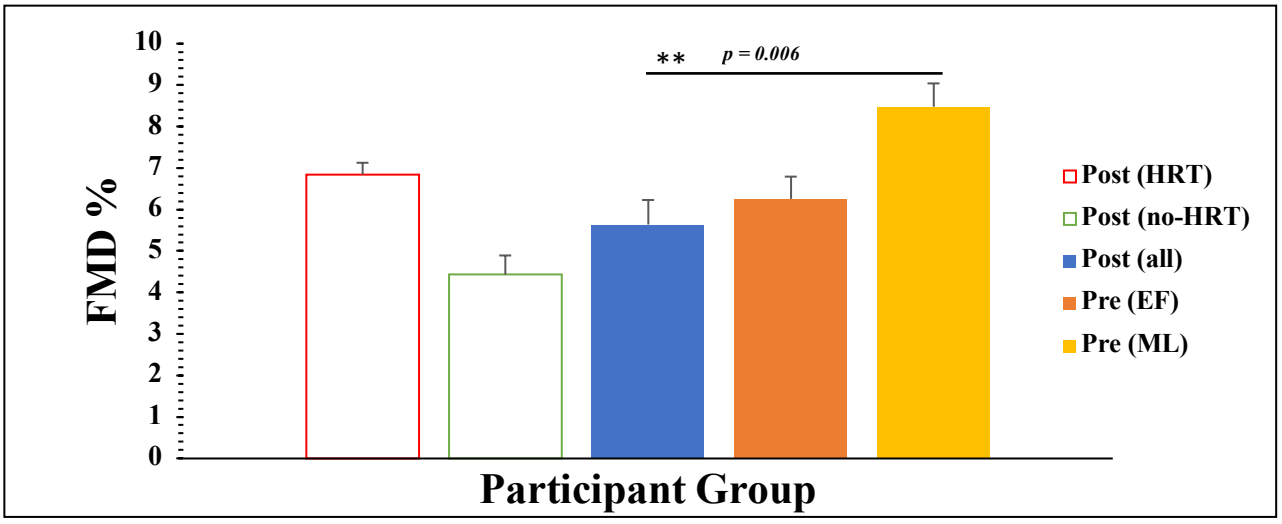

Figure 9.4. Mean flow-mediated dilation response (%) of the brachial artery in post-menopausal (all, HRT, no-HRT) and pre-menopausal (ML and EF) females. Values are presented as means and standard deviation bars. ** = p<0.05. when compared to pre-ML. Open bars represent sub-groups of POST females (HRT, red; no-HRT, green) where statistical analyses has not been carried out.

4.3.3 Hormone Replacement Therapy (HRT) observations

Out of the POST females, three were taking a form of hormone replacement therapy, yet due to the small sample size, statistical tests were unable to be carried out. However, data has been included in tables and graphs so comparisons and trends can be observed. MCAv and $CVCi_{MCAv}$ at baseline and over the CO_2 challenges appeared to be lower in POST females not taking any form of HRT when compared to PRE females and POST females taking HRT (the latter two appear comparable). However,$P_{ET}CO_2$ values appear to be comparable across all groups. Cerebrovascular reactivity values appeared to be affected by HRT, with a lower absolute CVR_{MCAv} observed in POST females not taking any form of HRT compared to PRE females. Values in POST females that were taking HRT appeared similar to PRE females for CVR_{MCAv}.

When comparing vascular measures, FMD% appeared to be higher in POST females taking HRT when compared to POST females that were not taking HRT. Furthermore, POST(HRT) females had a similar FMD% response as the PRE-EF females. However, POST (no-HRT) FMD% values appeared to be lower than PRE females in both phases of the menstrual cycle.

5. Discussion

The aim of this study was to compare cerebral and peripheral vascular function over the course of the menstrual cycle in pre-menopausal females, and compare these responses to post-menopausal females. The main findings show that in pre-menopausal females, CVR responses were similar between EF and ML phases of their menstrual cycles. The current study also showed that CVR and FMD measures were higher in pre-menopausal females when compared to post-menopausal females. This appeared dependent on menstrual cycle phase and possibly whether post-menopausal females were taking hormone replacement therapy (HRT), although data is limited.

5.1 The Menstrual Cycle: The influence of short term natural cyclic oscillations in female sex hormones

Cerebrovascular Reactivity Protocol

Measures taken over two phases of the menstrual cycle (low (EF) and high (ML) hormone phases) in pre-menopausal females were used to analyse the effect of natural cyclic fluctuations in female sex hormones on cerebral and peripheral vascular function measures. In the current study, no significant differences in baseline MCAv or PCAv were found between the ML and EF phases. This is in agreement with other studies (Diomedi et al., 2001; Krejza et al., 2013) that both show no significant change in MCAv over the course of the menstrual cycle, despite significant increases in oestrogen and progesterone levels. There is currently no literature looking at the PCA across the menstrual cycle. The current study also showed no differences in CVR_{MCAv} or CVR_{PCAv} to hypercapnia or hypocapnia between pre-menopausal females in the EF or ML phases of their menstrual cycle. This was contrary to our hypothesis, which was that

in pre-menopausal females, higher oestrogen and progesterone levels during the ML phase of the menstrual cycle would increase CVR, as compared to the EF phase. However, in agreement with our findings, a previous study also showed no differences in CVR_{MCAv} (measured by increased CO_2 inhalation) when comparing the EF, late follicular (LF) and ML phases (Favre et al., 2019). However, it is important to note that Favre et al., (2019) found no significant change in salivary oestradiol levels across the cycle, which may have been the reason for the lack of significance in results. Though, it should be noted that salivary oestradiol measures have been shown to be unreliable and not reflect the true change in female sex hormones across the cycle (Chatterton et al., 2005). In the current study, venous blood samples were taken to ascertain hormone levels, however, these samples have not been analysed at this time. Therefore, we are unable to confirm the hormone levels of the participants in the current study. Previous studies have shown an increase in CVR_{MCAv} in the LF or the ovulatory (O) phase of the menstrual cycle when compared to the EF phase (Peltonen et al., 2016; Diomedi et al., 2001). These findings indicate that the increase in oestrogen levels in the LF and O phases (78 pg/ml increase from EF to LF phase; Peltonen et al., 2016 and 150 pg/ml increase from EF to O; Diomedi et al., 2001) may be responsible for the increase in CVR. As far as aware, there are no studies looking at CVR_{PCAv} across the phases of the menstrual cycle, therefore the currently study's finding that CVR_{PCAv} did not change between menstrual cycle phases is a novel result. Higher oestrogen levels are associated with increased cerebral blood flow velocity (CBFV), enhanced cerebral vasodilation and a reduction in cerebrovascular resistance (Opsina et al., 2003), which would all increase CVR. The current study also found that MCAv and PCAv were similar between EF and ML phases of the menstrual cycle. In addition to the increased oestrogen levels found in these phases, progesterone levels reach their peak in the ML after being very low in the EF phase of the menstrual cycle. Progesterone has been shown

to antagonise the beneficial effects of oestrogen on the cerebral vessels in animal studies (Miller et al., 2013) and increase the resistance of cerebral arteries in humans (Souza et al., 2013). The increase in progesterone levels typically found in the ML phase of the menstrual cycle may have counteracted the protective mechanisms of the higher oestrogen levels found at this phase. This may be the reason for the similar blood flow velocities and CVR responses found between the ML and EF phases found in this study. More research is needed to look into the relationship and influences of fluctuating oestrogen and progesterone levels on the cerebrovasculature.

Differences in Cerebral Circulation in pre-menopausal females

There is a lack of data comparing different phases of the menstrual cycle and cerebrovascular reactivity in the posterior circulation. However, in the current study, when looking at the EF phase of the menstrual cycle, CVR in the PCA and MCA were significantly different. Absolute CVR to CO_2 was shown to be higher in the MCA compared with the PCA, yet no difference was found when comparing relative values, which is in agreement with other studies (Bruce et al., 2016; Skow et al., 2013) In one study, CVR_{MCAv} was reported as being 70% higher than CVR_{PCAv} (Bruce et al., 2016). The MCA has a greater diameter and therefore higher flow compared to the PCA, and this may be the reason for the higher absolute CVR but no difference in relative CVR (Willie et al., 2012). Furthermore, there may be a greater influence of vasodilators in the MCA when compared to the PCA, although this is largely unreported in the literature. Measuring the posterior circulation can give a more accurate reflection of global cerebrovascular reactivity, as the different circulations may react differently. In particular, the posterior circulation (i.e. PCA) has a lower absolute CVR. Given that the posterior circulation controls breathing, a measure of ventilatory reactivity and sensitivity to CO_2 could be

undertaken in future studies as changes in this factor may have influenced CVR . Despite, due to a smaller sample size, no significant differences were found between the two phases of the menstrual cycle in the current study, it is unclear as to whether female sex hormone levels influence different circulations in different ways.

