The Effect of Oestrogen on Cerebrovascular Regulation in Eumenorrheic Women

A thesis presented in partial fulfilment of the requirements for the degree of

Master of Science

Massey University, Wellington, New Zealand

Stephanie Korad BHlthSc.

2020

Abstract

Women experience fluctuating sex hormone concentrations throughout their lifetime and while their role in reproduction is well documented, there is little knowledge of the effects of the changing hormone concentrations on women's cerebrovascular health. Therefore, this study examined dynamic cerebral autoregulation (dCA) in 10 healthy eumenorrheic women (28 ± 7 years) volunteers. The participants dCA was examined at three different phases of the menstrual cycle: early follicular (EF; when oestrogen and progesterone concentrations are low), late follicular (LF; oestrogen concentrations are high, while progesterone remains steady), and mid-luteal (ML; oestrogen and progesterone concentrations are high). The dCA was assessed using the autoregulatory index (AI) of forced changes in blood pressure (BP) and mean middle cerebral blood velocity (MCAv) response, induced during phases of the Valsalva manoeuvre (VM). The VM is a four-phase manoeuvre that produces hyper- and hypotensive changes to blood pressure (BP): phase I (initial increase in BP), phase IIa (initial decrease in BP), phase IIb (stabilisation of BP), phase III (decrease in BP after cessation of breath-hold), and phase IV (overshoot in BP recovery). Resting mean arterial blood pressure (MAP, *P= 0.409)*, MCAv (*P= 0.635),* and cerebrovascular conductance index (CVCi, *P= 0.984)* were not different throughout all trials. The partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$) was unchanged between the trials (*P = 0.907)*. The VM induced middle cerebral artery velocity mean ($MCAv_{mean}$) differences between trials (interaction: *P = 0.039)*, MCAv during mid-luteal (ML; 58 ± 15 cm/s) showed a significant difference to early follicular (EF; 51 ± 14 cm/s, *P* = 0.013) and late follicular (LF; 49 ± 15 cm/s, *P* = 0.024) during phase IIb of the VM. There were no differences in MAP (*P* = 0.233) and CVCi (*P* = 0.808) during the VM throughout the trials. AI presented no difference during phase II of the VM (*P= 0.354)*, however, phase IV did show a trend (*P= 0.086).* The results of this study indicate that circulating ovarian hormone concentrations may regulate responses to dynamic cerebrovascular challenges.

Preface

This thesis report is submitted for the requirement of the degree of Master of Science (MSc) at Massey University, Wellington. This thesis was completed in April 2020 under the supervision of Dr Blake Perry from the School of Health Sciences, Associate Professor Toby Mundel from the School of Sport, Exercise and Nutrition, and Dr Sally Lark, previously of School of Sport, Exercise and Nutrition. This research was put forward to investigate and gain further knowledge on the effects of oestrogen on cerebrovascular regulation in eumenorrheic women.

There were complications with data collection, as the equipment required for the study was not available at the beginning of the study. Furthermore, like many *in vivo* human studies, there were participants unable to complete all trials. This was impacted further by the COVID-19 virus, which ended any attempt to collect more data or recruit participants. If original participants completed all trials, our numbers would have been ten instead of seven.

Additional data such as posterior cerebral artery blood velocity, transfer function analysis of spontaneous (resting) and forced (sit-to-stand manoeuvres and thigh cuff release) blood pressure oscillation was recorded during the study. The aforementioned data will be analysed and presented in a more detailed manuscript submitted for publication at a later date. For the purpose of this thesis, the candidate and supervisors decided to focus on the MCA as previous studies have used this artery as their main cerebral vessel. Furthermore, the decision to examine the VM, which is a well-recognised method for challenging cerebral autoregulation, would allow comparisons with existing literature.

There have been few studies investigating the effects of oestrogen and the menstrual cycle on cerebrovascular regulation, despite the knowledge that ovarian hormones can alter arterial vascular tone. Existing literature had poor control in regard to the timing of data collection throughout the menstrual cycle. Timing is paramount given the variability in individual cycle duration and frequency of ovulation. This study was designed to investigate the effects of oestrogen on cerebrovascular regulation in healthy female participants. As the candidate, I prepared the entire ethics application and gained the ethical approval needed for the research. I was solely responsible for the recruitment participants from Massey University, Wellington, as well as the wider Wellington area. Data collection for this thesis,

and the additional measures not included herein, was completed by Dr Perry and I (candidate contribution = 75%), and took place in a laboratory at Massey University, Wellington. I completed data analysis (100%) and statistical analysis (100%) under guidance of the supervisory team. I wrote and completed the entire thesis report for this degree.

Acknowledgements

First and foremost, I would like to say a massive thank you to my supervisors Dr Blake Perry, Associate Professor Toby Mundel, and Dr Sally Lark. Thank you, Dr Perry for all your words of encouragement, support and your extensive knowledge. Your guidance, ability to help me understand difficult concepts, and think critically about the research is greatly appreciated. Thank you, Associate Professor Toby Mundel for jumping on to this research and providing a wealth of knowledge and expertise in women's health and the menstrual cycle. Your words of wisdom and ideas always stimulated discussions that helped improved the quality of the research. I am also very thankful for the guidance, encouragement, and support of Dr Sally Lark. The abundance of knowledge that you possess and were able to give was extremely helpful and deeply appreciated throughout the research. Your different point of view allowed us to see all angles of the research and improve what was being done. Without my supervisors I would have been lost and struggled more than I needed to. Words cannot express how grateful I am.

This study would be nothing without our participants, thank you to all the participants who took time out of their busy lives to partake in this research. Without your contribution, the study would not have been possible. Your contribution to this field of research is greatly appreciated and will assist in understanding women's health.

Finally, I would like to thank my family, especially my mum and dad for their endless support, words of encouragement, and unconditional love. I am forever grateful and lucky to know that no matter what I choose to pursue, I have their entire love and support.

Table of Content

List of Abbreviations

A

AFC	Activity flow coupling
α	Alpha
ACA	Anterior cerebral artery
ABP	Arterial blood pressure
ANOVA	Analysis of Variance
ANS	Autonomic nervous system

B

bpm	Beats per minute
β	Beta
BK_{Ca}	Big Calcium channel
BBB	Blood brain barrier
BP	Blood pressure

C

Ca^{2+}	Calcium
CO_2	Carbon Dioxide
CVD	Cardiovascular disease
cm	Centimetre
cm/sec	Centimetres per second
cm/sec/mm Hg	Centimetres per second per millimetre of Mercury
CA	Cerebral autoregulation
CBF	Cerebral blood flow
CBFv	Cerebral blood flow velocity
CVCi	Cerebrovascular conductance index
CCA	Common carotid artery
CPP	Cerebral perfusion pressure

CVR	Cerebrovascular resistance
ΔP	Change in pressure
η	Coefficient of viscosity

D

°C	Degrees Celsius
DBP	Diastolic blood pressure
DMCAv	Diastolic middle cerebral artery blood velocity
DHT	Dihydrotestosterone
dCA	Dynamic cerebral autoregulation

E

ECA	External carotid artery
EDHF	Endothelial-derived hyperpolarising factor
eNOS	Endothelial nitric oxide synthase
ECG	Electrocardiogram
EF	Early follicular
E2	Estradiol

G

g	Grams

H

HF	Heart failure
HR	Heart rate
Hz	Hertz
HRT	Hormone replacement therapy
H^+	Hydrogen ion

I

ICA	Internal carotid artery
ICP	Intracranial pressure
IUD	Intra uterine device

K

K^+	Potassium ion
kg	Kilogram

L

L	Length of vessel
L-LMMA	NG-monomethyl-L-arginine
LF	Late follicular

M

MAP	Mean arterial pressure
MCA	Middle cerebral artery
MCAv	Middle cerebral artery blood velocity
$MCAv_{mean}$	Mean middle cerebral artery blood velocity
min	Minute
ml	Millilitre
ML	Mid-luteal
ml/kg/min	Millilitre per kilogram per minute
mm Hg	Millimetre of Mercury
MI	Myocardial infarction
MRI	Magnetic resonance imaging

N

nmol/L	Nanomoles per litre

NO	Nitric oxide
n	Number

O

OLBNP	Oscillatory lower body negative pressure
O_2	Oxygen

Q

Q	Cardiac output

R

RI	Resistance Index
r	Vessel diameter

P

PCO_2	Partial pressure of carbon dioxide
P_aCO_2	Arterial partial pressure of carbon dioxide
$P_{ET}CO_2$	Partial pressure of end-tidal carbon dioxide
P_aO_2	Arterial partial pressure of oxygen
pmol/L	Picomoles per litre
PCA	Posterior cerebral artery
PCAv	Posterior cerebral artery blood velocity
p	Probability
P4	Progesterone

S

s	Second
SD	Standard deviation
sCA	Static cerebral autoregulation
SNA	Sympathetic nervous system activity

SNS	Sympathetic nervous system
SBP	Systolic blood pressure
SMCAv	Systolic middle cerebral artery blood velocity

T

T	Testosterone
TCD	Transcranial Doppler
TFA	Transfer function analysis

V

VM	Valsalva manouevre
VA	Vertebral artery

Y

y	Years

List of Tables

List of Figures

Chapter One: Introduction

The brain, like many other organs in the body, requires adequate blood flow to be able to carry out normal function. Despite the brain only making up 2 – 3% of the body's entire mass, it needs approximately 15% of total cardiac output and approximately 20% of total oxygen consumption (Willie, Tzeng, Fisher, & Ainslie, 2014). Furthermore, despite the high metabolic rate, the brain does not store any energy substrate (Brown & Ransom, 2007) and therefore requires a constant supply of blood that is tightly regulated to allow adequate nutrient supply and metabolic waste removal. Brain blood supply is indirectly measured as variation in blood pressure. Cerebral perfusion pressure (CPP) is defined as the difference between MAP and intracranial pressure (ICP; CPP = MAP – ICP) (Smith, Clayton, & Robertson, 2011). Given that simple manoeuvres such as standing can rapidly decrease MAP, and therefore CPP, it is imperative that the brain can respond quickly to changes in MAP to maintain adequate perfusion. Even short periods of reduced perfusion can lead to unconsciousness (Van Lieshout, Wieling, Karemaker, & Secher, 2003), brain damage, and death if CPP does not return to normal (Smith, Clayton, & Robertson, 2011). As a means of protection against stresses such as changes to arterial blood pressure (ABP), neuronal metabolism, and autonomic nervous system (ANS), the brain has an array of regulatory mechanisms that interact with each other to enable adequate blood flow (Willie et al., 2014). An important mechanism involved in maintaining adequate cerebral blood flow (CBF) is cerebral autoregulation (CA). This mechanism is important in defending the brain against changes in blood pressure and works by altering the diameter of arterial blood vessels, thus controlling blood flow in the brain (Fantini, Sassaroli, Tgavalekos, & Kornbluth, 2016). Lassen (1959) stated that CA could defend blood pressure changes between the MAP ranges of 60 – 150 mm Hg. However, Tan (2012) found that the effective range is much narrower than previously thought (Figure 3).

CA is not the only mechanism that can alter cerebral vessel diameter. Sex hormones can alter arterial vessel diameter under different physiological and pathological conditions (Robison, Gannon, Salinero, & Zuloaga, 2019) in both females and males. Due to the different concentrations of circulating sex hormones, there are differences in CBF and CA between females and males (Krause, Duckles, & Pelligrino, 2006). Reports have shown that male sex

hormones induce vasoconstriction in cerebral vessels, thus decreasing CBF (Geary, Krause, & Duckles, 1998). However, the effects of female ovarian hormones are complicated. Krejza et al. (2003) found that during the late follicular phase when oestrogen concentrations are at their highest and progesterone concentrations are unchanged (steady), they saw an increase in cerebral blood flow velocity (CBFv) in the internal carotid artery (ICA), thus highlighting the vasodilatory ability of oestrogen (when no other regulatory mechanism is acting upon the vessel). During mid-luteal phase a reduction in CBFv was seen, which implies that there is relationship between cerebrovascular resistance (CVR) and progesterone concentrations (Krejza, Mariak, Nowacka, Melhem, & Babikian, 2004). Therefore, the fluctuating sex hormone concentrations during menstrual cycle, could have adverse effects on the female cerebral circulation and CA.

Abidi et al. (2017), who examined the effects of the Valsalva manoeuvre (VM) and orthostasis on CA during the follicular phase (low oestrogen and progesterone concentrations) and the mid-luteal phase (high oestrogen and progesterone concentrations) of the menstrual cycle. The authors found that during the mid-luteal phase, women displayed a greater tendency towards vasoconstriction during the VM and orthostasis when compared to men. There were no differences between oral contraception users and non-oral contraceptive users. Furthermore, in contrast Favre and Serrador (2019) found that there was no difference in CA in healthy women who were not on any form of contraception across the menstrual cycle, see Section 2.15 for further discussion.