Flow-Mediated Dilation Protocol

The current study showed that flow-mediated dilation increased in the ML phase of the menstrual cycle when compared to the EF phase in pre-menopausal females, indicating greater NO-dependent vasodilation in the peripheral vasculature when oestrogen and progesterone levels are higher during the ML phase. This is in agreement with other studies (Hashimoto et al., 1995 ; Harris et al., 2012), where pre-menopausal females in the ML phase of the menstrual cycle showed increased endothelium-dependent vasodilation (FMD%) when compared to the EF phase. Both Hashimoto et al., and Harris et al., showed similar levels of oestrogen in the luteal phase when compared to the late-follicular phase. These higher oestrogen levels may be the reason for the higher FMD% as oestrogen has been shown to increase the vascular response to shear stress (Huang et al., 1998) resulting in increased vasodilation and greater FMD %. Increased oestrogen levels also correlate with increased ER-α receptors in the peripheral vasculature (Stirone et al., 2003). The EF phase of the menstrual cycle has been shown to have a 30% reduction in ER-α expression in vascular endothelial cells when compared to higher hormone phases (i.e. the ML phase). This lower level of ER-α receptors in the EF phase has been correlated with a lower brachial artery FMD (Gavin et al., 2009). As ER-α receptors mediate NO via eNOS activation (Tan et al., 1999), NO-dependent endothelial vasodilation is thought to increase following increased ER-α receptors (Gavin et al., 2009). FMD is thought to be NO dependent and therefore the relationship between ER-α receptors, oestrogen and

nitric oxide may explain the higher FMD% in the ML (high hormone) phase of the menstrual cycle in the current study. If the mechanism behind the increased FMD is the oestrogen-NO mechanism, this may explain the lack of change in CVR, as the reactivity of the cerebral vessels have been shown to be NO-independent. (Harris et al., 2010). Therefore despite the increase in NO in the ML phase, CVR would be unaffected.

Some studies have shown how FMD% returns back to 'normal' (i.e. back to levels in the EF phase) at the early-luteal phase, after an increase at ovulation (Adkisson et al., 2010; Brandao et al., 2014). In all of these studies, the correct female sex hormone levels corresponded to the correct menstrual cycle phase. These data indicate that participants in the current study were in their ML phase and did have elevated circulating oestrogen levels, despite the analysis of female sex hormone levels not being carried out for this thesis. However, the analysis of circulating female sex hormone levels were unable to be conducted for this thesis and therefore we do not know for certain that our participants were in the correct EF and ML phases. Despite this, the change in FMD% in the ML phase of the menstrual cycle in the current study can give an indication as the mechanisms of oestrogen on the vasculature and how this differs dependent upon the vascular bed. The increase in FMD% but not CVR is of importance and may highlight the relationship between NO and increased oestrogen levels and the implications of this on vascular health.

5.2 Menopause: the influence of the natural reduction of female sex hormones on vascular function

Cerebrovascular Reactivity Protocol

Females were measured post-menopause to assess the impact of the natural decline in female sex hormone levels on the cerebral and peripheral vasculature, when compared to females that

are pre-menopausal. Data from our study has shown that MCAv and $CVCi_{MCAv}$ was lower in pre-menopausal females when compared to post-menopausal females, which is in agreement with other studies (Brislane et al., 2019). The naturally lower oestrogen levels found in post-menopausal females has been shown to increase the resistance and impendence of the cerebral vessels (Penotti et al., 2002), which is negatively associated with CBFv. Conversely, higher oestrogen levels in pre-menopausal females are associated with a decrease in cerebrovascular resistance and a higher CBFv (Krejza et al., 2001). These differences are likely as a result of oestrogen-related upregulation of ER-α and ER-β receptors in the cerebral blood vessels enhancing vasodilator substances (such as NO, prostacyclin) and promoting vasodilation and increasing blood flow (Hayashi et al., 1995). The lower MCAv and $CVCi_{MCAv}$ in post-menopausal females, as compared to the pre-menopausal females could indicate that lower oestrogen levels were present in the post-menopausal females in the current study. However, MCAv has also been shown to be negatively associated with ageing (Miller et al., 2019; Chen et al., 2018). There was a large age difference (e.g. a 37 ± 3 year age gap) between pre- and post-menopausal females in the current study. Consequently, it is likely that cerebrovascular differences between pre- and post-menopausal females were influenced by age-related vascular changes in the post-menopausal group. A study that compared pre-menopausal females over the course of surgical menopause showed no differences in MCAv from pre- to post-menopausal females, yet did not compare CVR (Penotti et al., 2002). Furthermore, another study showed that CVR_{MCAv} was higher in pre-menopausal females when compared to post-menopausal females, yet when participants were age-matched (e.g. late pre-menopausal vs. post-menopausal), no significant difference in CVR_{MCAv} was found, implying that age is the greater factor in CVR_{MCAv} change (Brislane et al., 2019). Of note, females sex hormone concentrations were not measured in Brislane's study and pre-menopausal were not exclude if

they displayed menopausal symptoms. It may be possible that late pre-menopausal females were actually peri-menopausal and had already seen a decrease in female sex hormone levels. It is hard to tell whether the lack of CVR_{MCAv} in this comparison is entirely due to age and or is influenced by the already low female sex hormone levels in the late pre-menopausal females.

Other studies are in agreement with these results and have shown a lower CVR_{MCAv} in post-menopausal females when compared to pre-menopausal females (Matteis et al., 1998; Olah et al., 2000; Kastrup et al., 1998). A reduction in oestrogen at the onset of menopause decreases in the amount of oestrogen-dependent vasodilation in the cerebral arteries, and therefore may decrease CVR to CO_2 (Barnes et al., 2019). As well as a reduction in oestrogen levels with the onset of menopause, there is evidence that the abundance of ER-α receptors is decreased, which reduces the action of oestrogen-promoted vasodilation as previously discussed (Gavin et al., 2009). Since hypercapnia evokes a vasodilatory response in the cerebral vessels, the lack of oestrogen-related vasodilation may be the reason that CVR to hypercapnia was significantly different between pre- and post-menopausal females, yet no difference to hypocapnia was found (largely vasoconstriction) in the current study.

Flow-Mediated Dilation Protocol

In our study, brachial artery FMD was found to be higher in pre-menopausal females during the ML phase of their menstrual cycle when compared to post-menopausal females. This is in agreement with other studies, in that a reduction in FMD% occurs at the onset of menopause (Brislane et al., 2019; Green et al., 2016; Celermajer et al., 1995; McCrohen et al., 1996; Moreau et al., 2012). Conversely, there was no difference between post-menopausal and pre-

menopausal females during their EF phase, though mean FMD% values were similar to other studies (Brislane et al., 2019). Thus, it is likely that we were underpowered in the current study due to the small sample size and therefore, results should be interpreted with caution as individual variability is high. However, the higher levels of oestrogen and progesterone levels during the ML phase seemed to increase FMD % to an extent, where despite a lower n, there was a significant difference as compared to post-menopausal females. Lower levels of oestrogen at the onset of menopause can lead to a decreased response to shear stress (as indicated by the lower shear rate in post-menopausal females when compared to pre), which decreases NO production and reduces vasodilation. As FMD is thought to be largely NO dependent, the mediation of NO by increase oestrogen levels may explain the higher FMD response found in pre-menopausal females in the ML phase of their menstrual cycle. When oestrogen levels are dramatically reduced at the onset of menopause, several other vascular endothelium mechanisms are changed. The increase in ET-1(a vasoconstrictor) and decrease in BH_4 (a cofactor for eNOS) that occur at menopause are both related to the reduction in oestrogen levels and may lower FMD response (Moreau et al., 2012; Bourque et al., 2011). Furthermore, the reduction in oestrogen in post-menopausal females may increase levels of atherosclerosis in peripheral vessels via directly reducing its anti-atherogenic benefits (such as suppression of vascular smooth muscle migration; Geraldes et al., 2002). Given that atherosclerosis is directly associated with endothelial dysfunction, a lower FMD response in post-menopausal females (found in the current study) may also be due to higher levels of atherosclerosis. A decreased FMD response is also associated with ageing, with FMD% decreasing in the 6_{th} decade of life (Celermajer et al., 1994). Participants in the current study were not age-matched, yet post-menopausal females were all <60 years old. Therefore, it seems less likely that age had a strong effect in the current study as a previous study has shown a

stronger correlation was found between FMD response and the onset of menopause as oppose to FMD response and age, when females were measured over the course of the menopausal transition (Moreau et al., 2012).

Hormone Replacement Therapy Observations

Observations from the current study showed that post-menopausal females taking HRT had comparable cerebrovascular measures (MCAv, $CVCi_{MCAv}$, absolute and relative CVR_{MCAv}) to pre-menopausal females, highlighting a possible protective benefit of oestrogen/progesterone supplementation during post-menopause. Previous studies have shown CVR_{MCAv} to be higher (Kastrup et al., 1998) or unchanged (Bain et al., 2004) in post-menopausal females taking HRT, yet the literature varies in methodology, making results difficult to compare. Results in the current study were similar to previous studies when looking at the FMD. In the current study, there was a sustained increase in FMD % in post-menopausal females taking HRT when compared to pre-menopausal females, which is in agreement with other studies (Koh et al., 2001; Bush et al., 1987; Wakatsuki et al., 2001). It would be of interest to look at similar cohorts (post-menopausal females taking HRT vs. not taking HRT) but with a larger sample size and further research is needed to look at differences between timing/type of HRT and their influence on post-menopausal females.

5.3 Strengths and Limitations

This study presents preliminary evidence that different levels of female sex hormone levels can impact cardiovascular and cerebrovascular disease markers (CVR and FMD). The main limitation in this study is the small sample size, which underpowers the results and increases the likelihood of type II error. Future studies should use a larger cohort of pre- and post-

menopausal females to increase the reliability of results. The results from post-menopausal females using HRT present novel data on peripheral and cerebral vascular function and this would be strengthened by increasing the sample size. Furthermore, the cohort of post-menopausal females on HRT all varied in the type/timing of HRT use which ideally would also be controlled for. Other risk factors that can affect vascular function measures were not controlled for in the current study, for examples, LDL levels (Rosendorff et al., 2002), physical exercise (Siasos et al. 2013), time since menopause (Vitale et al., 2007) and age at menopause (Senese et al., 2020). However, a previous study has shown a strong association between menopause and FMD response (Moreau et al., 2012) independent of other risk factors. Therefore it could be anticipated that a menopause effect would be detectable despite other risk factors were not being fully controlled for. Although female sex hormone levels were collected, they were not analysed and presented in this thesis due to time constraints. Therefore, it is impossible to determine how much female sex hormones accounted for differences found between groups and what extent hormone levels varied between groups. Furthermore, analysis on other cardiovascular and respiratory measurements should be undertaken, such as the reactivity calculations of HR and ventilation to CO_2 inhalation and the influence of this on CBFV. The ventillatory data in the current study is unphysiologically lower and present an issue in equipment or calculation. Finally, caution should be applied when using TCD to measure CVR, as conflicting evidence has shown that vessel diameter may change during CO_2 inhalation and this is impacted by increased age (Hoiland et al., 2016; Coverdale et al., 2016). Despite this, in many studies that look at age, CVR values measured by TCD and MRI are similar (Wise et al., 2007). Future studies should include duplex doppler measurements on the carotid artery to measure exact diameter changes and get a better overall picture of blood flow velocity in the cerebral vessels.

5.4 Conclusion

Overall the current study has shown that markers of vascular function and overall cardiovascular and cerebrovascular health (CVR and FMD) vary depending on different levels of female sex hormones. Data found in the current study has shown how natural fluctuations of female sex hormones over the course of the menstrual cycle can influence the peripheral vasculature (i.e. FMD %) but not the cerebral vasculature. When comparing pre-menopausal females to post-menopausal females, absolute CVR_{MCAv} was found to be lower in post-menopausal females. FMD% in post-menopausal females was also significantly lower when compared to pre-menopausal females in the high female sex hormone phase of their menstrual cycle (PRE-ML females). Therefore, it can be speculated that increased levels of female sex hormones has a positive yet varying effect on different vasculatures. This also seems to be the case for females taking HRT during menopause, though more data needs to be collected. Research in this area can be used to provide insight into the cardioprotective mechanisms of female sex hormones, which may be used to aid interventions in post-menopausal females that are at increased risk of developing cardiovascular and cerebrovascular diseases.

6. References

Aaslid, R. (2006) Cerebral autoregulation and vasomotor reactivity. **Frontiers of neurology and neuroscience,** 21 216-228.

Adkisson, E.J., Casey, D.P., Beck, D.T., et al. (2010) Central, peripheral and resistance arterial reactivity: fluctuates during the phases of the menstrual cycle. **Experimental biology and medicine (Maywood, N.J.),** 235 (1): 111-118.

Ainslie, P.N., Murrell, C., Peebles, K., et al. (2007) Early morning impairment in cerebral autoregulation and cerebrovascular CO2 reactivity in healthy humans: relation to endothelial function. **Experimental physiology,** 92 (4): 769-777.

Aisha S S Meel-van den Abeelen, Joep Lagro, Arenda H E A van Beek, Jurgen A H R Claassen 1 (2014) **Impaired cerebral autoregulation and vasomotor reactivity in sporadic Alzheimer's disease**. **Curr Alzheimer's Res.,** 11 (1):.

Al-Safi, Z. and Santoro, N. (2014) Menopausal hormone therapy and menopausal symptoms. **Fertility and sterility,** 101 (4): 905-915.

Anderson, G.L., Limacher, M., Assaf, A.R., et al. (2004) Effects of Conjugated Equine Estrogen in Postmenopausal Women With Hysterectomy: The Women's Health Initiative Randomized Controlled Trial. **JAMA : the journal of the American Medical Association,** 291 (14): 1701-1712.

Ando, J. and Yamamoto, K. (2013) Flow detection and calcium signalling in vascular endothelial cells. **Cardiovascular research,** 99 (2): 260-268.

Bain C.A.L., Lees K.R., Lumsden M.A., et al. (2005) Effect of a gonadotropin releasing hormone analog on cerebral hemodynamics in premenopausal women. **Climacteric,** 8 (2): 193-197.

Bain, C.A.L., Walters, M.R., Lees, K.R., et al. (2004) The effect of HRT on cerebral haemodynamics and cerebral vasomotor reactivity in post-menopausal women. **Human Reproduction,** 19 (10): 2411-2414.

Baird, D. and Fraser, I. (1974) Blood production and ovarian secretion rates of esuadiol-17β and estrone in women throughout the menstrual cycle. **J Clin Endocrinol Metab,** 38 1009-1017.

Bakker, S.L.M., de Leeuw, F., den Heijer, T., et al. (2004) Cerebral Haemodynamics in the Elderly: The Rotterdam Study. **Neuroepidemiology,** 23 (4): 178-184.

Barnes, J.N., Harvey, R.E., Eisenmann, N.A., et al. (2019) Cerebrovascular reactivity after cessation of menopausal hormone treatment. **Climacteric : the journal of the International Menopause Society,** 22 (2): 182-189.

Barrett-Connor, E. (1997) Sex Differences in Coronary Heart Disease: Why Are Women So Superior? The 1995 Ancel Keys Lecture. **Circulation (New York, N.Y.),** 95 (1): 252-264..

Battisti-Charbonney, A., Fisher, J. and Duffin, J. (2011) The cerebrovascular response to carbon dioxide in humans. **The Journal of physiology,** 589 (12): 3039-3048.

Benjamin, E.J., Larson, M.G., Keyes, M.J., et al. (2004) Clinical correlates and heritability of flow-mediated dilation in the community: the Framingham Heart Study. **Circulation (New York, N.Y.),** 109 (5): 613-619.

Betik, A.C., Luckham, V.B. and Hughson, R.L. (2004) Flow-mediated dilation in human brachial artery after different circulatory occlusion conditions. **American Journal of Physiology - Heart and Circulatory Physiology,** 286 (1): 442-448.

Bhogal, A.A., Siero, J.C.W., Fisher, J.A., et al. (2014) Investigating the non-linearity of the BOLD cerebrovascular reactivity response to targeted hypo/hypercapnia at 7T. **NeuroImage,** 98 296-305.

Black, M.A., Cable, N.T., Thijssen, D.H.J., et al. (2009) Impact of age, sex, and exercise on brachial artery flow-mediated dilatation. **American journal of physiology.Heart and circulatory physiology,** 297 (3): H1109-H1116.

Boardman, H.M.P., Hartley, L., Eisinga, A., et al. (2015) Hormone therapy for preventing cardiovascular disease in post-menopausal women. **Cochrane library,** (3): CD002229.

Brackley, K.J., Ramsay, M.M., Broughton Pipkin, F., et al. (1999) The effect of the menstrual cycle on human cerebral blood flow: studies using Doppler ultrasound. **Ultrasound in Obstetrics and Gynecology,** 14 (1): 52-57.

BrandaO A.H.F., Serra P.J., Zanolla K., et al. (2014) Variation of endothelial function during the menstrual cycle evaluated by flow-mediated dilatation of brachial artery. **Jornal Brasileiro de Reproducao Assistida,** 18 (4): 148-150.

Brislane, A., Low, D., Carter, S., et al. (2019) Cerebral and peripheral vascular differences between pre- and postmenopausal women. **Menopause,** 27 (2):.

British Heart Foundation (BHF) (July 2020) **BHF analysis of European Cardiovascular Disease Statistics.** [Online]. Available from: https://www.bhf.org.uk/what-we-do/our-research/heart-statistics 2020].

Bruce, C.D., Steinback, C.D., Chauhan, U.V., et al. (2016) Quantifying cerebrovascular reactivity in anterior and posterior cerebral circulations during voluntary breath holding. **Experimental physiology,** 101 (12): 1517-1527.

Brugniaux, J.V., Hodges, A.N.H., Hanly, P.J., et al. (2007) Cerebrovascular responses to altitude. **Respiratory physiology & neurobiology,** 158 (2): 212-223.

Brunser, A.M., Lavados, P.M., Hoppe, A., et al. (2009) **Accuracy of transcranial Doppler compared with CT angiography in diagnosing arterial obstructions in acute ischemic strokes**. **Stroke,** 40 (6): 2037-2041.

Burgess, K.R., Fan, J., Peebles, K., et al. (2010) Exacerbation of obstructive sleep apnea by oral indomethacin.