The lack of research is due to the additional difficulty of requiring testing to be completed at more than one time point to account for the changes in hormones. Furthermore, external factors such as stress, diet, and exercise can modify the menstrual cycle. A factor that further complicates the studies on the menstrual cycle is that a significant proportion of women of reproductive age are using contraception, which can alter hormone concentrations. Research with women on contraception provides important information, however, it does not provide information in regard to the natural occurring variations in women ovarian hormones.

Increasing the understanding of the effects of female ovarian hormones is of particular importance because there is a significant increase in the incidence of stroke in women after menopause (Lisabeth & Bushnell, 2012). Due to menopausal women having low oestrogen

concentrations, it is plausible that oestrogen has a regulatory role and a possible protective mechanism in cerebrovascular function. There is very little scientific investigation on cerebrovascular function in healthy eumenorrheic women. A previous study by Abidi et al. (2017) reported that female ovarian hormones could potentially play a role in cerebrovascular function in response to changes in ABP. However, the authors of this study only investigated the low oestrogen and progesterone phase against the high oestrogen and progesterone phase, therefore, it is difficult to isolate which hormone is eliciting the response seen in the study.

The objective of this thesis was to investigate the potential regulatory role of female ovarian hormones in cerebrovascular regulation by comparing cerebrovascular responses throughout the menstrual cycle in eumenorrheic women. This thesis aims to assist in improving the understanding of female ovarian hormones on CBF regulation by examining healthy female individuals who are not using oral contraceptives, hormonal contraception, and implant or intra uterine devices (IUD), during the VM. By furthering our understanding on the role that these hormones play on cerebrovascular function in healthy eumenorrheic women, future studies can investigate the physiological impact when endogenous hormones are altered naturally or pharmacologically. The hypothesis for this investigation was that during the high oestrogen phase (late follicular) of the menstrual cycle, female participants would demonstrate a blunted response in dynamic cerebrovascular autoregulation compared to the early follicular phase of the menstrual cycle.

Chapter Two: Literature Review

2.1 Cerebral Blood Flow

The human brain accounts for ~2% of total body mass but receives close to 15% of cardiac output (Q) and is responsible for ~20% of the body's oxygen consumption (Willie et al., 2011) due to an extraordinarily high metabolism. As there are no energy stores in the brain (Brown & Ransom, 2007), energy substrate needs to be continuously delivered to the brain, thus resulting in precise CBF regulation (Willie et al., 2011; Willie et al., 2014). Any significant changes to CBF such as inadequate perfusion (hypoperfusion) or excess perfusion (hyperperfusion) can result in severe brain injury (Fantini et al., 2016).

The brain receives blood via two pairs of arteries: the internal carotid arteries and the vertebral arteries (VA). The internal carotid artery then bifurcates into the middle cerebral artery (MCA) and the anterior cerebral artery (ACA), with the middle cerebral artery being the larger division of the ICA (Makowicz, Poniatowska, & Lusawa, 2013). The MCA provides blood flow to portions of the frontal lobe and the lateral surface of both the temporal and parietal lobes of the brain by distributing blood to the anterior circulation (Chandra, Li, Stone, Geng, & Ding, 2017). The vertebral arteries anastomose to form a singular basilar artery, which then bifurcates into the left and right posterior cerebral arteries which supply blood to the occipital lobe and the brain stem (posterior circulation) (Chandra et al., 2017). The posterior circulation is connected by the posterior communicating arteries, while the anterior communicating artery connects the left and right anterior cerebral arteries, which forms an arterial anastomotic ring called the circle of Willis (Figure 1). In human brains, the circle of Willis comprises the anterior cerebral arteries, anterior communicating artery, posterior cerebral arteries, and the posterior communicating arteries (Vrselja, Brkic, Mrdenovic, Radic, & Curic, 2014) and due to the anastomotic arrangement, the circle of Willis forms collateral circulation for the cerebral circulation (see Figure 1). There are many structural variations of the circle of Willis, and though the collateral circulation function stays intact, an abnormal circle of Willis will determine the occurrence, manifestation, and treatment of cerebrovascular disease (CVD) (Iqbal, 2013).

Figure 1 Diagram of the arteries forming the circle of Willis (Vrselja et al., 2014).

CBF can be defined as "the blood volume that supplies the brain at a given time", written in the units of ml blood / min (Fantini et al., 2016). Poiseuille's law is a fundamental law in haemodynamics and states that the rate of blood flow is determined by the change in pressure (ΔP), vessel diameter (r) to the fourth power, and is inversely related to the length of the vessel (L) and the coefficient of viscosity (η);

$$Blood\ flow = \frac{\Delta P \pi r^4}{8\eta L}$$

Changes in blood pressure (BP), cardiac output, neural metabolism, arterial partial pressure of carbon dioxide ($PaCO_2$), and sympathetic nerve activity all alter CBF (Ainslie & Duffin, 2009).

Figure 2 The directional effects of physiological factors involved with CBF regulation. BP, blood pressure; SNA, sympathetic nerve activity; PCO_2, partial pressure of carbon dioxide (Ainslie & Duffin, 2009).

Poiseuille's law is important in determining blood flow through a vascular bed. In the cerebral circulation, what drives blood through the vasculature of the brain is the CPP and inversely the CVR of the entire vascular tree (Willie et al., 2014). As mentioned earlier CPP is the difference between MAP and ICP (Tameem & Krovvidi, 2013). ICP tends to deviate during everyday activities such as breath holding (Valsalva manoeuvre) (Greenfield, Rembert, & Tindall, 1984), coughing and sneezing (Dunn, 2002), and postural changes, (Andresen, Hadi, Petersen, & Juhler, 2015; Schneider, Helden, Franke, Lanksch, & Unterberg, 1993) but also brain injuries can increase ICP, due to the minimal space to expand from being confined in the skull (Haykowsky, Eves, Warburton, & Findlay, 2003). Due to variations in MAP occurring more often and at a greater magnitude, this makes MAP the largest contributing factor affecting CPP. Small arteries and the pial arterioles contribute greatest to CVR (Kulik, Kusano, Aronhime, Sandler, & Winn, 2008). From Poiseuille's law, it is evident that any changes in radius (i.e., vasodilation or vasoconstriction) will significantly affect CBF (Tameem & Krovvidi, 2013).

The brain has a poor tolerance for ischaemia (Van Lieshout et al., 2003), with tissue remaining viable without adequate oxygen and nutrients for only a couple of minutes (Lee, Grabb, Zipfel, & Choi, 2000) and if oxygen levels are not restored to adequate levels, brain injury and brain damage can occur (Tameem & Krovvidi, 2013). If there is an excess CBF, it could lead to a breakdown of the brain-blood barrier (BBB) which leads to hyperperfusion syndromes such as seizures, headaches, encephalopathy, and stroke (van Mook et al., 2005). Therefore, understanding CBF regulation will help us better understand pathologies caused by a compromised CBF.

2.2 Regulation of CBF

2.2.1 Arterial Partial Pressure of Carbon Dioxide

The discovery that arterial partial pressure of carbon dioxide (P_aCO_2) has a significant effect on CBF was first made by Kety and Schmidt (1945), with more recent data indicating beP_aCO_2 is the most potent regulator of CBF (Figure 2) (Ainslie, Ashmead, Ide, Morgan, & Poulin, 2005; Verbree et al., 2014). Kety and Schmidt (1948) found that an elevation in P_aCO_2 (hypercapnia) produces vasodilation which leads to an increase in CBF. In contrast, a low level (hypocapnia) leads to a reduction in CBF (Kety & Schmidt, 1946; Wasserman & Patterson, 1961). Therefore, P_aCO_2 is seen to have an effect on vascular resistance by altering vessel diameter. How CO_2 initiates changes in vascular tone is still unknown (Battisti-Charbonney, Fisher, & Duffin, 2011), however it is believed that carbon dioxide produces a change in H^+ ions which activates the voltage potassium channels leading to hyperpolarisation of the endothelial cells, thus altering the vascular tone of the vessel (Ainslie & Duffin, 2009; Kitazono, Faraci, Taguchi, & Heistad, 1995; Nelson & Quayle, 1995).

Fortune et al. (1992) demonstrated an increase in CBF by 3% when they increased P_aCO_2 by 1 mm Hg. Battisti-Charbonney et al. (2011); Ide, Eliasziw, and Poulin (2003); Skow et al. (2013); Willie et al. (2012) showed that for every 1 mm Hg increase in $PaCO_2$ there was a 3 – 6% increase in CBF, whilst for every 1 mm Hg decrease in $PaCO_2$ there was an approximate 1 – 3% decrease in CBF. Any changes in $PaCO_2$ are rapidly followed by changes in CBF. Whilst CBF may be more sensitive to hypercapnia compared to hypocapnia, Wasserman and Patterson (1961) found that only a 2 mm Hg decrease in $PaCO_2$ is required to initiate vasoconstriction of vessels. In contrast, a 4.5 mm Hg increase is needed to initiate vasodilation. Wasserman and Patterson (1961) also discovered that once the threshold of 4.5 mm Hg increase is reached, the overall CBF pattern to prevailing hypercapnia is sigmoidal. Once chemoreceptor activation is sufficient to increase MAP then it also becomes a pressure-dependent response (Battisti-Charbonney et al., 2011). Conversely, unlike hypercapnia, once the 2 mm Hg decrease in $PaCO_2$ is reached, there is not a linear response to a hypocapnia, but a smaller response in CBF per unit decrease in arterial carbon dioxide (Wasserman & Patterson, 1961).

2.2.2 Arterial Partial Pressure of Oxygen

Although carbon dioxide may be the most potent regulator of CBF, arterial partial pressure of oxygen (PaO_2) still contributes to the regulation and can have a significant effect on CBF (Kety & Schmidt, 1948). Hyperventilation (arising from hypoxia) would produce hypocapnia and reduction in CBF, reflecting the greater influence of CO_2 on CBF. However, isocapnic hypoxia has been seen to increase cerebral vasodilation (Ainslie & Poulin, 2004). Vasodilation is achieved through the process of activating the membrane of the potassium (K^+) channels which decreases calcium (Ca^{2+}) levels, thus producing a relaxation effect on vessel tone (Pearce, 1995). Hypoxia-induced increases in CBF are complex, and there are many contributing factors to the increase seen in CBF. For example, to observe an increase in CBF, PaO_2 will need to be at ~45 mm Hg and oxygen (O_2) saturation at ~80% (Ainslie & Poulin, 2004; Willie et al., 2012).

Adenosine is a metabolite that has been seen as a mediator of hypoxic cerebral vasodilation (Willie et al., 2014), due to the release of adenosine with hypoxaemia (Meno, Ngai, & Winn, 1993). Furthermore, in vitro, it has been demonstrated that adenosine obstructs signals for vasoconstriction within the parenchyma (Gordon, Choi, Rungta, Ellis-Davies, & MacVicar, 2008). Bowton et al. (1988) investigated the effects of adenosine on humans and found that there was a decrease in CBF as well as cerebral oxygen delivery during normoxia when adenosine was inhibited by the administration of aminophylline. Furthermore, the authors also reported that although CBF decreased during normoxia, it increased during hypoxaemia with aminophylline administration. This indicates that adenosine is associated with hypoxic cerebral vasodilation response, however, it cannot be the only mediator of hypoxic-induced increases in CBF. Nitric oxide (NO) is another metabolite that is commonly associated with L-arginine (L-LMMA) infusion on the regulation of cerebrovascular vessels during normoxia and hypoxia. They found that administration of L-LMMA during normoxia did not alter CBF. However, during hypoxia CBF increased and after L-LMMA was administered CBF and CVR returned to baseline measures.

2.2.3 Arterial Blood Pressure

Cerebral autoregulation is a homeostatic mechanism that contributes to maintaining constant CBF despite changes to CPP. Lassen (1959) came up with the concept of cerebral

autoregulation when he compiled data sets from seven different studies and calculated the now well-known autoregulatory curve (Figure 3). The classic autoregulatory curve showed that CA was extremely effective, with CBF staying at a constant level when MAP was between 60 and 150 mm Hg. CA works by altering CVR in response to the change in CPP (Whittaker et al., 2017; Willie et al., 2014). CA acts as a negative feedback loop, reacting to the changes in the MAP to stabilise CBF (Tzeng & Ainslie, 2014). A decrease in MAP would elicit vasodilation of the cerebral vessel thus increasing CBF. Conversely, hypertension would induce vasoconstriction, increase CVR and decrease CBF (Fantini et al., 2016).

One of the first studies demonstrating the influence of blood pressure on cerebrovascular arteries was completed by Fog (1939a, 1939b). He found that a significant decrease in blood pressure caused the pial arteries of the brain to dilate within seconds of the decrease. This inferred that a decrease in blood pressure caused dilation of cerebral arteries and, therefore, increased CBF. Furthermore, Kontos et al. (1978) reported that during changes in CPP the smaller arteries and arterioles of the brain dilated within seconds, thus reflecting that changing CVR of smaller cerebral vessels contributes to defending against arbitrary changes to CPP (Aaslid, Lindegaard, Sorteberg, & Nornes, 1989). Static cerebral autoregulation (sCA) and dynamic cerebral autoregulation (dCA) are the same mechanism that responds to the changes in MAP occurring in the cerebral circulation, with the only difference being the time frame in which they respond (Tan & Taylor, 2014; Zhang, Zuckerman, Giller, & Levine, 1998). sCA is engaged when change in MAP is gradual (minutes to hours) and/or is sustained over a period of time and buffers lower frequencies (<0.5 Hz) MAP changes, whilst dCA regulates rapid (seconds) changes to MAP in the higher frequency range (>0.5 Hz).