Bush, D., Jones, C., Bass, K., et al. (1998) **Estrogen Replacement Reverses Endothelial Dysfunction in Postmenopausal Women American Journ. Medicine,** 104 (6): 552-558.

C Ratnatunga , M Adiseshiah (1990) **Increase in middle cerebral artery velocity on breath holding: a simplified test of cerebral perfusion reserve**. **Eur J Vasc. Surg.,** 4 (5): 519-523.

CACCIATORE, B., PAAKKARI, I., TOIVONEN, J., et al. (1998) Randomized Comparison of Oral and Transdermal Hormone Replacement on Carotid and Uterine Artery Resistance to Blood Flow. **Obstetrics and gynecology (New York.1953),** 92 (4): 563-568.

Canderelli, R., Leccesse, L., Miller, N., et al. (2007) **Benefits of hormone replacement therapy in postmenopausal women**. **J Am Acad Nurse Pract.,** 19 (12): 635-641.

Carlos Henrique, F.C., Eduardo, A.M., Marcos, C.L., et al. (2015) Abnormal Cerebrovascular Reactivity in Patients with Parkinson's Disease. **Parkinson's disease,** 2015 523041-5.

Carrasquilla, G.D., Frumento, P., Berglund, A., et al. (2017) Postmenopausal hormone therapy and risk of stroke: A pooled analysis of data from population-based cohort studies. **PLoS medicine,** 14 (11): e1002445.

Celermajer, D.S. (1997) Endothelial Dysfunction: Does It Matter? Is It Reversible? **Journal of the American College of Cardiology,** 30 (2): 325-333.

Celermajer, D.S., Sorensen, K.E., Spiegelhalter, D.J., et al. (1994) Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. **Journal of the American College of Cardiology,** 24 (2): 471-476.

Chandra, A., Li, W.A., Stone, C.R., et al. (2017) The cerebral circulation and cerebrovascular disease I: Anatomy. **Brain circulation,** 3 (2): 45-56.

Chen, J.J. (2018) Cerebrovascular-Reactivity Mapping Using MRI: Considerations for Alzheimer's Disease. **Frontiers in aging neuroscience,** 10 170.

Chen, J., Ye, Z., Wang, X., et al. (2018) Nitric oxide bioavailability dysfunction involves in atherosclerosis. **Biomedicine & pharmacotherapy,** 97 423-428.

Chen, Y.W., Gurol, M.E., Rosand, J., et al. (2006) Progression of white matter lesions and hemorrhages in cerebral amyloid angiopathy. **Neurology,** 67 (1): 83-87.

Chen, Y.J., Rosario, B.L., Mowrey, W., et al. (2015) Relative 11C-PiB Delivery as a Proxy of Relative CBF: Quantitative Evaluation Using Single-Session 15O-Water and 11C-PiB PET. **The Journal of nuclear medicine (1978),** 56 (8): 1199-1205.

Cipolla, M.J. (2013) The Adaptation of the Cerebral Circulation to Pregnancy: Mechanisms and Consequences. **Journal of Cerebral Blood Flow & Metabolism,** 33 (4): 465-478.

Coverdale, N.S., Badrov, M.B. and Shoemaker, J.K. (2017) Impact of age on cerebrovascular dilation versus reactivity to hypercapnia. **Journal of cerebral blood flow and metabolism,** 37 (1): 344-355.

D'Urzo, K.A., King, T.J., et al. (2018) The impact of menstrual phase on brachial artery flow-mediated dilatation during handgrip exercise in healthy premenopausal women. **Experimental physiology,** 103 (2): 291-302.

Dahl, A., Russell, D., Nyberg-Hansen, R., et al. (1994) Cerebral vasoreactivity in unilateral carotid artery disease. A comparison of blood flow velocity and regional cerebral blood flow measurements. **Stroke (1970),** 25 (3): 621-626.

Dan, P., Joyce, C.Y.C., David, R.L.S., et al. (2003) Epitope-dependent localization of estrogen receptorα, but not -β, in en face arterial endothelium. **American Journal of Physiology - Heart and Circulatory Physiology,** 284 (4): 1295-1306.

Davis, S. (2000) **Drugs for the treatment of menopausal symptoms Expert Opinion on Pharmacotherapy,** 11 (8): 1329-1341.

De Leo, V., la Marca, A., Orlandi, R., et al. (2003) Effects of estradiol alone or in combination with cyproterone acetate on carotid artery pulsatility index in postmenopausal women. **Maturitas,** 46 (3): 219-224.

De Souza, M.A.,M., De Souza, B.,M. and Geber, S. (2013) Progesterone increases resistance of ophthalmic and central retinal arteries in climacteric women. **Climacteric,** 16 (2): 284-287.

Deanfield, J.E., Halcox, J.P. and Rabelink, T.J. (2007) Endothelial function and dysfunction: testing and clinical relevance. **Circulation (New York, N.Y.),** 115 (10): 1285-1295.

Debert, C.T., Ide, K. and Poulin, M.J. (2012) Effects of estrogen and progesterone on cerebrovascular responses to euoxic hypercapnia in women. **Climacteric,** 15 (6): 621-631.

Dhandapani, K.M. and Brann, D.W. (2007) Role of astrocytes in estrogen-mediated neuroprotection. **Experimental gerontology,** 42 (1): 70-75.

Diomedi, M., Cupini, L.M., Rizzato, B., et al. (2001) Influence of physiologic oscillation of estrogens on cerebral hemodynamics. **Journal of the neurological sciences,** 185 (1): 49-53.

Doshi, S. and Agarwal, A. (2013) The role of oxidative stress in menopause. **Journal of Mid-life Health,** 4 (3): 140-146.

Duffin, J. (2011) Measuring the respiratory chemoreflexes in humans. **Respiratory Physiology & Neurobiology,** 177 (2): 71-79.

Edvinsson, L. (2002) Sensory nerves in the human cerebral circulation and trigeminal ganglion: role in primary headaches. **The Journal of Headache and Pain,** 3 (1): 7-14.

Favre, M.E. and Serrador, J.M. (2019) Sex differences in cerebral autoregulation are unaffected by menstrual cycle phase in young, healthy women. **American journal of physiology.Heart and circulatory physiology,** 316 (4): H920-H933.

Fierstra, J., Sobczyk, O., Battisti-Charbonney, A., et al. (2013) Measuring cerebrovascular reactivity: what stimulus to use? **The Journal of physiology,** 591 (23): 5809-5821.

Findlay, B.B., Gupta, P., Szijgyarto, I.C., et al. (2013) Impaired brachial artery flow-mediated vasodilation in response to handgrip exercise-induced increases in shear stress in young smokers. **Vascular Medicine,** 18 (2): 63-71.

Fortune J.B., Bock D., Kupinski A.M., Stratton H.H., Shah D.M. and Feustel, P.J. (1992) "Human cerebrovascular response to oxygen and carbon dioxide as determined by internal carotid artery duplex scanning", Journal of Trauma; Lippincott Williams and Wilkins (351 West Camden Street, Baltimore MD 21201-2436, United States) pp. 618.

Franklin, S.S. (1999) Ageing and hypertension: the assessment of blood pressure indices in predicting coronary heart disease. **Journal of hypertension.Supplement,** 17 (5): S29.

Gangar, K.F., Vyas, S., Whitehead, M., et al. (1992) 91294607 Pulsatility index in internal carotid artery in relation to transdermal oestradiol and time since menopause. **Maturitas,** 15 (1): 79-79.

Gavin, K.M., Seals, D.R., Silver, A.E., et al. (2009) Vascular Endothelial Estrogen Receptor α Is Modulated by Estrogen Status and Related to Endothelial Function and Endothelial Nitric Oxide Synthase in Healthy Women. **The Journal of clinical endocrinology and metabolism,** 94 (9): 3513-3520.

Geary, G.G., Krause, D.N. and Duckles, S.P. (2000) Estrogen reduces mouse cerebral artery tone through endothelial NOS- and cyclooxygenase-dependent mechanisms. **American Journal of Physiology - Heart and Circulatory Physiology,** 279 (2): 511-519.

Geraldes, P., Sirois, M.G., Bernatchez, P.N., et al. (2002) Estrogen regulation of endothelial and smooth muscle cell migration and proliferation: role of p38 and p42/44 mitogen-activated protein kinase. **Arteriosclerosis, Thrombosis, and Vascular Biology,** 22 (10): 1585-1590.

Gerhard, M., Walsh, B.W., Tawakol, A., et al. (1998) Estradiol Therapy Combined With Progesterone and Endothelium-Dependent Vasodilation in Postmenopausal Women. **Circulation (New York, N.Y.),** 98 (12): 1158-1163.

Gold, E. (2011) **The Timing of the Age at Which Natural Menopause Occurs**. **Obstet.Gynecol. Clin North AM,** 38 (3): 425-440.

Green, D.J., Dawson, E.A., Groenewoud, H.M., et al. (2014) Is flow-mediated dilation nitric oxide mediated?: A meta-analysis. **Hypertension (Dallas, Tex.1979),** 63 (2): 376-382.

Green, D.J., Jones, H., Thijssen, D.H.J., et al. (2011) Flow-mediated dilation and cardiovascular event prediction: does nitric oxide matter? **Hypertension (Dallas, Tex.1979),** 57 (3): 363-369.

Green, D.J., Hopkins, N.D., Jones, H., et al. (2016) Sex differences in vascular endothelial function and health in humans: impacts of exercise: Sex, exercise and the endothelium. **Experimental physiology,** 101 (2): 230-242.

Guetta, V. and Cannon, R.O. (1996) Cardiovascular Effects of Estrogen and Lipid-Lowering Therapies in Postmenopausal Women. **Circulation (New York, N.Y.),** 93 (10): 1928-1937.

Guo, J., Krause, D.N., Horne, J., et al. (2010) Estrogen-Receptor-Mediated Protection of Cerebral Endothelial Cell Viability and Mitochondrial Function after Ischemic Insult in vitro. **Journal of Cerebral Blood Flow & Metabolism,** 30 (3): 545-554.

Hamelin, B., Méthot, J., Arsenault, M., et al. (2003) **Influence of the menstrual cycle on the timing of acute coronary events in premenopausal women**. **American Journal of Medicine,** 114 (7): 599-602.

Harris, R.A., Nishiyama, S.K., Wray, D.W., et al. (2010) Ultrasound Assessment of Flow-Mediated Dilation. **Hypertension (Dallas, Tex.1979),** 55 (5): 1075-1085.

Hashimoto M., Akishita M., Eto M., et al. (1995) Modulation of endothelium-dependent flow-mediated dilatation of the brachial artery by sex and menstrual cycle. **Circulation,** 92 (12): 3431-3435.

Herrington, D.M., Fan, L., Drum, M., et al. (2001) Brachial flow-mediated vasodilator responses in population-based research: methods, reproducibility and effects of age, gender and baseline diameter. **Journal of cardiovascular risk,** 8 (5): 319.

Hoiland, R.L., Tymko, M.M., Bain, A.R., et al. (2016) Carbon dioxide-mediated vasomotion of extra-cranial cerebral arteries in humans: a role for prostaglandins? **The Journal of physiology,** 594 (12): 3463-3481.

Holder, S.M., Brislane, Á., Dawson, E.A., et al. (2019) Relationship Between Endothelial Function and the Eliciting Shear Stress Stimulus in Women: Changes Across the Lifespan Differ to Men. **Journal of the American Heart Association,** 8 (4): e010994.

Huang, A., Sun, D., Koller, A., et al. (2000) 17β-Estradiol Restores Endothelial Nitric Oxide Release to Shear Stress in Arterioles of Male Hypertensive Rats. **Circulation (New York, N.Y.),** 101 (1): 94-100.

Hurtado, R., Celani, M. and Geber, S. (2016) Effect of short-term estrogen therapy on endothelial function: a double-blinded, randomized, controlled trial. **Climacteric,** 19 (5): 448-451.

Iadecola, C. (2004) Neurovascular regulation in the normal brain and in Alzheimer's disease. **Nature reviews.Neuroscience,** 5 (5): 347-360.

Ikdip Brar Andrew Robertson Richard L Hughson (2013) The relationship between peripheral endothelial function and cerebrovascular reactivity to CO2 in older adults. **The FASEB Journal,** 27 (S1):.

Inge Dørup, Skajaa, K. and Keld E. Sørensen (1999) Normal pregnancy is associated with enhanced endothelium-dependent flow-mediated vasodilation. **American Journal of Physiology - Heart and Circulatory Physiology,** 276 (3): 821-825.

Jackson, W.F. (2005) Potassium Channels in the Peripheral Microcirculation. **Microcirculation,** 12 (1): 113-127.

Jiang, C., Sarrel, P.M., Lindsay, D.C., et al. (1991) Endothelium-independent relaxation of rabbit coronary artery by 17β-oestradiol in vitro. **British journal of pharmacology,** 104 (4): 1033-1037.

Jochmann, N., Müller, S., Kuhn, C., et al. (2009) Chronic Smoking Prevents Amelioration of Endothelial Function in the Course of the Menstrual Cycle. **Circulation journal : official journal of the Japanese Circulation Society,** 73 (3): 568-572.

Jones-Muhammad M, Warrington JP. (2019) Cerebral Blood Flow Regulation in Pregnancy, Hypertension, and Hypertensive Disorders of Pregnancy. **Brain Sci.** 9(9):224.

Junejo, R.T., May, S., Alsalahi, S., et al. (2020) Cerebrovascular carbon dioxide reactivity and flow-mediated dilation in young healthy South Asian and Caucasian European men. **American journal of physiology.Heart and circulatory physiology,** 318 (4): H756-H763.

Juonala, M., Kahonen, M., Laitinen, T., et al. (2008) Effect of age and sex on carotid intima-media thickness, elasticity and brachial endothelial function in healthy adults: The Cardiovascular Risk in Young Finns Study. **European heart journal,** 29 (9): 1198-1206.

Kannel, W.B., Hjortland, M.C., McNamara, P.M., et al. (1976) Menopause and risk of cardiovascular disease: the Framingham study. **Annals of Internal Medicine,** 85 (4): 447.

Kastrup, A., Dichgans, J., Niemeier, M., et al. (1998) Changes of cerebrovascular CO2 reactivity during normal aging. **Stroke (1970),** 29 (7): 1311.

Kastrup, A., Thomas, C., Hartmann, C., et al. (1997) Sex dependency of cerebrovascular CO2 reactivity in normal subjects. **Stroke (1970),** 28 (12): 2353.

Kawano, H., Motoyama, T., Ohgushi, M., et al. (2001) Menstrual Cyclic Variation of Myocardial Ischemia in Premenopausal Women with Variant Angina. **Annals of Internal Medicine,** 135 (11): 977.

Kety S.S. and Schmidt, C.F. (1948) The effects of altered arterial tensions of carbon dioxide and oxygen on cerebral blood flow and cerebral oxygen consumption of normal young men. **The Journal of clinical investigation,** 27 (4): 484-492.

Khalil, R.A. (2013) Estrogen, vascular estrogen receptor and hormone therapy in postmenopausal vascular disease. **Biochemical pharmacology,** 86 (12): 1627-1642.

Kharitonov, S.A., Logan-Sinclair, R., Busset, C.M., et al. (1994) Peak expiratory nitric oxide differences in men and women: relation to the menstrual cycle. **British heart journal,** 72 (3): 243-245.

Kisler, K., Nelson, A.R., Montagne, A., et al. (2017) Cerebral blood flow regulation and neurovascular dysfunction in Alzheimer's disease. **Nature reviews.Neuroscience,** 18 (7): 419-434.

Kleiser, B. and Widder, B. (1992) Course of carotid artery occlusions with impaired cerebrovascular reactivity. **Stroke (1970),** 23 (2): 171-174.

Koh, K.K., Ahn, J.Y., Jin, D.K., et al. (2002) Effects of Continuous Combined Hormone Replacement Therapy on Inflammation in Hypertensive and/or Overweight Postmenopausal Women. **Arteriosclerosis, Thrombosis, and Vascular Biology,** 22 (9): 1459-1464.

Koh, K.K., Won Son, J., Yeal Ahn, J., et al. (2001) Effect of hormone replacement therapy on nitric oxide bioactivity and monocyte chemoattractant protein-1 levels. **International journal of cardiology,** 81 (1): 43-50.

Krause D.N., Duckles S.P. and Pelligrino, D.A. (2006) Influence of sex steroid hormones on cerebrovascular function. **Journal of applied physiology,** 101 (4): 1252-1261.

Krejza, J., Rudzinski, W., Arkuszewski, M., et al. (2013) Cerebrovascular reactivity across the menstrual cycle in young healthy women. **Journal of the neurological sciences,** 333 e490-e491.

Krejza, J., Siemkowicz, J., Sawicka, M., et al. (2003) Oscillations of cerebrovascular resistance throughout the menstrual cycle in healthy women. **Ultrasound in Obstetrics and Gynecology,** 22 (6): 627-632.

Krejza, J., Mariak, Z., Nowacka, A., et al. (2004) Influence of 17-beta-estradiol on cerebrovascular impedance during menstrual cycle in women. **Journal of the neurological sciences,** 221 (1): 61-67.

Krejza, J., Szydlik, P., Liebeskind, D.S., et al. (2005) Age and sex variability and normal reference values for the V(MCA)/V(ICA) index. **American journal of neuroradiology : AJNR,** 26 (4): 730.

Lakatta E.G. and Levy, D. (2003) Arterial and cardiac aging: Major shareholders in cardiovascular disease enterprises: Part II: The aging heart in health: Links to heart disease. **Circulation,** 107 (2): 346-354.

Lavi, S., Egbarya, R., Lavi, R., et al. (2003) Role of nitric oxide in the regulation of cerebral blood flow in humans: chemoregulation versus mechanoregulation. **Circulation (New York, N.Y.),** 107 (14): 1901-1905.

Lieberman, E.H. (1994) Estrogen Improves Endothelium-Dependent, Flow-Mediated Vasodilation in Postmenopausal Women. **Annals of Internal Medicine,** 121 (12): 936.