Although the exact mechanism/s are not clear, it has been suggested that CA comprises of three physiological mechanisms that contribute to maintaining stable CBF, the myogenic, metabolic, and neurogenic mechanisms (Tzeng & Ainslie, 2014; Willie et al., 2014). The myogenic mechanism is how vascular smooth muscles respond to a change in transmural pressure (Ibrahim, McGee, Graham, McGrath, & Dominiczak, 2006). Changes in transmural pressure induce a change in arterial diameter through constriction or dilation of the smooth muscles (Osol & Halpern, 1985). Metabolic mechanism refers to the changes in the concentrations in metabolites that influence changes in the vasoactive properties of blood

vessels. These metabolites can change the permeability of the vessel, which can cause contraction of the smooth muscles, thus changing the vessel properties (Panerai, 2008). The neurogenic mechanism of CA is characterised by the neurons secreting neurotransmitters with the ability to decrease and increase small to moderate vessel diameters to regulate CBF (Rivera-Lara et al., 2017). Furthermore, the neurogenic mechanism is also referred to as neurovascular coupling. As discussed in Section 2.10, neurovascular coupling is the increase in blood flow to local or global brain regions in response to increased neuronal activity (Belanger, Allaman, & Magistretti, 2011). The mechanisms that contribute to CA are all chemically regulated, which infers that a set amount of time is needed to induce the necessary changes in CVR in response to changes in the MAP (Fantini et al., 2016). dCA processes occur rapidly and therefore, is the contribution of the myogenic response (Bevan & Hwa, 1985). dCA is regulated by the stretch-activated ion channels (Peterson, Wang, & Britz, 2011), where the stretch of the vessel wall is caused by changes in blood pressure (Bevan & Hwa, 1985). Voltage-gated Ca^{2+} channels are sensitive to mechanical stimuli (Hill, Zou, Potocnik, Meininger, & Davis, 2001; Schubert & Brayden, 2005), therefore, intracellular Ca^{2+} concentrations can be altered due to stretch stimuli (Davis & Hill, 1999; Hill et al., 2001; Schubert & Mulvany, 1999). Tzeng, Chan, Willie, and Ainslie (2011) reported that blocking Ca^{2+} channels of the brain cause large cerebral vessels to dilate, thus decreasing middle cerebral artery blood velocity (MCAv) and increasing pressure-driven blood flow, highlighting the importance of Ca^{2+} in dynamic autoregulation.

2.2.4 Dynamic Cerebral Autoregulation

Dynamic cerebral autoregulation quantifies the changes in middle MCAv in response to abrupt alterations in CVR due to rapid changes in ABP (Aaslid et al., 1989). Technological advances such as the development of transcranial Doppler (TCD) ultrasonography and the finger photoplethysmography (Finapres) enabled the examination of the dynamics of the pressure-flow relationship of CBF by allowing beat-to-beat observations of CA responses to BP of different magnitude and duration (van Beek, Claassen, Rikkert, & Jansen, 2008). Aaslid et al. (1989) conducted the first experiment looking at dCA using the TCD. They induced a sudden change in ABP using a thigh-cuff deflation method in which they inflated the thigh cuff 20 mm Hg above the participant's systolic pressure for 2 minutes, then deflated the thigh cuff causing instantaneous hypotension. The authors found that dynamic cerebral

autoregulatory capacity is more efficient at compensating transient hypertension rather than transient hypotension. This phenomenon is referred to as hysteresis (Brassard, Ferland-Dutil, et al., 2017). Tzeng, Lucas, Atkinson, Willie, and Ainslie (2010) found there is a compensatory relationship between baroreflex and dCA. If an individual possesses greater dCA response, then they have lesser baroreflex sensitivity and vice versa. This indicates there is an interaction between dCA and baroreflex sensitivity to regulate any abrupt changes in blood pressure.

2.2.4.1 Effects of $PaCO_2$ on Cerebral Autoregulation

Carbon dioxide is the most potent regulator of CBF (Ainslie et al., 2005), however the effects of $PaCO_2$ on CA can vary. Aaslid et al. (1989) and Czosnyka, Harris, Pickard, and Piechnik (1993) reported that dCA was compromised during hypercapnia. The plateau seen in the autoregulation curve shortens and is shifted to the right, whilst the upper limit of the curve is shifted left, thus decreasing the range and efficiency of the autoregulatory mechanism (Meng & Gelb, 2015). The brain can become pressure-passive when the hypercapnic stimulus produces chemoreceptor-induced increases in MAP (Battisti-Charbonney et al., 2011). During severe hypercapnia, the cerebral vessels are fully dilated, which results in the plateau in the autoregulatory mechanism to be lost, thus leading to a linear relationship between pressure and flow (Meng & Gelb, 2015). McCulloch, Visco, and Lam (2000) showed that in patients under general anaesthesia, graded hypercapnia impaired sCA. Furthermore, McCulloch et al. (2000) reported that to significantly impair CA during sevoflurane anaesthesia, , the $PaCO_2$ threshold averaged 56 mm Hg, whilst during propofol anaesthesia the $PaCO_2$ threshold averaged 61 mm Hg. Hypercapnic impairments in sCA seen in humans under anaesthesia are also seen in conscious humans. Perry et al. (2014) saw that in hypercapnic conditions (+5% CO_2), during a steady-state increase in MAP (+ 40 mm Hg), sCA was impaired with increases in MAP translated into the cerebral circulation.

Conversely, hypocapnia is reported to have a restorative effect on dCA (Aaslid et al., 1989) and sCA during isoflurane anaesthesia (McCulloch, Boesel, & Lam, 2005). Meng and Gelb (2015) illustrated a widening of the autoregulatory plateau and the upper limit of the CA. Furthermore, the authors reported that any changes in the lower limits of the autoregulatory curve were insignificant. The downward shift seen by Meng and Gelb (2015) could be a concern as lower CBF can lead to hypoperfusion, and therefore ischaemia and

tissue damage. The vasomotor tone of cerebral vessels is the main factor regulating CA (Aaslid et al., 1989), with the dilatory effect of $PaCO_2$ contributing to the impairment of CA (McCulloch et al., 2000). dCA has been seen to be impaired by hypercapnia and restored by hypocapnia (Aaslid et al., 1989); however, more studies are needed to look at these effects on conscious subjects.

2.2.4.2 Effects of PaO_2 on Cerebral Autoregulation

The arterial partial pressure of oxygen may not have a significant effect on the regulation of CBF compared to carbon dioxide; however, hypoxia does influence the regulation of CA and therefore cerebral homeostasis (Ogoh et al., 2014; Ogoh et al., 2013). Ogoh et al. (2014); Ogoh et al (2013) reported that during hypoxia, cerebral CO_2 reactivity is weakened and therefore cerebral vasoconstriction is restricted, which may aid in oxygen delivery. Moreover, the authors found that the impaired dCA seen in hypercapnia and normoxia, was less during concurrent acute hypoxia, indicating that the effects of $PaCO_2$ may be weakened during hypoxia to aid in regulation of CBF and O_2 homeostasis. However, the effects of hypoxia are often confused due to the acute ventilatory response (hyperventilation) that occurs, which induces hypocapnia; thus, the hypoxic effect seen may be in fact caused by the hypocapnic mechanism.

As mentioned above, hypocapnia improves dCA (Aaslid et al., 1989). Ogoh, Nakahara, Ainslie, and Miyamoto (2010) investigated the effects of hypoxia on dCA during isocapnic conditions. The authors reported that dCA was impaired during isocapnic hypoxia, however, they saw an improvement in dCA during mild hypocapnia. This highlights the dominance of carbon dioxide during regulation of dCA. Furthermore, Querido et al. (2013) investigated the effects of poikilocapnic and isocapnic hypoxia on dCA during both hypotensive and hypertensive changes to ABP. The authors found that the efficiency of dCA remained during poikilocapnic hypoxia due to the poikilocapnic induced hypocapnia via hyperventilation, which provided the dCA with a protective mechanism. However, during isocapnic hypoxia Querido et al. (2013) reported an impaired dCA, caused by the vasodilatory effects of hypoxia which agrees with Ogoh et al. (2010). Other studies (Jansen, Krins, Basnyat, Odoom, & Ince, 2007; Nishimura, Iwasaki, Ogawa, & Aoki, 2010; Subudhi, Panerai, & Roach, 2009, 2010) have also reported no changes in CBF during poikilocapnic hypoxia, which indicates that PCO_2 is

the superior chemical moderator of CBF than PO_2, which explains why hyperventilation offsets the effects of isocapnic hypoxia.

Impairments seen in dCA due to hypoxia can be alleviated by hyperoxia (Ainslie et al., 2008; Jansen et al., 2007; Nishimura, Iwasaki, Ogawa, & Shibata, 2007; Subudhi et al., 2010). Hyperoxia does not directly improve dCA (Ogoh et al., 2010), but hyperoxia decreases CBF by increasing CVR (Subudhi et al., 2010) and produces hypocapnia through the ventilatory response (Becker, Polo, McNamara, Berthon-Jones, & Sullivan, 1996). Aaslid et al. (1989) found that the effects that improved or impaired dCA induced by PaO_2 are comparable to the effects produced by $PaCO_2$, due to the influence they have over vascular tone. For example, hypocapnia and hyperoxia induce vasoconstriction of the cerebral vessels which improve CA, whilst hypercapnia and hypoxia induce vasodilation which impairs CA.

2.2.5 Static Cerebral Autoregulation

The static cerebral autoregulation curve proposed by Lassen (1959) has been the accepted model for CA for years, and the findings proved to be monumental for the time. However, the CA curve was generated from a combination of data sets from seven different studies, in which there were a total of 11 diverse participant cohorts. Thus, the CA curve was a representation of inter-subject values of individuals with different health conditions, instead of being a representation of MAP-CBF relationship in a group with the same or similar health status. A reanalysis of the data from Lassen's study was done by Heistad and Kontos (1983), but they did not include all the data, excluding the clinical cohorts to attain a more accurate result. The results from the reanalysis demonstrated that CBF decreased by 2% to 7% for every 10 mm Hg drop in the MAP and increased 7% per rise of 10 mm Hg of the MAP. Lucas et al. (2010) also reinvestigated the CA curve by investigating the effects of intravenous infusion of sodium nitroprusside and phenylephrine to elicit changes in MAP. The authors found that for every 1 mm Hg change in MAP a 0.8% change in MCAv was apparent, indicative of a more pressure-passive MAP-CBF relationship. Furthermore, Tan (2012) reported that the autoregulatory range might only be 5 mm Hg either side of baseline measures of blood pressure (Figure 3), which is much narrower than the range suggested by Lassen (1959). Zhang, Behbehani, and Levine (2009) conducted a study looking at the effects of steady state increases in ABP on MCAv and found that an increase in blood pressure by ~37% (~30 mm Hg)

above baseline increased MCAv by ~11%. Thus, contrasting the results by Lassen (1959) claiming that CBF will only increase when MAP is above 150 mm Hg.

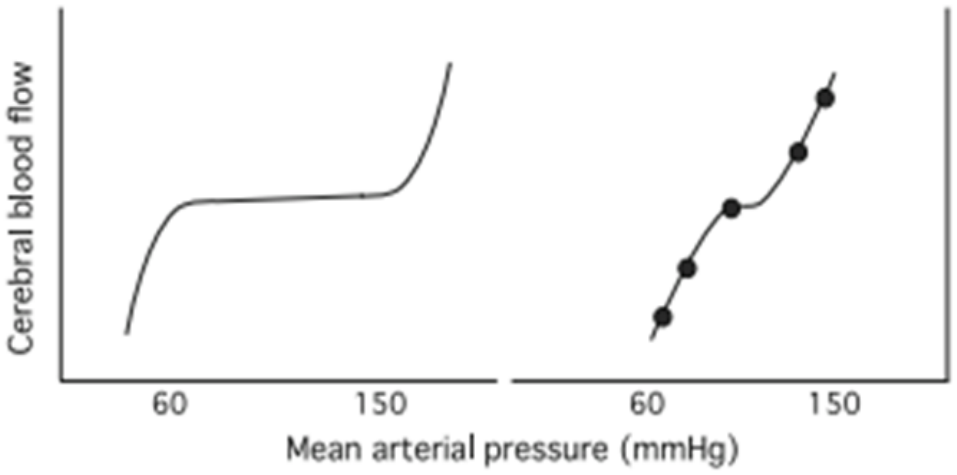

Figure 3 Lassen (1959) classic static cerebral autoregulation curve (left), Tan (2012) limited plateau cerebral autoregulation curve (right) demonstrating the inefficiency of CA. Figure from Tzeng and Ainslie (2014).