Liu, P., De Vis, J.,B. and Lu, H. (2019) Cerebrovascular reactivity (CVR) MRI with CO2 challenge: A technical review. **NeuroImage (Orlando, Fla.),** 187 104-115.

Lizarelli, P.M., Martins, W.P., Vieira, C.S., et al. (2009) Both a combined oral contraceptive and depot medroxyprogesterone acetate impair endothelial function in young women. **Contraception,** 79 (1): 35-40.

Lloyd-Jones, D., Leip, E.P., Larson, M.G., et al. (2006) Prediction of Lifetime Risk for Cardiovascular Disease by Risk Factor Burden at 50 Years of Age. **Circulation (New York, N.Y.),** 113 (6): 791-798.

Løkkegaard, E., Nielsen, L.H. and Keiding, N. (2017) Risk of Stroke With Various Types of Menopausal Hormone Therapies: A National Cohort Study. **Stroke (1970),** 48 (8): 2266-2269.

Ma, Q., Sun, X., Chen, Y., et al. (2009) Progesterone levels and carotid intima-media thickness: a negative association in older northern Chinese men. **Texas Heart Institute journal,** 36 (4): 303-308.

MacKay, C.M., Skow, R.J., Tymko, M.M., et al. (2016) Central respiratory chemosensitivity and cerebrovascular CO2 reactivity: a rebreathing demonstration illustrating integrative human physiology. **Advances in Physiology Education,** 40 (1): 79-92.

Madureira, João,M.Sc, M.D., Castro, P., M.D. and Azevedo, Elsa,PhD., M.D. (2016) Demographic and Systemic Hemodynamic Influences in Mechanisms of Cerebrovascular Regulation in Healthy Adults. **Journal of Stroke and Cerebrovascular Diseases,** 26 (3): 500-508.

Magness, R.R., Phernetton, T.M., Gibson, T.C., et al. (2005) Uterine blood flow responses to ICI 182 780 in ovariectomized oestradiol-17β-treated, intact follicular and pregnant sheep: Oestrogen receptor antagonist and uterine blood flow. **The Journal of physiology,** 565 (1): 71-83.

Majmudar, N.G., Robson, S.C. and Ford, G.A. (2000) Effects of the Menopause, Gender, and Estrogen Replacement Therapy on Vascular Nitric Oxide Activity. **The Journal of clinical endocrinology and metabolism,** 85 (4): 1577-1583.

Mandell, D.M., Han, J.S., Poublanc, J., et al. (2008) Mapping cerebrovascular reactivity using blood oxygen level-dependent MRI in Patients with arterial steno-occlusive disease: comparison with arterial spin labeling MRI. **Stroke (1970),** 39 (7): 2021-2028.

Manson, J.E., Aragaki, A.K., Rossouw, J.E., et al. (2017) Menopausal Hormone Therapy and Long-term All-Cause and Cause-Specific Mortality: The Women's Health Initiative Randomized Trials. **JAMA : the journal of the American Medical Association,** 318 (10): 927-938.

Markus H. and Cullinane, M. (2001) Severely impaired cerebrovascular reactivity predicts stroke and TIA risk in patients with carotid artery stenosis and occlusion. **Brain,** 124 (3): 457-467.

Maruhashi, T., Soga, J., Fujimura, N., et al. (2013) Relationship between flow-mediated vasodilation and cardiovascular risk factors in a large community-based study. **Heart,** 99 (24): 1837-1842.

Matsuzawa, Y., Kwon, T., Lennon, R.J., et al. (2015) Prognostic Value of Flow-Mediated Vasodilation in Brachial Artery and Fingertip Artery for Cardiovascular Events: A Systematic Review and Meta-Analysis. **Journal of the American Heart Association,** 4 (11):.

Matteis, M., Troisi, E., Monaldo, B.C., et al. (1998) Age and Sex Differences in Cerebral Hemodynamics: A Transcranial Doppler Study. **Stroke (1970),** 29 (5): 963-967.

McCrohon, J.A., Adams, M.R., McCredie, R.J., et al. (1996) Hormone replacement therapy is associated with improved arterial physiology in healthy post-menopausal women. **Clinical endocrinology,** 45 (4): 435-441.

McDonald, D.A. (1960) **Blood flow in arteries / Donald A. McDonald.** London : Edward Arnold, London.

McNeill, A.M., Zhang, C., Stanczyk, F.Z., et al. (2002) Estrogen increases endothelial nitric oxide synthase via estrogen receptors in rat cerebral blood vessels: effect preserved after concurrent treatment with medroxyprogesterone acetate or progesterone. **Stroke (1970),** 33 (6): 1685-1691.

Meendering, J.R., Torgrimson, B.N., Miller, N.P., et al. (2008) Estrogen, medroxyprogesterone acetate, endothelial function, and biomarkers of cardiovascular risk in young women. **American Journal of Physiology - Heart and Circulatory Physiology,** 294 (4): 1630-1637.

Mendelsohn, M.E. (2000) "Mechanisms of estrogen action in the cardiovascular system", Journal of Steroid Biochemistry and Molecular Biology; Elsevier Ltd (Langford Lane, Kidlington, Oxford OX5 1GB, United Kingdom) pp. 337.

Miller, K.B., Howery, A.J., Rivera-Rivera, L., et al. (2019) Age-Related Reductions in Cerebrovascular Reactivity Using 4D Flow MRI. **Frontiers in aging neuroscience,** 11 281.

Miller, V.M., Garovic, V.D., Kantarci, K., et al. (2013) Sex-specific risk of cardiovascular disease and cognitive decline: pregnancy and menopause. **Biology of sex differences,** 4 (1): 6-6.

Miner, J.A., Martini, E.R., Smith, M.M., et al. (2011) Short-term oral progesterone administration antagonizes the effect of transdermal estradiol on endothelium-dependent vasodilation in young healthy women. **American journal of physiology.Heart and circulatory physiology,** 301 (4): H1716-H1722.

Molinari, C., Battaglia, A., Grossini, E., et al. (2001) The effect of progesterone on coronary blood flow in anaesthetized pigs. **Experimental physiology,** 86 (1): 101-108.

Moreau, K. and Hilderth, K. (2014) Vascular Aging across the Menopause Transition in Healthy Women. **Adv. Vasc. Med.,** 17.

Moreau, K.L., Hildreth, K.L., Meditz, A.L., et al. (2012) Endothelial Function Is Impaired across the Stages of the Menopause Transition in Healthy Women. **The Journal of clinical endocrinology and metabolism,** 97 (12): 4692-4700.

Navarro-Orozco, D. and Sánchez-Manso, J. (2020) **Neuroanatomy, Middle Cerebral Artery**. **StatPearls,** .

Nevo, O., Soustiel, J.F. and Thaler, I. (2007) Cerebral blood flow is increased during controlled ovarian stimulation. **American Journal of Physiology - Heart and Circulatory Physiology,** 293 (6): 3265-3269.

Novella, S., Dantas, A.P., Segarra, G., et al. (2012) Vascular Aging in Women: is Estrogen the Fountain of Youth? **Frontiers in physiology,** 3 165.

Nyberg, M., Gliemann, L. and Hellsten, Y. (2015) Vascular function in health, hypertension, and diabetes: effect of physical activity on skeletal muscle microcirculation. **Scandinavian Journal of Medicine & Science in Sports,** 25 60-73.

Oikonomou, E., Siasos, G., Chrysohoou, C., et al. (2013) THE IMPACT OF PHYSICAL ACTIVITY ON TOTAL ANTIOXIDANT CAPACITY AND ENDOTHELIAL FUNCTION: IKARIA STUDY. **JACC (Journal of the American College of Cardiology),** 61 (10): E1614-E1614.

Oláh, L., Valikovics, A., Bereczki, D., et al. (2000) Gender-Related Differences in Acetazolamide-Induced Cerebral Vasodilatory Response: A Transcranial Doppler Study. **Journal of neuroimaging,** 10 (3): 151-156.

Olufsen, M.S., Nadim, A. and Lipsitz, L.A. (2002) Dynamics of cerebral blood flow regulation explained using a lumped parameter model. **American Journal of Physiology - Regulatory, Integrative and Comparative Physiology,** 282 (2): 611-622.

Ospina, J.A., Duckles, S.P. and Krause, D.N. (2003) 17β-Estradiol decreases vascular tone in cerebral arteries by shifting COX-dependent vasoconstriction to vasodilation. **American Journal of Physiology - Heart and Circulatory Physiology,** 285 (1): 241-250.

Oz, F., Elitok, A., Bilge, A.K., et al. (2012) Relationship Between Brachial Artery Flow-Mediated Dilation, Carotid Artery Intima-Media Thickness and Coronary Flow Reserve in Patients With Coronary Artery Disease. **Cardiology research,** 3 (5): 214-221.

Peebles K., Celi L., Mcgrattan K., et al. (2007) Human cerebrovascular and ventilatory CO2 reactivity to end-tidal, arterial and internal jugular vein PCO2 . **Journal of Physiology,** 584 (1): 347-357.

Pelligrino D.A. and Galea, E. (2001) Estrogen and cerebrovascular physiology and pathophysiology. **Japanese journal of pharmacology,** 86 (2): 137-158.