2.2.6 Measuring cerebral autoregulation

Activities such as standing up (Sorond, Serrador, Jones, Shaffer, & Lipsitz, 2009; Thomas et al., 2009), the Valsalva manoeuvre (Aaslid et al., 1989; Perry, Cotter, Mejuto, Mundel, & Lucas, 2014; Pott, van Lieshout, Ide, Madsen, & Secher, 2000; Tiecks et al., 1995), body weight squat-stand manoeuvre (Claassen et al., 2009), resistance exercise (Edwards, Martin, & Hughson, 2002; Perry, Schlader, et al., 2014; Romero & Cooke, 2007), and rowing (Pott et al., 1997) inflict a significant ABP oscillations that challenge dCA and are used experimentally to assess dCA effectiveness. The methods used to assess CA are ambiguous as there is no gold standard (Tzeng & Ainslie, 2014; Tzeng, MacRae, Ainslie, & Chan, 2014). One method of assessing CA is to assess the linear relationship between ABP and CBF at rest (Zhang et al., 1998), as normal respiration and cyclic change sin autonomic tone generate spontaneous fluctuations in MAP. Conversely, ABP can be manipulated experimentally, so called driven or forced change sin MAP. Such forced oscillations can be elicited by the manoeuvres described above, with two common modes being repeated squat stands and the

Valsalva manoeuvre, (Claassen, Levine, & Zhang, 2009; Tan, 2012) to challenge the regulatory mechanisms. Hamner et al. (2019) examined CA at very low frequencies (<0.03 Hz) and low frequencies (0.03 – 0.10 Hz) using oscillatory lower body negative pressure (OLBNP) and determined that CA is most effective at buffering changes in ABP at frequencies below 0.03 Hz. Whilst some argue that as the brain is counteracting spontaneous fluctuations in MAP more frequently, assessing dCA at rest provides a more accurate representation of cerebrovascular function (Tzeng & Panerai, 2018). However, the counterargument is that everyday life activities cause changes in ABP far greater than those experienced during rest, therefore it is important to examine how CBP responds to these insults (Simpson & Claassen, 2018). There is an obvious decrease in CA variability observed when CA is challenged by increasing ABP (Birch, Neil-Dwyer, & Murrills, 2001; Claassen et al., 2009). Eliciting changes to ABP allows confirmation that there is a correlation between ABP and CBF, which is a prerequisite for assessing CA. Irrespective of how the change in in the input (MAP) is produced, dCA can be assessed in several ways and three methods are described below

2.2.6.1 Autoregulatory Index

Dynamic cerebral autoregulation can also be assessed using Tiecks et al. (1995) autoregulatory index (AI). Tiecks's AI evaluated dCA by measuring the relative change in CBFv (output) in response to rapid (dynamic) changes in BP (input). The CBFv response generated is compared with 10 theoretical curves that was created using the changes in BP and exact combinations of its parameters to evaluate autoregulation (Tiecks et al., 1995). The effectiveness of dCA can be quantified by comparing the actual CBFv response with one of the 10 curves generated. The autoregulatory index that ranges from 0 (which indicates an absence of autoregulation) to 9 (which indicates the best autoregulation). AI is one of the straightforward methods to measure dCA and can be implemented during the Valsalva manoeuvre (VM) (Tiecks et al., 1995). As mentioned in Section 2.5 below, the VM is an effective technique which induces both increases and decreases in BP. The AI values for the VM are generated for Phase II and Phase IV of the manoeuvre through the equations seen in Section 3.9.1.

2.2.6.2 Rate of Regulation

Rate of regulation (RoR) is another method to examine dCA and uses the equation: RoR = $(\Delta CVR/\Delta t)/\Delta ABP$ (Aaslid et al., 1989). This method has been used to determine the efficiency of dCA following thigh cuff occlusion and more recently by Labrecque et al. (2019) to examine dCA during the sit-to-stand manoeuvre. A critical aspect of the RoR equation is the time in which the changes to CVR occur without the arterial baroreflex regulation (Aaslid et al., 1989). Aaslid et al. (1989) found that the time in which CVR is strictly influenced by dCA to be approximately 1 – 3.5s. The linear regression slope demonstrates the rate of change in CVR, which is dependent on ABP. Theoretically, CBF would be maintained if CVR and ABP were the same, however, that is unlikely, therefore ABP largely influences the RoR (Aaslid et al., 1989). The lower the RoR the more efficient the dCA response to changes in ABP.

2.2.6.3 Transfer Function Analysis

Transfer function analysis (TFA) is a frequency analysis that describes the linear relationship between the input signal (blood pressure) and the output signal (CBF or a proxy e.g. MCAv), with dCA being the regulator (Tzeng & Ainslie, 2014). Resultant metrics include Gain (magnitude), phase shift (timing), and coherence (dependence) are three factors in which dCA describes the transfer function between fluctuations in BP and MCAv. The gain calculates how well CA reduces the transfer of input (BP) to output (MCAv). The dampening effect (gain) between BP and MCAv is observed in the size of fluctuations in BP. An efficient CA is represented by a low gain, while conversely, a high gain illustrates an ineffective CA process. Phase shift describes the movement of 'in phase' waveforms comparative to a different waveform with the same period. The phase shift is used to identify the time lag of the autoregulatory response. The differences are described in degrees or radians. An efficient CA is seen as a 'phase lead' where the phase shift between input and output is a positive number. Conversely, if the number is closer to zero, then CA can be described as inefficient. The linear relationship between the input and output signals is referred to as the coherence. If the frequency of both input and output are almost in sync at a particular frequency, then this would indicate that there is a linear relationship. No relationship between input and output signals can be described when there is a coherence of zero. When quantifying gain and phase shift, the cut-off of coherence >0.5 is used (Zhang et al., 1998).

2.2.7 Neurovascular Coupling

Neurovascular coupling (NVC) involves a matching increase in CBF to local or global regions of the brain to sustain neuronal function, due to an increase in neuronal activity in that area (Attwell et al., 2010; Belanger et al., 2011). During NVC, arterioles in the area of increased neuronal activity dilate within seconds, to sufficiently supply active neurons with oxygen and substrates (Carmignoto & Gomez-Gonzalo, 2010). Astrocytes play a critical role in the mediation of the coupling of neuronal function and vascular response (Zonta & Carmignoto, 2002), via the glutamatergic-mediated pathways. One of these glutamate-mediated pathways is Ca^{2+} dependent. An increase in intracellular Ca^{2+} concentration in astrocytes initiates the activation of voltage-gated Ca^{2+} channels, stimulating vasodilation (Filosa & Blanco, 2007). Astrocyte end feet are in close proximity to the vessel walls, which allows for intracellular Ca^{2+} signalling to the vessel walls, thus triggering a vascular response (Chow et al., 2020; Simard, Arcuino, Takano, Liu, & Nedergaard, 2003). Rosengarten, Spiller, Aldinger, and Kaps (2003) investigated the effects of hypercapnia and normocapnia on neurovascular coupling using visual stimuli. The authors found that hypercapnia impaired dCA by slowing down the initial component. However, hypercapnia enhanced the NVC to the area of the brain being stimulated, thus increasing CBF in that area. This indicates that the initial slow blood flow velocity at the start of dCA aided in distributing blood flow to the activated areas. Further information on neurovascular coupling and the influences it has on CBF can be found in reviews such as Attwell et al. (2010); Filosa and Blanco (2007); Iadecola and Nedergaard (2007).

2.2.8 Autonomic Nervous System (ANS)

Cerebral vessels are greatly innervated by adrenergic and cholinergic fibres (Edvinsson, 1975; Gulbenkian, Uddman, & Edvinsson, 2001), however, the role of the autonomic nervous system in the regulation of cerebral circulation remains contentious (Brassard, Tymko, & Ainslie, 2017; Gulbenkian et al., 2001; Heistad & Marcus, 1978; Strandgaard & Sigurdsson, 2008). Due to the difficulties of methods, there have been limited studies examining the role of the autonomic nervous system in the regulation of CA and the results are inconsistent (Zhang et al., 2002). Only a handful of studies managed to examine the sympathetic nervous systems (SNS) contribution to CA in humans via pharmacological blockade. Zhang et al. (2002) induced a ganglionic autonomic blockade by administering

trimethaphan to examine the cross-spectral relationship between ABP and the fluctuations in CBF via OLBNP. The authors found that ganglionic autonomic blockade altered dCA, as ABP and CBF both decreased, whilst TFA gain was doubled and phase was decreased at low frequencies. Thus, indicating that CA had been reduced. However, the ganglionic autonomic blockade stopped both the sympathetic and parasympathetic nervous control, therefore the effect on dCA could have been from either nervous system or both (Tan & Taylor, 2014). Hamner, Tan, Lee, Cohen, and Taylor (2010) conducted a similar study using OLBNP to examine the cross-spectral relationship between ABP and low-frequency fluctuations in CBF. The authors induced an α-adrenergic blockade using phentolamine. Hamner et al. (2010) results were similar to Zhang et al. (2002), with the sympathetic blockade increased gain and coherence and decreased phase, thus highlighting that there is sympathetic nervous influence on CA. This notion is further supported by a study by Ogoh, Brothers, Eubank, and Raven (2008) and Zhang, Crandall, and Levine (2004). Ogoh et al. (2008) showed that α_1-adrenoreceptor block with prazosin weakens CBF recovery from transient hypotension. Zhang et al. (2004) found that after autonomic ganglion blockade with trimethaphan, dCA was unable to prevent the significant phase IV overshoot in MCAv in the VM (see Section 2.14 for descriptions of VM phases). Although MAP rapidly increased from phase III, no Phase IV BP overshoot was observed. Despite the reduced phase IV response, MCAv increased by 55%, indicative of a loss of vasoconstrictor ability, indicative that the sympathetic nervous system restrains MCAv during rising CPP.

Data available for the effects of cholinergic control in CA was scarce in humans, however, cholinergic nerves are found distributed throughout intracranial vessels (Hamel, 2004; Heistad, Marcus, Said, & Gross, 1980; Sato, Sato, & Uchida, 2001). There has only been one study examining the effects of the parasympathetic nervous control on cerebrovascular control. Hamner, Tan, Tzeng, and Taylor (2012) studied the effect of cholinergic blockade using glycopyrrolate on the pressure-flow relationship during OLBNP at six different frequencies (0.03 – 0.08 Hz). The authors reported that systemic cholinergic blockade impaired CA, as an increase in transfer function coherence was shown between MAP and CBF. This demonstrates that the parasympathetic nervous control has an active and special role in CA. Moreover, Hamner and Tan (2014) reported that the cholinergic nervous control

contributes to a small but significant role in CA, especially in the gain and upper pressure limit in the active regions of CA.

2.3 Cerebral blood flow regulation differences: Males versus Females

Regulation of CBF differs between males and females due to the different sex hormones circulating in the blood (Krause et al., 2006; Robison et al., 2019). Sex hormones are produced in the gonads but can be produced in extragonadal sites such as vasculature and the brain (Simpson et al., 2000). This is important, especially for menopausal women (Simpson et al., 2000). The three main cholesterol-derived sex steroid hormones are androgens (testosterone (T) and dihydrotestosterone (DHT)), oestrogens (estrodiol (E2), estrone, estriol), and progestogens (progesterone (P4)) (Pelligrino & Galea, 2001; Robison et al., 2019). Oestrogen is often perceived as the female sex hormone, while androgens are seen as purely male sex hormones. However, females and males have both oestrogens and androgens, albeit at varying concentrations (Robison et al., 2019).

The different concentrations of androgens and oestrogens in females and males influence their cerebral vasculature reactivity and therefore CBF (Krause et al., 2006). Due to having higher oestrogen concentrations, females are seen to have greater CBF throughout their life compared to their male counterparts. This is because oestrogen increases cerebral vasculature production and/or sensitivity to vasodilatory factors (Chrissobolis & Sobey, 2004; Geary et al., 1998; Geary, Krause, & Duckles, 2000a; Ospina, Duckles, & Krause, 2003; Ospina, Krause, & Duckles, 2002; Skarsgard, van Breemen, & Laher, 1997) and counteract the effects of vasoconstrictive mechanisms (Chrissobolis, Budzyn, Marley, & Sobey, 2004). Oestrogen has been reported to increase nitric oxide production by activating multiple mechanisms that increase endothelial NO synthase (eNOS) (Orshal & Khalil, 2004), such as increasing oestrogen receptor (α) density, which stimulates eNOS gene expression (Mendelsohn & Karas, 1999). This mechanism is referred to as a genomic mechanism because it takes hours to days to initiate vasodilation. However, oestrogen also stimulates non-genomic mechanisms such as increasing the activation of the oestrogen receptor pathway. This pathway activates phosphoinositide-3 kinases-Akt signalling to phosphorylate eNOS at serine-1177/1179, which increases the enzyme activity of eNOS and makes the enzyme more sensitive to Ca^{2+} stimulation (Ho & Liao, 2002; Stirone, Boroujerdi, Duckles, & Krause, 2005). Figure 4 below

shows both the non-genomic and genomic mechanisms in which oestrogen increases cerebral vasodilation and thus increases CBF in females.