Peltonen, G.L., Harrell, J.W., Aleckson, B.P., et al. (2016) Cerebral blood flow regulation in women across menstrual phase: differential contribution of cyclooxygenase to basal, hypoxic, and hypercapnic vascular tone. **American journal of physiology.Regulatory, integrative and comparative physiology,** 311 (2): R222-R231.

Peltonen, G.L., Harrell, J.W., Rousseau, C.L., et al. (2015) Cerebrovascular regulation in men and women: stimulus-specific role of cyclooxygenase. **Physiological reports,** 3 (7): e12451-n/a.

Peng, S., Chen, X., Li, Y., et al. (2018) Age-related changes in cerebrovascular reactivity and their relationship to cognition: A four-year longitudinal study. **NeuroImage (Orlando, Fla.),** 174 257-262.

Penotti, M., Sironi, L., Amicarelli, F., et al. (2002) Surgically induced menopause and blood flow in cerebral arteries. **Fertility and sterility,** 77 (5): 1086-1087.

Penotti, Farina, Castiglioni, et al. (1996) Alteration in the pulsatility index values of the internal carotid and middle cerebral arteries after suspension of postmenopausal hormone replacement therapy: A randomized crossover study. **American Journal of Obstetrics and Gynecology,** 175 (3): 606-611.

Perko, D., Pretnar-Oblak, J., Šabovič, M., et al. (2011) Cerebrovascular reactivity to l-arginine in the anterior and posterior cerebral circulation in migraine patients. **Acta Neurologica Scandinavica,** 124 (4): 269-274.

Phippard AF, H.J., Glynn EM, Garner MG, et al. (1986) **Circulatory adaptation to pregnancy--serial studies of haemodynamics, blood volume, renin and aldosterone in the baboon (Papio hamadryas)**. **J Hypertens,** 6 (4): 773-779.

Portegies, M.L.P., de Bruijn, R.,F.A.G., Hofman, A., et al. (2014) Cerebral vasomotor reactivity and risk of mortality: the Rotterdam Study. **Stroke (1970),** 45 (1): 42-47.

Pretnar-Oblak, J., Sabovic, M., Sebestjen, M., et al. (2006) Influence of atorvastatin treatment on L-arginine cerebrovascular reactivity and flow-mediated dilatation in patients with lacunar infarctions. **Stroke (1970),** 37 (10): 2540-2545.

Purkayastha, S. and Sorond, F. (2012) **Transcranial Doppler Ultrasound: Technique and Application**. **Semin Neurol.,** 32 (4): 411-420.

Pyke, K.E., Poitras, V. and Tschakovsky, M.E. (2008) Brachial artery flow-mediated dilation during handgrip exercise: evidence for endothelial transduction of the mean shear stimulus. **American Journal of Physiology - Heart and Circulatory Physiology,** 294 (6): 2669-2679.

Quinn, U., Tomlinson, L.A. and Cockcroft, J.R. (2012) Arterial stiffness. **JRSM cardiovascular disease,** 1 (6): 1-8.

Qureshi, A., Malik, A., Saeed, O., et al. (2016) Hormone replacement therapy and the risk of subarachnoid hemorrhage in postmenopausal women. **J neurosurg.,** 124 (1): 45-50.

Read, D.C. (1967) A CLINICAL METHOD FOR ASSESSING THE VENTILATORY RESPONSE TO CARBON DIOXIDE. **Australasian Annals of Medicine,** 16 (1):.

Ringelstein, E.B., Sievers, C., Ecker, S., et al. (1988) Noninvasive assessment of CO2-induced cerebral vasomotor response in normal individuals and patients with internal carotid artery occlusions. **Stroke (1970),** 19 (8): 963-969.

Riskin-Mashiah, S., Belfort, M.A., Saade, G.R., et al. (2002) Transcranial doppler measurement of cerebral velocity indices as a predictor of preeclampsia. **American Journal of Obstetrics and Gynecology,** 187 (6): 1667-1672.

Ritz, K., Denswil, N.P., Stam, O.C.G., et al. (2014) Cause and Mechanisms of Intracranial Atherosclerosis. **Circulation (New York, N.Y.),** 130 (16): 1407-1414.

Ritz, K., Denswil, N.P., Stam, O.C.G., et al. (2014) Cause and Mechanisms of Intracranial Atherosclerosis. **Circulation (New York, N.Y.),** 130 (16): 1407-1414.

Robert T Chatterton, J., Esnar, T.M., Hou, N., et al. (2005) Characteristics of salivary profiles of oestradiol and progesterone in premenopausal women. **Journal of Endocrinology,** 186 (1): 77-84.

Rodriguez-Miguelez, P., Seigler, N. and Harris, R.A. (2016) Ultrasound Assessment of Endothelial Function: A Technical Guideline of the Flow-mediated Dilation Test. **Journal of visualized experiments : JoVE,** (110):.

Roger, V.L., Go, A.S., Lloyd-Jones, D., et al. (2012) Executive Summary: Heart Disease and Stroke Statistics—2012 Update: A Report From the American Heart Association. **Circulation (New York, N.Y.),** 125 (1): 188-197.

Rosano, G.M.C., Vitale, C., Marazzi, G., et al. (2009) Menopause and cardiovascular disease: the evidence. **Climacteric,** 10 19-24.

Rosendorff, C. (2002) Effects of LDL cholesterol on vascular function. **Journal of human hypertension,** 16 S26-S28.

Sacco, S., Merki-Feld, G., Ægidius, K.L., et al. (2017) Hormonal contraceptives and risk of ischemic stroke in women with migraine: a consensus statement from the European Headache Federation (EHF) and the European Society of Contraception and Reproductive Health (ESC). **Journal of headache and pain,** 18 (1): 1-20.

Sarrel, P.M. (1999) The differential effects of oestrogens and progestins on vascular tone. **Human reproduction update,** 5 (3): 205-209.

Sato, K., Sadamoto, T., Hirasawa, A., et al. (2012) Differential blood flow responses to CO2 in human internal and external carotid and vertebral arteries. **The Journal of physiology,** 590 (14): 3277-3290.

Schechter, A.N. and Gladwin, M.T. (2003) Hemoglobin and the Paracrine and Endocrine Functions of Nitric Oxide. **The New England journal of medicine,** 348 (15): 1483-1485.

Schnabel, R.B., Biener, M.P., Wilde, S., et al. (2013) Sex differences in noninvasive vascular function in the community. **Journal of hypertension,** 31 (7): 1437-1446.

Scicchitano, P., Cortese, F., Gesualdo, M., et al. (2019) The role of endothelial dysfunction and oxidative stress in cerebrovascular diseases. **Free radical research,** 53 (6): 579-595.

Senese, K.A., Corkery, A.T., Howery, A.J., et al. (2020) The Influence of Age at Natural Menopause on Cerebrovascular Reactivity. **The FASEB journal,** 34 1-1.

Serrador, J.M., Picot, P.A., Rutt, B.K., et al. (2000) MRI Measures of Middle Cerebral Artery Diameter in Conscious Humans During Simulated Orthostasis. **Stroke (1970),** 31 (7): 1672-1678.

Sessa, W.C. (2004) eNOS at a glance. **Journal of cell science,** 117 (12): 2427-2429.

Shamma, F.N., Fayad, P., Brass, L., et al. (1992) Middle cerebral artery blood velocity during controlled ovarian hyperstimulation. **Fertility and sterility,** 57 (5): 1022-1025.

Shatri, J., Bexheti, D., Bexheti, S., et al. (2017) Influence of Gender and Age on Average Dimensions of Arteries Forming the Circle of Willis Study by Magnetic Resonance Angiography on Kosovo's Population. **Open access Macedonian journal of medical sciences,** 5 (6): 714-719.

Shenouda, N., Skelly, L.E., Gibala, M.J., et al. (2018) Brachial artery endothelial function is unchanged after acute sprint interval exercise in sedentary men and women. **Experimental physiology,** 103 (7): 968-975.

Silvestrini, M., Vernieri, F., Pasqualetti, P., et al. (2000) Impaired Cerebral Vasoreactivity and Risk of Stroke in Patients With Asymptomatic Carotid Artery Stenosis. **JAMA : the journal of the American Medical Association,** 283 (16): 2122-2127.

Simoncini, T., Mannella, P., Fornari, L., et al. (2004) Differential Signal Transduction of Progesterone and Medroxyprogesterone Acetate in Human Endothelial Cells. **Endocrinology (Philadelphia),** 145 (12): 5745-5756.

Skow, R.J., MacKay, C.M., Tymko, M.M., et al. (2013) Differential cerebrovascular CO2 reactivity in anterior and posterior cerebral circulations. **Respiratory Physiology & Neurobiology,** 189 (1): 76-86.

Smith, K.J. and Ainslie, P.N. (2017) Regulation of cerebral blood flow and metabolism during exercise. **Experimental physiology,** 102 (11): 1356-1371.

Somani, Y.B., Moore, D.J., Kim, D.J., et al. (2019) Retrograde and oscillatory shear increase across the menopause transition. **Physiological Reports,** 7 (1): e13965-n/a.