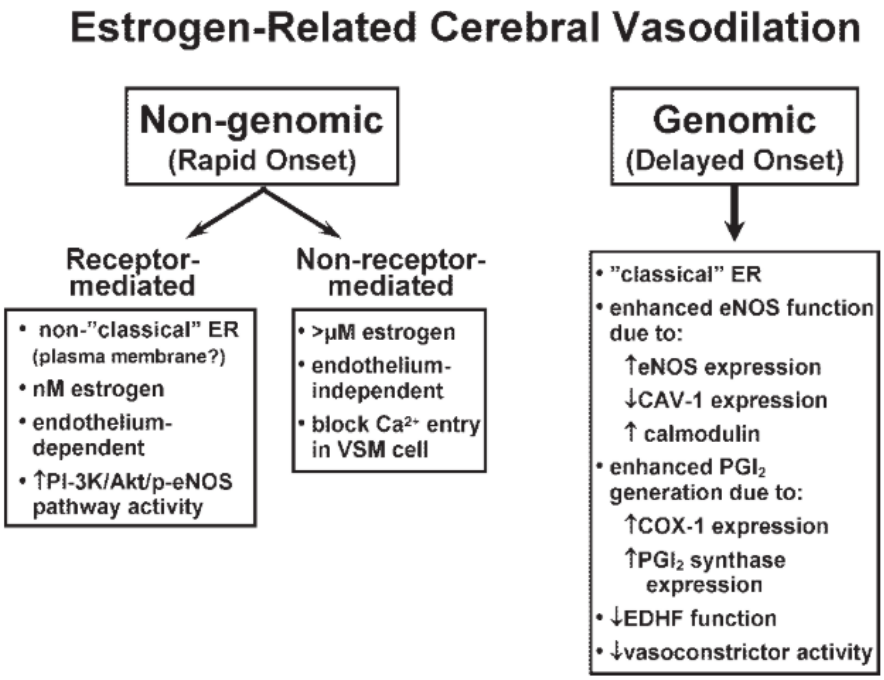

Figure 4 Summary of supposed mechanisms facilitating genomic and non-genomic effects of oestrogen on vasodilating function in cerebral vessels from Krause et al. (2006). ER, oestrogen receptors; Ca^{2+}, calcium; PI-3K, phosphoinositide-3 kinase Akt; p-eNOS, serine-1177/ 1179-phosphorylated endothelial NO synthase (eNOS); CAV-1, caveolin-1; VSM, vascular smooth muscle; COX, cyclooxygenase, PGI_2, prostacyclin; EDHF, endothelial derived hyperpolarising factor.

Conversely, androgens such as testosterone have the opposite effect to that of oestrogen. Testosterone is seen to induce vasoconstriction in the cerebral vessels as a response to transmural pressure (Geary et al., 1998). In vivo studies have investigated the effects of constant exposure to androgens in male rats (Geary, Krause, & Duckles, 2000b; Gonzales, Krause, & Duckles, 2004). The authors found that androgens vasoconstricted the MCA through the endothelial BK_{Ca}–dependent/NO-independent mechanism. In male rats a gonadectomy was seen to elicit vasodilation, however; chronic exposure to testosterone in male rats with a gonadectomy was seen to decrease vasodilator endothelial-derived hyperpolarising factor (EDHF) expression in conjunction with increasing the potent vasoconstrictor thromboxane expression in the MCA (Gonzales, Ghaffari, Duckles, & Krause,

2005; Gonzales et al., 2004). Vascular relaxation has been reported in acute androgen treatments; however, it has not yet been tested on cerebral vessels. Therefore, the effects of androgen hormones on cerebral vessels may be dose-dependent (Robison et al., 2019).

2.4 Sex Hormone levels in Women

Women experience different sex hormone concentrations reflecting which stage of their life they are currently in, what phase of the menstrual cycle they are in and if they use contraception. There is an increase in oestrogen concentrations during pregnancy (Brackley, Ramsay, Pipkin, & Rubin, 1998), whilst during menopause there is a substantial decrease in oestrogen concentrations (Su & Freeman, 2009). Oestrogen (17β-estradiol) and progesterone concentrations also fluctuate during the menstrual cycle (Brackley, Ramsay, Broughton Pipkin, & Rubin, 1999; Diomedi et al., 2001; Krejza et al., 2004; Krejza et al., 2003). These undulating hormone concentrations have been reported to have different effects on the cerebral circulation of women. As seen in Figure 5, during the late follicular phase, when oestrogen concentrations are highest and progesterone concentrations remain steady, cerebrovascular reactivity to CO_2 increases (Diomedi et al., 2001). 17β-estradiol concentration increases in the late follicular phase and reaches a peak on day ~14 (Krejza et al., 2003). During this time there is also a decrease in resistance in the internal carotid arteries, thus leading to an increase CBF (Krejza, Mariak, Huba, Wolczynski, & Lewko, 2001). Alternatively, during the mid-luteal phase, both oestrogen and progesterone concentrations are high, however there is an increase in CVR conjointly with the increase in progesterone (Krejza et al., 2004). Furthermore, studies have shown that progesterone has the ability to counteract the vasodilatory effects of oestrogen, through competitive blockage in the vascular tree of oestrogen receptors by progesterone (Miller & Vanhoutte, 1991; Willekes, Hoogland, Keizer, Hoeks, & Reneman, 1999). An increase in progesterone also initiates hypocapnia via an increase in ventilation, producing the observed increase in CVR (Brackley et al., 1999; Krejza et al., 2004).

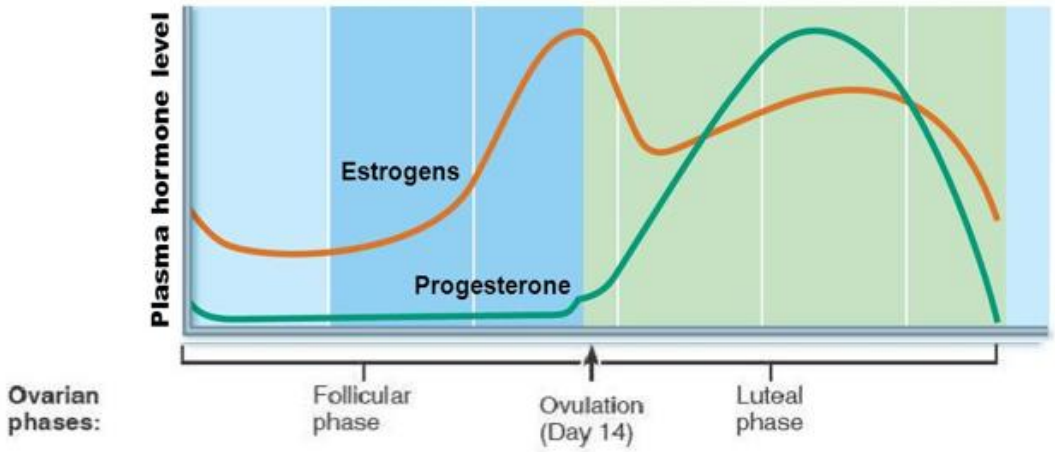

Figure 5 Oestrogen and progesterone levels throughout the menstrual cycle from Marieb and Hoehn (2018)

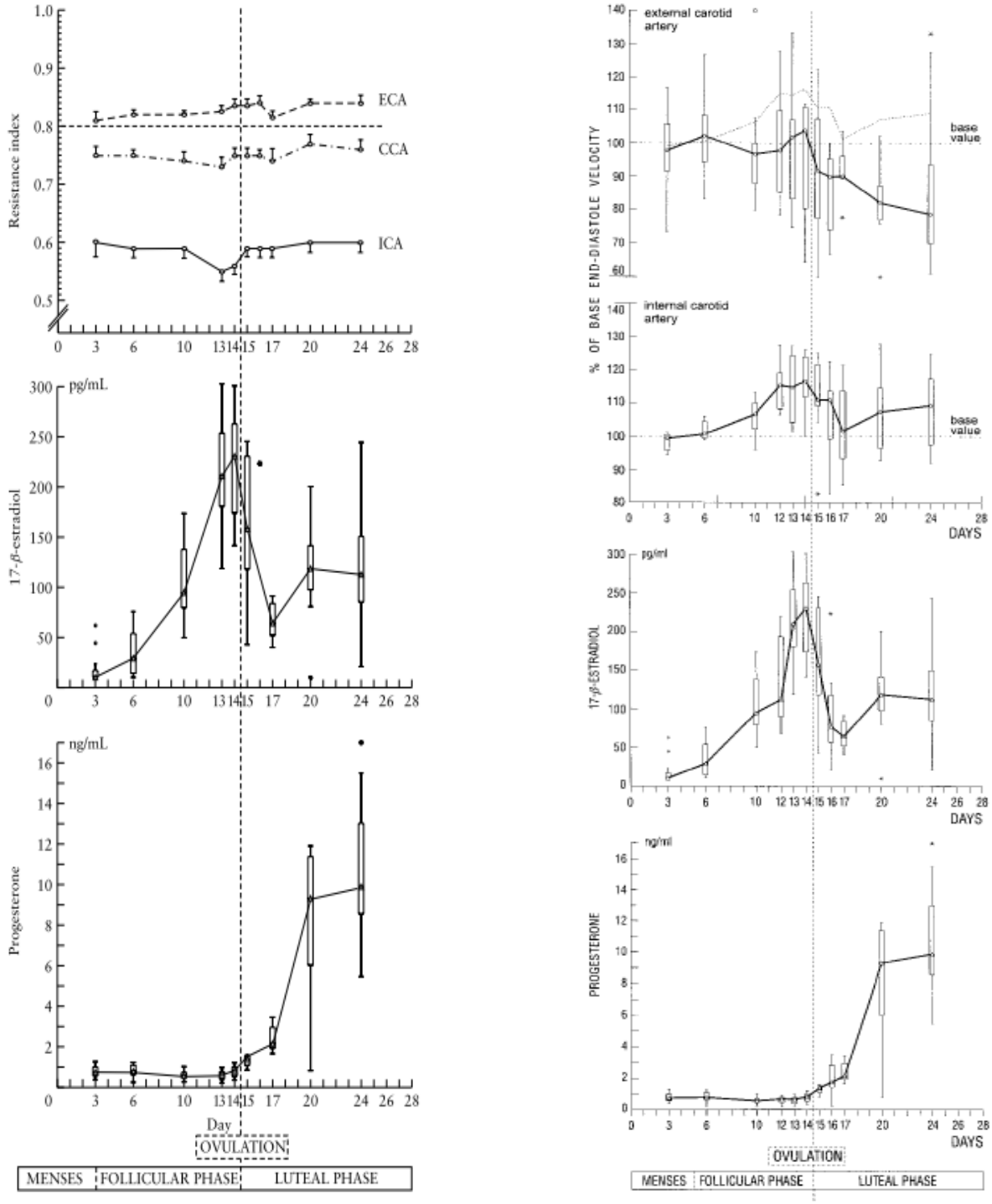

Figure 6 *Left*: Resistance Index (RI) in Internal (ICA) and external (ECA) and common (CCA) carotid arteries and 17β-estradiol and progesterone concentrations during the menstrual cycle (Krejza et al., 2004). *Right*: 17β-estradiol and progesterone concentrations and end-diastolic velocity in ICA and ECA during the menstrual cycle (Krejza et al., 2001).

As described in the previous paragraph, the changing hormone concentrations throughout the menstrual cycle can alter the haemodynamics of cerebral vessels and therefore can influence CBF (Krause et al., 2006). However, some studies have reported an increase in internal carotid artery blood velocity as a consequence of increased endogenous (Krejza et al., 2004) and exogenous oestrogen (Ohkura et al., 1995), while one has reported no change in CBF (Bain, Lees, Lumsden, & Walters, 2005). Furthermore, during the luteal phase, progesterone has a more dominant effect, producing vasoconstriction in the ICA and reducing mean ICA velocity (Krejza et al., 2004). The authors found that from base measures (pulsatility index (PI) measures from day 3 and day 6), late follicular day 13 and day 14 showed a decrease in resistance and increase in ICA blood velocity which is greater than day 17 when progesterone and oestrogen concentrations are both high. The oscillating changes in hormones during the menstrual cycle could contribute to women experiencing greater orthostatic hypotension compared to men (Ali et al., 2000; Cheng, Vyas, Hymen, & Perlmuter, 2011). It has been reported that during the early follicular phase, there is a higher incidence of light-headedness (Muppa et al., 2013; Peggs et al., 2012), which could be caused by increased vasodilation of cerebral vessels and the inability to vasoconstrict peripherally promptly during orthostasis.

2.5 Valsalva Manoeuvre

An essential aspect of the assessment of dCA is to look at the response of cerebral blood flow to changes in blood pressure. There are numerous methods to cause oscillations or sudden increases and decreases in blood pressure. For the purposes of this thesis the VM was the intervention of choice to challenge cerebrovascular regulation.

The VM is a simple but effective technique to induce characteristic changes in blood pressure and CBFv (Tiecks et al., 1995). There are four distinct phases of the VM that reflects the changes in ABP, which can be seen in Figure 9 in Section 3.10. Phase I is defined as the initial increase in blood pressure that occurs due to the increase in intrathoracic and intra-abdominal pressure, which occurs at the onset of straining (breath hold). Phase IIa is characterised by the first decrease in blood pressure seen in Figure 9. The decrease in blood pressure is because the reduction in venous return and decreased stroke volume. The decrease in ABP activates the baroreflex which initiates vasoconstriction and increases HR

(phase IIb) (Remmen, Aengevaeren, Verheugt, & Jansen, 2005). Once the strain is alleviated, there is a rapid decrease in MAP (phase III) (Tiecks et al., 1995), and the overshoot seen in MAP is phase IV of the VM, as cardiac output has been restored and being ejected into the constricted vasculature. Williams (1981) observed that an increase in intrathoracic pressure could translate into an increase in intracranial pressure, which consequently can decrease CPP more than what is reflected by BP. Pott, van Lieshout, Ide, Madsen, and Secher (2000) observed a decrease in $PaCO_2$ during the standing VM. This indicates that during a 15s VM, the decrease in $PaCO_2$ contributes to the 10 – 15% decrease in MCAv observed. Moreover, the VM was being performed standing up, indicating that orthostatic factors such as stroke volume and cardiac output contributed to the decrease in MCAv.

2.6 Previous Literature the effects of hormone concentration on cerebrovascular regulation

Previous studies by Abidi et al. (2017) and Favre and Serrador (2019) both investigated the sex differences in CA across the menstrual cycle. Abidi et al. (2017) investigated women on the oral contraceptive pill (*n* = 14) and women who were not on the contraceptive (*n* = 12) during the low hormone days (days 2 – 5) and high hormone days (day 18 – 24) of the menstrual cycle. Low hormone was defined as early follicular, when circulating oestrogen and progesterone was low and high hormone was identified as mid-luteal, when circulating oestrogen and progesterone was high (also see Figure 5). To challenge CA, the participants partook in the VM and a supine-sit-stand protocol. The authors found that women have superior CA when compared to their male counterparts. Abidi et al. (2017) also reported that during the high hormone phase of the menstrual cycle, there was a greater tendency towards vasoconstriction during the VM and the supine-sit-stand protocol, however there were no significant differences in CA between the different menstrual cycle phases. Abidi et al. (2017) compared low oestrogen and progesterone concentrations to high oestrogen and progesterone concentrations, which made it difficult to distinguish which hormone was eliciting the effect seen in cerebrovascular response.

Favre and Serrador (2019) examined the difference in CA between males and females, but, also the difference in CA across three different stages of the menstrual cycle; early follicular (~day 3), late follicular (~day 13), and the mid-luteal (~day 23). The female

participants of this study were not on any form of contraception. The sit-to-stand manoeuvre and repeated squat-to-stand manoeuvre were used to challenge CA. Similar to Abidi et al. (2017), Favre and Serrador (2019) did not find any significant differences in cerebral autoregulation across the menstrual cycle in women. Furthermore, young women demonstrated improved CA than men. The authors measured MCAv and anterior cerebral artery blood velocity, which both branch off the ICA. Changes seen, or lack of changes seen in CA could be due to the fact that the vessels of interest both stem from the same artery. The study did not look at the posterior cerebral artery blood velocity (PCAv), which stems from the VA. The VA is smaller than the ICA, therefore any changes in CBF as a result in changing ABP could be more evident due to the smaller vessels. Moreover, Favre and Serrador (2019) only challenged dCA using the sit-to-stand and repeated squat-to-stand manoeuvre. Favre and Serrador (2019) were more specific with the menstrual cycle, investigating the early follicular, late follicular and the mid-luteal phase, which permitted a more in-depth examination of both oestrogen and progesterone. However, the authors reported that the salivary estradiol concentrations for 39% of the participants were lower than what was expected in late follicular, suggesting that oestrogen levels were low when it was expected to be highest. Furthermore, individual cycles vary, and direct plasma hormone measurement is the most robust method to adequately confirm a participant's position with the menstrual cycle. Furthermore, there are some factors that can contaminate salivary hormone levels. Drinks such as coffee can interfere with the test and cause a transient shift in hormone levels (Lipson & Ellison, 1989). Chewing gum can increase salivary production, however, it could cause bleeding gum, which would contaminate the sample with blood and blood can cause significant changes in hormone levels (Dabbs Jr, 1991). Over the years salivary samples have become more prominent in identifying hormone levels, however, there are many factors that are overlooked which can produce unreliable results. The unreliable results of salivary sampling suggest that direct measurement of plasma hormone concentrations is a more suitable method to measure oestrogen concentrations.

2.7 Summary

This literature review discussed the cerebral circulation and the abundant factors that contribute to its regulation to ensure optimal brain function. There are many factors that contribute to the regulation of CBF, however, the interactions between these factors and how

much they contribute to the overall CBF regulation is less understood. Furthermore, the role of sex steroid hormones on cerebral circulation has not been examined extensively. It is known that oestrogen can alter reactivity of the cerebral vasculature via vasodilation and that progesterone initiates a vasoconstrictive response, and that these different cerebral reactivity responses can translate to CBF. Moreover, in women, hormone concentrations change throughout their life, such as during the menstrual cycle, pregnancy, and menopause. Changes in hormone concentrations during the menstrual cycle can have adverse effects, especially when women are more prone to orthostatic hypotension than men. Furthermore, women's hormones can be manipulated by oral contraceptives, therefore the full effect of the hormones especially oestrogen, has not been fully studied and understood. Therefore, the main objective of this Masters thesis was to examine effect of oestrogen on cerebrovascular regulation in eumenorrheic women.

Chapter Three: Materials and Methodology

3.1 Ethics and Informed Consent

Participation in this experiment was entirely voluntary. The participants were informed about the experimental procedures and were aware of the purpose of this study, as well as all potential risks associated with participating. All participants provided written informed consent prior to partaking in the research. The study was approved by the Massey University Human Ethics Committee (SOA 18/77) and conformed to the Declaration of Helsinki.

3.2 Participants

Based on results from Abidi et al. (2017) of low and high sex hormone effect on middle cerebral artery blood flow the effect sizes were calculated, with significance (alpha) set to p=0.05 (5%) and ß set at 90%. Using the tables from Thomas, Nelson, and Silverman (2001, pp 118), the sample size estimated to 12. Allowing for up to 20% drop out the total number of participants to be recruited is 14. A total of 10 healthy female individuals (mean ± SD: age, 28 ± 7 years; height 168 ± 6 cm, weight 75 ± 19 kg, BMI 26 ± 6 kg/m^2) were recruited for the study. However, data was reported for seven participants as one withdrew, one did not ovulate (see Section 3.7 for ovulatory criteria), and one participant is still currently active in the research. All participants were healthy and free of any medical conditions, were not taking any form of medication (including any form of oral contraceptives and intrauterine devices), were non-smokers, and had no history or symptoms of cardiovascular, pulmonary, metabolic, or neurological disease. A general health questionnaire was completed by the participants for screening purposes.

3.3 Experimental Design

All participants visited the temperature-controlled laboratory four times, once to receive a full familiarisation session of the experimental protocol and three times to complete the experimental trial at the different stages of the menstrual cycle. Demonstrations, explanations, and potential risks were clarified to the participants during the familiarisation session and experimental sessions to ensure a full understanding of the trial. During the familiarisation, the participant became familiar with the equipment used for data collection

in the trial and the sequence of procedures for the experimental trial. In addition, the participant practiced generating a mouth pressure of 40 mm Hg for the VM arm of the study. The MCA and posterior cerebral artery (PCA) were insonated using techniques described in section 3.4.2. The participants were also given an ovulation kit to track their high oestrogen phase (see Section 3.7). The participants could volunteer for the study at any day of the menstrual cycle then begin tracking from the last known day 1 (onset of menses). If the participant does not remember their last menses, then it was encouraged to start tracking from the next menses to avoid any confusion. The completion of the study was in random order, as the first trial for each participant was not always the early follicular phase.

The familiarisation occurred > 1 week before the tracking period of the experiment. Participants arrived at the laboratory having refrained from caffeinated beverages for 12 hours, and vigorous exercise and alcohol consumption for ≥ 24 hours prior to testing. Once consent was gained and the familiarisation session was completed, participants were instructed to track their menses for two cycles before their first experimental trial. The purpose of tracking menses before the first experimental trial was to determine the length of the menstrual cycle and menses as well as the approximate number of day/s high oestrogen concentrations occurred. This was done to ensure that when the participants came in for the experimental trials, they were in the phase that was intended, and that oestrogen and progesterone concentrations were reflective of those phases. The sequence of trials is outlined in Figure 7 below.

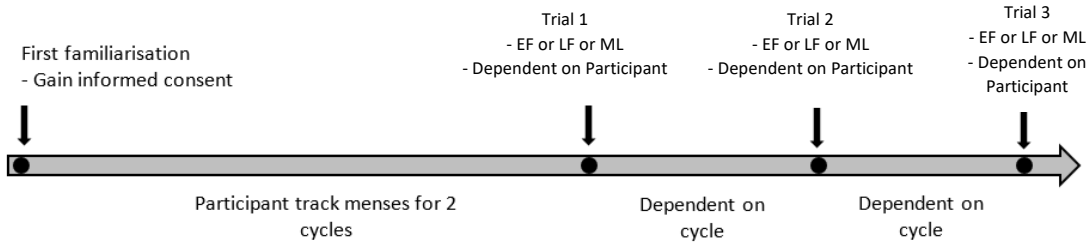

Figure 7 Experimental trial sequence. The participants first came to the lab for the familiarisation session. The trials began after tracking menses. The trials were completed in a random order, which is dependent on when during their cycle they entered the study and when they began tracking. Early Follicular; EF, Late Follicular; LF, Mid-Luteal; ML.

3.4 Measurements of Cerebral Blood Flow velocity, Arterial Blood Pressure, and Heart Rate

3.4.1 Systemic Haemodynamics

Heart rate (HR) was measured using a three-lead electrocardiogram (ECG; ADInstrument, Australia). Non-invasive beat-to-beat ABP was measured by finger photoplethysmography (Finapres Medical Systems, The Netherlands). The cuff was placed on the right hand on either the middle finger or the index finger of the participant and referenced to the level of the heart using the height correction unit of the machine and was checked against a manual sphygmomanometer. Cerebrovascular conductance index (CVCi) was then calculated using the equation:

$$MCAv_{mean} / MAP$$

3.4.2 Middle Cerebral Artery Blood Velocity

Blood flow velocity in the MCA (MCAv) was measured using transcranial Doppler ultrasonography (DWL, Compumedics, Germany). Blood velocity in the M1 segment of the MCA was measured using a 2 MHz probe, fixed in position via an adjustable headband. The probe was fixed over the temporal window, above the zygomatic arch, known as the trans-temporal approach using search techniques described elsewhere (Aaslid, Markwalder, & Nornes, 1982; Willie et al., 2011). Bathala, Mehndiratta, & Sharma (2013) found that the M1 MCA is found approximately at the depth of 45 – 60mm. The average depth where MCA was found in the current study was 59mm, with a range of 55 – 66mm. Ultrasound gel (Tensive, Parker Laboratory, Fairfeild, NY, USA) was placed between the transducer probe and the skin to ascertain the highest quality image.

3.5 Partial Pressure of End-tidal Carbon Dioxide

The partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$) was measured using a breath-by-breath online gas analyser (ADInstruments, Australia) and was collected throughout the experimental trial using a nasal cannula. The gas analyser was calibrated to known gas concentrations before each experimental trial. $P_{ET}CO_2$ was not measured during the VM, as the participants were instructed to blow into a tube with a mouth pressure of 40 mm Hg to induce the VM.

3.6 Data Acquisition

All data were collected continuously using an analogue to digital converter (PowerLab, ADInstruments, Australia) interfaced with a computer and then analysed using the LabChart software (v8.1.12 ADInstruments, Australia).