Sørensen, M.B., Rosenfalck, A.M., Højgaard, L., et al. (2001) Obesity and Sarcopenia after Menopause Are Reversed by Sex Hormone Replacement Therapy. **Obesity research,** 9 (10): 622-626.

Sorensen, K.E., Dorup, I., Hermann, A.P., et al. (1998) Combined Hormone Replacement Therapy Does Not Protect Women Against the Age-Related Decline in Endothelium-Dependent Vasomotor Function. **Circulation (New York, N.Y.),** 97 (13): 1234-1238.

Sorond, F.A., Khavari, R., Serrador, J.M., et al. (2005) Regional Cerebral Autoregulation During Orthostatic Stress: Age-Related Differences. **The journals of gerontology.Series A, Biological sciences and medical sciences,** 60 (11): 1484-1487.

Staszewski, J., Skrobowska, E., Piusińska-Macoch, R., et al. (2018) Cerebral and Extracerebral Vasoreactivity in Patients With Different Clinical Manifestations of Cerebral Small-Vessel Disease: Data From the Significance of Hemodynamic and Hemostatic Factors in the Course of Different Manifestations of Cerebral Small-Vessel Disease Study. **Journal of ultrasound in medicine,** 38 (4): 975-987.

Steinback, C., Usselman, C. and Davenport, M. (2015) Sympathetic Neurovascular Regulation During Pregnancy: A Longitudinal Case Study. **The FASEB Journal,** 29 (1):.

Stirone, C., Duckles, S.P., Krause, D.N., et al. (2005) Estrogen Increases Mitochondrial Efficiency and Reduces Oxidative Stress in Cerebral Blood Vessels. **Molecular pharmacology,** 68 (4): 959-965.

Strohm, J., Duffin, J. and Fisher, J.A. (2014) Circadian cerebrovascular reactivity to CO2. **Respiratory Physiology & Neurobiology,** 197 15-18.

Sudhir, K., Ko, E., Zellner, C., et al. (1997) Physiological Concentrations of Estradiol Attenuate Endothelin 1–Induced Coronary Vasoconstriction In Vivo. **Circulation (New York, N.Y.),** 96 (10): 3626-3632.

Tachibana, H., Washida, K., Kowa, H., et al. (2016) Vascular Function in Alzheimer's Disease and Vascular Dementia. **American Journal of Alzheimer's Disease and Other Dementias,** 31 (5): 437-442.

Taddei, S., Virdis, A., Ghiadoni, L., et al. (1996) Menopause Is Associated With Endothelial Dysfunction in Women. **Hypertension (Dallas, Tex.1979),** 28 (4): 576-582.

Tan, E., Gurjar, M.V., Sharma, R.V., et al. (1999) Estrogen receptor-alpha gene transfer into bovine aortic endothelial cells induces eNOS gene expression and inhibits cell migration. **Cardiovascular research,** 43 (3): 788-797.

Thijssen, D.H.J., Black, M.A., Pyke, K.E., et al. (2011) Assessment of flow-mediated dilation in humans: a methodological and physiological guideline. **American journal of physiology.Heart and circulatory physiology,** 300 (1): H2-H12.

Thosar, S.S., Berman, A.M., Herzig, M.X., et al. (2019) Circadian Rhythm of Vascular Function in Midlife Adults. **Arteriosclerosis, Thrombosis, and Vascular Biology,** 39 (6): 1203-1211.

van Bussel, B.,C., Schouten, F., Henry, R.M., et al. (2011) Endothelial Dysfunction and Low-Grade Inflammation Are Associated With Greater Arterial Stiffness Over a 6-Year Period. **Hypertension (Dallas, Tex.1979),** 58 (4): 588-595.

Vink, A.S., Clur, S.B., Wilde, A.A.M., et al. (2018) Effect of age and gender on the QTc-interval in healthy individuals and patients with long-QT syndrome. **Trends in cardiovascular medicine,** 28 (1): 64-75.

Vitale, C., Mercuro, G., Cerquetani, E., et al. (2008) Time Since Menopause Influences the Acute and Chronic Effect of Estrogens on Endothelial Function. **Arteriosclerosis, Thrombosis, and Vascular Biology,** 28 (2): 348-352.

Vriens, E.M., Kraaier, M., Musbach, M., et al. (1989) Transcranial pulsed Doppler measurements of blood velocity in the middle cerebral artery: reference values at rest and during hyperventilation in healthy volunteers in relation to age and sex. **Ultrasound Med. Biol,** 15 1-8.

Vrselja Z, Brkic H, Mrdenovic S, Radic R, Curic G. (2014) Function of circle of Willis. **J Cereb Blood Flow Metab.** 34(4):578-84.

Wakatsuki, A., Okatani, Y., Ikenoue, N., et al. (2001) Effect of medroxyprogesterone acetate on endothelium-dependent vasodilation in postmenopausal women receiving estrogen. **Circulation (New York, N.Y.),** 104 (15): 1773-1778.

Wander, K., Brindle, E., O&apos, et al. (2008) C-reactive protein across the menstrual cycle. **American Journal of Physical Anthropology,** 136 (2): 138-146.

Wander, K., Brindle, E. and O'Connor, K.,A. (2008) C-reactive protein across the menstrual cycle. **American Journal of Physical Anthropology,** 136 (2): 138-146.

Weissgerber, T.L., Milic, N.M., Milin-Lazovic, J., et al. (2016) Impaired Flow-Mediated Dilation Before, During, and After Preeclampsia: A Systematic Review and Meta-Analysis. **Hypertension (Dallas, Tex.1979),** 67 (2): 415-423.

White, R.P., Deane, C., Vallance, P., et al. (1998) Nitric oxide synthase inhibition in humans reduces cerebral blood flow but not the hyperemic response to hypercapnia. **Stroke (1970),** 29 (2): 467-472.

White, R.P., Deane, C., Vallance, P., et al. (1998) Nitric oxide synthase inhibition in humans reduces cerebral blood flow but not the hyperemic response to hypercapnia. **Stroke (1970),** 29 (2): 467-472.

Williams, J.K., Honoré, E.,K., Washburn, S.A., et al. (1994) Effects of hormone replacement therapy on reactivity of atherosclerotic coronary arteries in cynomolgus monkeys. **Journal of the American College of Cardiology,** 24 (7): 1757-1761.

Williams, L.R. and Leggett, R.W. (1989) Reference values for resting blood flow to organs of man. **Clinical physics and physiological measurement,** 10 (3): 187-217.

Williams, M.R.I., Westerman, R.A., Kingwell, B.A., et al. (2001) Variations in Endothelial Function and Arterial Compliance during the Menstrual Cycle. **The Journal of clinical endocrinology and metabolism,** 86 (11): 5389-5395.

Willie, C.K., Macleod, D.B., Shaw, A.D., et al. (2012) Regional brain blood flow in man during acute changes in arterial blood gases. **The Journal of physiology,** 590 (14): 3261-3275.

Willie, C.K., Tzeng, Y., Fisher, J.A., et al. (2014) Integrative regulation of human brain blood flow. **The Journal of physiology,** 592 (5): 841-859.

Wilson, M.H., Edsell, M.E.G., Davagnanam, I., et al. (2011) Cerebral Artery Dilatation Maintains Cerebral Oxygenation at Extreme Altitude and in Acute Hypoxia—An Ultrasound and MRI Study. **Journal of cerebral blood flow and metabolism,** 31 (10): 2019-2029.

Wise, R.G., Pattinson, K.T.S., Bulte, D.P., et al. (2007) Dynamic forcing of end-tidal carbon dioxide and oxygen applied to functional magnetic resonance imaging. **Journal of Cerebral Blood Flow & Metabolism,** 27 (8): 1521-1532.

World Health Organisation (WHO) (May, 2017) **Fact Sheets: Cardiovascular Diseases (CVDs).** [Online]. [Accessed 09/03 2020].

Xie, A., Skatrud, J.B., Morgan, B., et al. (2006) Influence of cerebrovascular function on the hypercapnic ventilatory response in healthy humans. **The Journal of physiology,** 577 (1): 319-329.

Yang, T., Sun, Y., Lu, Z., et al. (2017) The impact of cerebrovascular aging on vascular cognitive impairment and dementia. **Ageing research reviews,** 34 15-29.

Yao, F., Liu, Y., Liu, D., et al. (2014) Sex Differences Between Vascular Endothelial Function and Carotid Intima-Media Thickness by Framingham Risk Score. **Journal of Ultrasound in Medicine,** 33 (2): 281-286.

Yen SSC (1991) "The human menstrual cycle. Neuroendocrine modulation" In Eds SSC Yen & RB Jaffe (ed.) **Reproductive Endocrinology: Physiology, Pathology and Clinical Management,** Philadelphia: WB Saunders. pp. 273-308.

Zarrinkoob, L., Ambarki, K., Wåhlin, A., et al. (2015) Blood Flow Distribution in Cerebral Arteries. **Journal of cerebral blood flow and metabolism,** 35 (4): 648-654.

Žegura, B., Keber, I., Šebeštjen, M., et al. (2003) Orally and transdermally replaced estradiol improves endothelial function equally in middle-aged women after surgical menopause. **American Journal of Obstetrics and Gynecology,** 188 (5): 1291-1296.