3.7 Measurement of Oestrogen and Progesterone

Participants were instructed to monitor and record key events of their menstrual cycle for two cycles prior to the experiment. As seen in Figure 5, to identify if the participant was in early follicular (EF), EF was defined as the duration of menses, which included the onset of menses to the end (full cessation of menses). Mid-luteal phase (ML) of the menstrual cycle was defined as the midpoint between ovulation and the start of menses. In a 28-day cycle, ML would be on day 21, however, due to the length of menstruation being different for all individuals, this was calculated using the information gathered from two previous cycles as described below. A urinary-based digital ovulation kit that detects both luteinizing hormone (LH) and oestrogen concentrations (Clearblue Fertility Monitor, SPD Swiss Precision Diagnostics) was given to the participants following menstruation. Participants were instructed to use it daily in the morning of the day following the cessation of menstruation (see paragraph below for more information). The participant used the ovulation kit until high fertility (rise in oestrogen concentration) is indicated on the ovulation kit. Once high fertility is identified, participants would record this in reference to the first day of their menstrual cycle (onset of menstruation = day 1). The participant continued to use the ovulation kit until peak fertility (LH surge) was identified and use of the ovulation kit would cease for that cycle. The recorded timings would serve as a guideline for use of the ovulation kit and in preparation for the experimental trial. In the next menstrual cycle, two days prior to the estimated onset of high oestrogen participants would use the ovulation kit. Once high fertility was confirmed participants were instructed to schedule an appointment that would coincide with their late follicular phase. The participant would use the ovulation kit in the morning of the experimental trial to confirm high fertility. If the high fertility symbol did not appear on the day of the scheduled trial, the trial was postponed until the ovulation kit showed high fertility.

The Clearblue Advanced Digital Ovulation Test detects both LH and oestrogen, thus providing a larger window for the late follicular phase (high oestrogen) phase. Su, Yi, Wei,

Chang, and Cheng (2017) found that both LH urinary ovulation kits and digital monitors kits for LH and oestrogen had high accuracy for ovulation, with accuracies of 97% and 95.8 – 97% respectively. Furthermore, a study conducted by Tanabe et al. (2001) found that the peak in urinary LH detected by the fertility monitor strongly correlated to LH peak detected by laboratory assay. Behre et al. (2000) study showed that ovulation was correctly predicted within two days of urinary LH peak day in 123 of 135 ovulatory cycles. Urinary-based ovulation kits to detect a surge in LH and therefore detect ovulation has been used in many studies (Berglund, Hirschberg, & Scherwitzl, 2015; Ceric, Silva, & Vigil, 2005; Fehring, 2002) because of the accuracy, cost effectiveness, and non-invasiveness of the test, when compared to ultrasonography.

Blood samples were also taken from the participants on the day of each experimental trial to determine oestrogen and progesterone concentrations and confirm which phase of the menstrual cycle that they were in. The defined range for the trials were: EF, oestrogen <200 pmol/L; progesterone 0 – 1 nmol/L, LF, oestrogen >201 pmol/L; progesterone 0 – 2 nmol/L, and ML, oestrogen <120 pmol/L; progesterone >9.5 nmol/L. Hatcher, Breedlove, Judy, and Martin (1988) stated that progesterone concentrations over 9.5 nmol/L indicate that ovulation has occurred. Therefore, data collected from participants with progesterone concentrations below 9.5 nmol/L were excluded, and the participant was required to complete a re-trial. The participants were instructed to complete blood sampling = within 1 hour of the experimental trial. All trials were completed at the same time of day.

3.7.1 Blood Sample Extraction Method

The blood extraction was done by a trained phlebotomist at a Southern Community Laboratory (SCL) location in Wellington. The phlebotomist verbally confirmed the participants details and the reason for the blood samples. Then the participant was asked to be seated as the phlebotomist inspected the participant's arm for a suitable vein, this is often the medial cubital vein located in the antecubital fossa. When the vein had been located, the surface of the skin was cleaned with an alcohol wipe. A tourniquet was placed 5 – 10 cm above the site of venous puncture to engorge the vein with blood. The blood sample was collected using a closed vacutainer system. The needle was inserted into the vein and a yellow serum separator tube (SST) was inserted into the vacutainer that allowed self-filling of the blood. Two yellow SSTs were collected. Once the blood was collected, the tourniquet was undone with the

needle still in place. A cotton swab was placed over the venepuncture site and the needle was gently removed. Pressure was applied at the site of venepuncture to stem any bleeding.

3.7.2 Blood Sample Analysis

The blood analysis for oestrogen and progesterone concentrations was completed on-site using electrochemiluminescence immunoassay analysis, using the Roche Cobas e602 machine. The results were then emailed to the lead researcher.

3.8 Experimental Procedures

Assessment of dCA was done throughout the VM as it is a manoeuvre that induces both hypotensive and hypertensive changes to blood pressure. The intention of using forced oscillation in MAP was to increase the power (i.e. MAP) input which would allow for more significant analysis of the relationship between BP and MCAv (Smirl, Hoffman, Tzeng, Hansen, & Ainslie, 2015). The experimental design is outlined in Figure 8 below.

3.8.1 Valsalva Manoeuvre

During the VM, the participant was seated and remained seated for baseline measures. Once baseline measures were taken, the participant performed the VM by blowing in a tube with a mouth pressure of 40 ± 5 mm Hg for 15 s, as described previously by Perry, Cotter, Mejuto, Mundel, and Lucas (2014) and Junqueira (2008). The participants had an opportunity to practice the VM during the familiarisation and before the VM was performed the participants were reminded of what pressure and duration were needed. The pressure measure was on the screen in front of the participants so that they were to achieve a mouth pressure of 40 mm Hg with real time visual feedback. The gas analyser was turned off during this manoeuvre, and the participant was provided with a nose clip to ensure that they were performing the VM correctly. HR, MAP and MCA_v were measured continuously throughout this manoeuvre. Once the VM was performed the gas analyser was turned back on and the nose peg was removed, the participant was instructed to breath normally through their nose. The participant remained seated until systemic haemodynamic measures returned to baseline, then the VM was performed again.

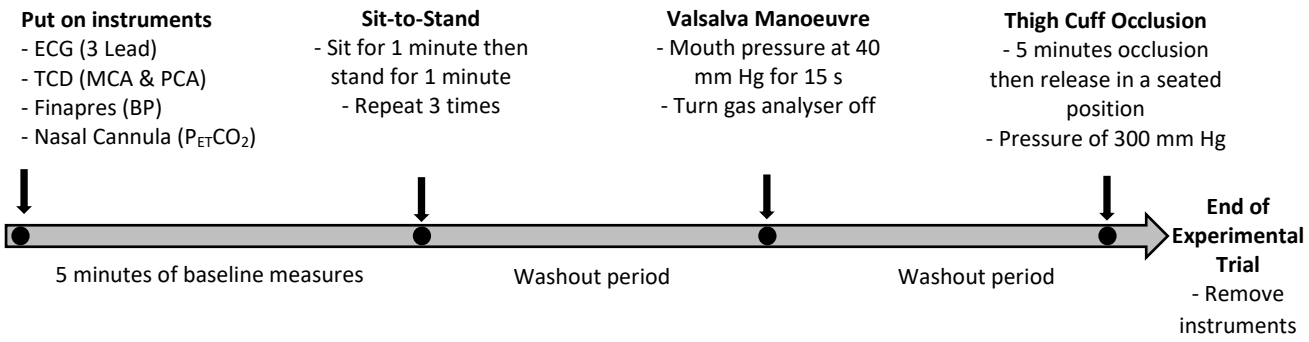

Figure 8 Experimental Protocol. Each participant completed the sit-to-stand manoeuvre, Valsalva manoeuvre, and the thigh cuff occlusion for each trial. Circulatory (MAP and HR) and respiratory ($P_{ET}CO_2$) variables returned to baseline levels prior to the next intervention. **Please note** for the purposes of this thesis only the Valsalva manoeuvre data was presented.

3.9 Data Analysis

3.9.1 Assessment of the dynamic relationship between MAP and MCAv

Tieck's AI method was used to assess the dCA in the thesis (also see section 2.2.6.1). Two different equations were used to calculate the AI for the VM. The first equation was used to calculate dCA during phase II of the VM is as follows:

$$AI - II = \frac{\text{CBFV (phase IIb} - \text{phase II a) / CBFV (phase IIb)}}{\text{ABP (phase IIb} - \text{phase II a) / ABP (phase IIb)}}$$

The equation used to calculate dCA during phase IV of the VM is as follows:

$$AI - IV = \frac{\text{CBFV (phase IV) / CBFV (phase III)}}{\text{ABP (phase IV) / ABP (phase III)}}$$

The equation above has been modified from the original calculation from Tiecks et al. (1995), as phase I has been replaced with phase III to reflect post strain regulation. Furthermore, the magnitude of the response to the strain is being assessed, therefore standardising the response using the phase immediately before presents a more valid result.

Values that are greater than 1.00 indicate that autoregulation is present, while values less than 1.00 indicate that autoregulation is absent.

3.9.2 Dependent Measures

Mean MCAv ($MCAv_{mean}$) and MAP were calculated by the cardiac cycle divided by the pulse interval. The cerebrovascular conductance index (CVCi) for the MCA was calculated by using the equation MCAv/MAP. The Gosling pulsatility index (PI) for the MCA was calculated via $\frac{\text{systolic MCAv (SMCAv)} - \text{diastolic MCAv (DMCAv)}}{\text{MCAvmean}}$ (Gosling & King, 1974).

3.10 Statistical Analysis

All data were analysed using SPSS statistical software version 26 (IBM Corp., Armonk, NY, USA). Statistical significance was set at $P \leq 0.05$. A one-way analysis of variance (ANOVA) was performed to compare baseline measures of all three trials. A repeated measures two-way ANOVA was used to analyse dependent variables of interest for all conditions (phases x trials, 5 x 3). Additionally, the relative changes in MCAv, MAP, and CVCi for all conditions were analysed using a repeated measures two-way ANOVA (phases x trials, 5 x 3). The *post-hoc* pairwise comparisons were used to isolate main effects in the data. The Bonferroni correction factor was used when necessary. Partial eta square (partial η2) is reported for the interaction effect only, with large effect sizes identified as > 0.1379, medium 0.0588 – 0.1379, and small < 0.0099 (Cohen, 2013). All data are displayed as the mean ± SD.

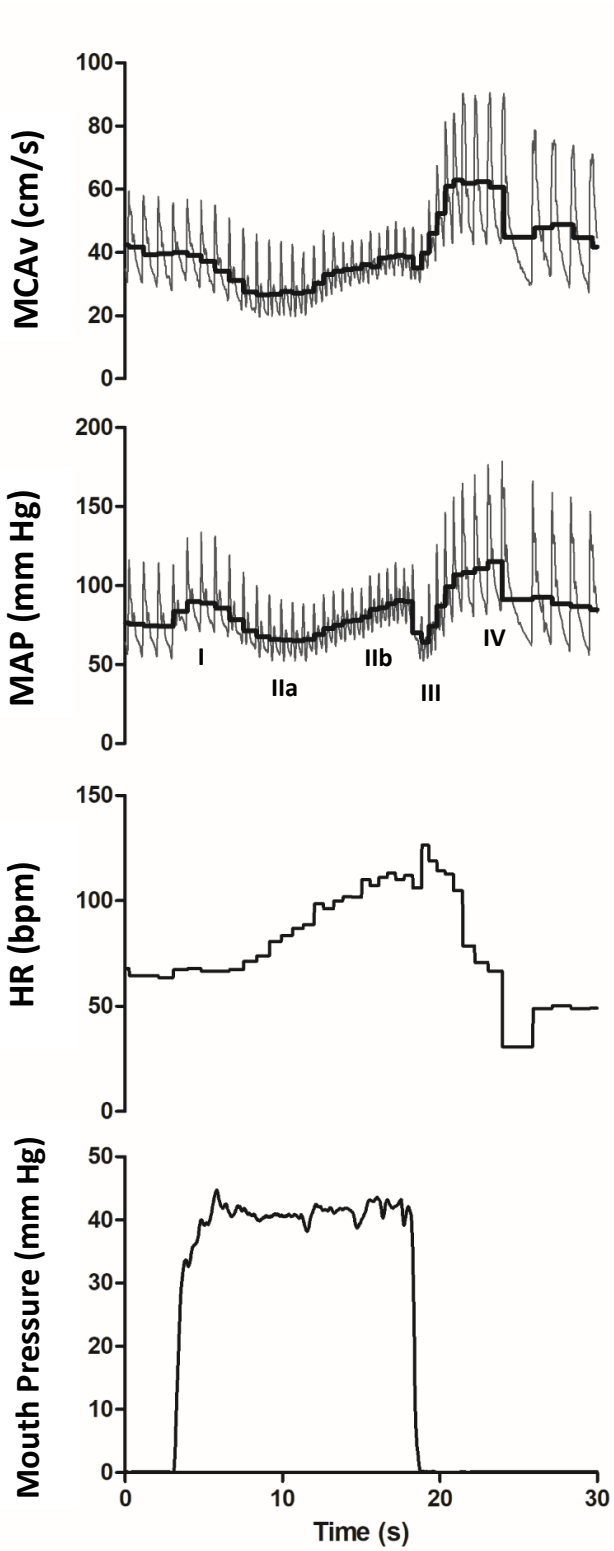

Figure 9 Haemodynamic variables for one participant's 40 mm Hg Valsalva manoeuvre, MCAv; middle cerebral artery velocity, MAP; mean arterial pressure, HR; heart rate, and Mouth pressure as a surrogate for intrathoracic pressure. Thick black line represents the mean value for each cardiac cycle within the MAP and MCAv trace.

Chapter Four: Results

4.1 Baseline Values

Baseline cardiovascular and cardiorespiratory measures are shown in Table 1 below. All resting values were not different between EF, LF, and ML and yielded no significant statistical differences between the trials.

Table 1 Participant baseline cerebral blood flow and cardiovascular measurements

Variables	Early Follicular	Late Follicular	Mid Luteal	*P*-value
$MCAv_{mean}$ (cm/s)	63 ± 13	56 ± 13	62 ± 12	0.635
MAP (mm Hg)	73 ± 8	66 ± 9	67 ± 14	0.409
HR mean (bpm)	69 ± 16	73 ± 12	73 ± 13	0.876
CVCi (cm/s/mm Hg)	0.87 ± 0.18	0.89 ± 0.26	0.89 ± 0.26	0.984
PI	0.76 ± 0.18	0.77 ± 0.14	0.77 ± 0.14	0.912
PP (mm Hg)	64 ± 4	65 ± 15	66 ± 7	0.943
$PETCO_2$ (mm Hg)	42 ± 4	42 ± 3	40 ± 3	0.525

Data are means ± SD. $MCAv_{mean}$, mean middle cerebral artery blood flow velocity; MAP, mean arterial pressure; HR, heart rate; CVCi, cerebrovascular conductance index; PI, pulsatility index; PP, pulse pressure.

4.2 Oestrogen and Progesterone Concentrations

Ovarian hormone concentrations are shown in Table 2.

Table 2 Participant sex hormone concentrations and ratio

Variables	Early Follicular	Late Follicular	Mid Luteal	*P*-value
Oestrogen (pmol/L)	129 ± 31	485 ± 208 *	425 ± 230 *	**0.003**
Progesterone (nmol/L)	1 ± 0	2.2 ± 2	28 ± 13*‡	**<0.001**
Progesterone/Oestrogen Ratio	8 ± 2	2 ± 1*	80 ± 47*‡	**<0.005**

Data are means ± SD. **, significantly different from early follicular (*P < 0.025*). ‡, significantly different from late follicular (*P ≤ 0.001*).*

4.3 Partial Pressure of End-Tidal CO_2

There were no statistically significant differences in $PETCO_2$ between the first full breath taken post VM between EF (40 ± 4 mm Hg), LF (40 ± 4 mm Hg), and ML (40 ± 3 mm Hg, *P = 0.907)*.

4.4 Cerebrovascular and cardiovascular response to the Valsalva manoeuvre

Dynamic cerebrovascular and cardiovascular responses to the VM are shown in Table 3. Additionally, the percentage change from the preceding baseline is shown in Figure 10, and autoregulatory indices for phase II and IV are shown in Table 4. Briefly, a significant interaction effect for MCAv was identified (*P = 0.039)*. MCAv in phase IIb of the VM was greater for ML (58 ± 15 cm/s) compared to EF (51 ± 14 cm/s, *P* = 0.013) and LF (49 ± 15 cm/s, *P* = 0.024). No other significant interaction effects were found for absolute or relative data (Table 3 and Figure 10, respectively).

Table 3 Cerebrovascular and cardiovascular responses to the Valsalva manoeuvre (VM)

Variables	Trial	Valsalva Manoeuvre Phase					*P*-Values			Partial η^2
		I	IIa	IIb	III	IV	Trial	VM Phase	Trial x Phase	
$MCAv_{mean}$ (cm/s)	EF	64 ± 12	43 ± 13	51 ± 14‡	63 ± 15	89 ± 13	*0.058*	**<0.0001**	**0.039**	0.274
	LF	65 ± 16	39 ± 11	49 ± 15‡	52 ± 9	74 ± 17				
	ML	66 ± 10	44 ± 11	58 ± 15	58 ± 14	82 ± 13				
MAP (mm Hg)	EF	100 ± 22	70 ± 18	82 ± 23	67 ± 12	106 ± 11	0.206	**<0.0001**	0.097	0.233
	LF	101 ± 11	67 ± 12	79 ± 15	57 ± 11	100 ± 10				
	ML	102 ± 17	76 ± 16	94 ± 12	68 ± 8	101 ± 11				
CVCi (cm/s/mm Hg)	EF	0.67 ± 0.21	0.67 ± 0.32	0.68 ± 0.34	0.95 ± 0.21	0.85 ± 0.14	0.565	**<0.0001**	0.808	0.085
	LF	0.65 ± 0.19	0.58 ± 0.20	0.63 ± 0.19	0.94 ± 0.27	0.74 ± 0.18				
	ML	0.66 ± 0.14	0.61 ± 0.20	0.63 ± 0.19	0.92 ± 0.32	0.83 ± 0.19				
HR (bpm)	EF	82 ± 16	96 ± 11	108 ± 12	116 ± 15	94 ± 23	0.245	**<0.0001**	0.360	0.159
	LF	81 ± 11	93 ± 11	104 ± 9	117 ± 13	81 ± 15				
	ML	83 ± 16	92 ± 19	105 ± 16	111 ± 18	79 ± 26				
PI	EF	0.71 ± 0.20	1.04 ± 0.38	0.54 ± 0.11	0.92 ± 0.53	0.63 ± 0.08	0.412	**0.003**	0.354	0.160
	LF	0.67 ± 0.14	0.86 ± 0.21	0.58 ± 0.08	0.79 ± 0.23	0.74 ± 0.14				
	ML	0.69 ± 0.15	0.94 ± 0.32	0.54 ± 0.10	1.16 ± 0.72	0.73 ± 0.18				
PP (mm Hg)	EF	69 ± 10	47 ± 7	45 ± 7	54 ± 19	84 ± 14	**0.040**	**<0.0001**	0.942	0.055
	LF	68 ± 5	45 ± 10	42 ± 10	53 ± 14	88 ± 17				
	ML	72 ± 8	57 ± 14	51 ± 8	59 ± 17	92 ± 13				

Data are means ± SD. EF; early follicular, LF; late follicular, ML; mid luteal, $MCAv_{mean}$, mean middle cerebral artery blood flow velocity; MAP, mean arterial pressure; HR mean, heart rate mean; CVCi, cerebrovascular conductance index**.,** PI, pulsatility index; PP, pulse pressure. ‡ Statistically significant difference in this VM phase from ML (P <0.025).

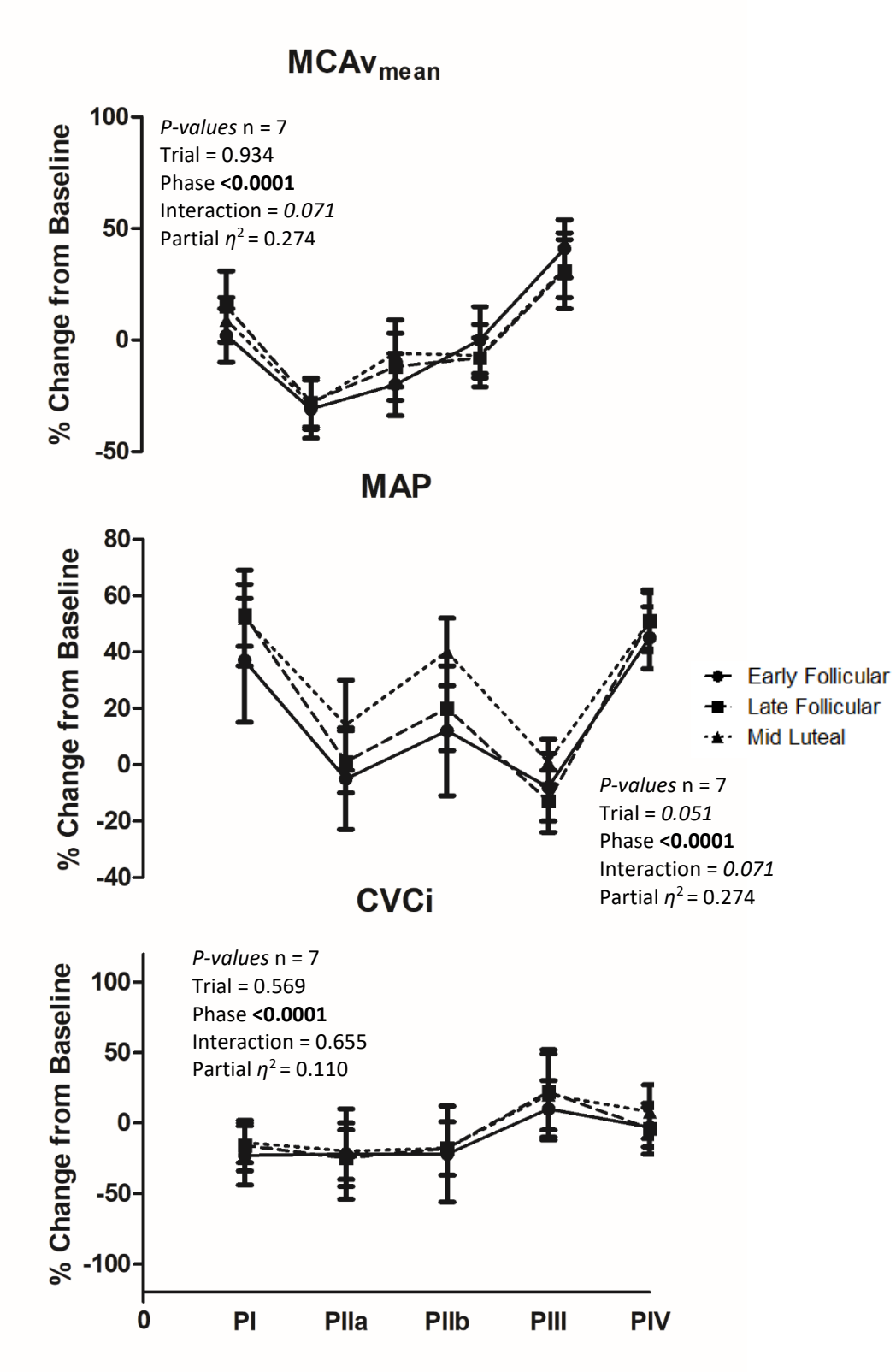

Figure 10 Mean middle cerebral artery blood velocity ($MCAv_{mean}$), mean arterial pressure (MAP), and cerebrovascular conductance index (CVCi) relative changes from baseline (%). Data are means ± SD.

Table 4 Autoregulatory index

Variable	Trial	Mean ± SD	*P*-Values
AI-II	EF	2.14 ± 2.16	0.354
	LF	1.78 ± 0.85	
	ML	1.07 ± 0.55	
AI-IV	EF	0.90 ± 0.12	0.086
	LF	0.81 ± 0.11	
	ML	0.96 ± 0.11	

Data are means ± SD. AI; Autoregulatory Index. The AI scale range is 0 – 9, the greater the value is over 1, the greater CA. AI – II; autoregulatory index for phase II (initial strain regulation) , AI – IV; autoregulatory index for phase IV (post strain regulation); EF, early follicular; LF, late follicular; ML, mid luteal.

Chapter Five: Discussion

The aim of this study was to investigate the effects of oestrogen on cerebrovascular regulation in eumenorrheic women. The main findings from the present study are that the MCAv response to the VM varied throughout the menstrual cycle. A greater phase IIb MCAv was observed in ML compared to EF and LF. There were no statistically significant differences in resting cerebrovascular haemodynamics measures during rest across the menstrual cycle and are in agreement with Favre and Serrador (2019). Indices of cerebrovascular function (e.g. AI, Table 4) and blood pressure were also trending towards significance. Importantly, plasma hormone concentrations corroborated the expected position of the participants within the menstrual cycle. These data indicate that it is probable that circulating ovarian hormones modulate the cerebrovascular response to dynamic blood pressure challenges.

5.1 Plasma Ovarian Hormone Measures

As seen in Table 2, there were significant differences in ovarian hormone concentrations and progesterone to oestrogen ratio across the menstrual cycle, confirming the position of the participants within their cycle(Figure 5). Others investigating dCA across the menstrual cycle did not include the LF phase (Abidi et al., 2017) or measured salivary ovarian hormone concentrations (Favre & Serrador, 2019). Favre and Serrador (2019) reported no significant change in salivary oestrogen despite targeting similar timing across the menstrual to this study (EF, LF and ML). Despite progesterone having a larger influence on the vascular tree due to its ability to offset the vasodilatory effects of oestrogen (see

Section 2.14) (Miller & Vanhoutte, 1991; Willekes, Hoogland, Keizer, Hoeks, & Reneman, 1999)there were no significant differences in resting arterial blood pressures or MCAv across the menstrual cycle. Furthermore, as seen in the current study, participants ovarian hormone concentrations can vary, and they may not ovulate during their cycle. Indeed, two participants had to repeat the ML trial as progesterone concentrations did not meet the ≥9.5 nmol/L criteria for ovulation (Hatcher et al., 1988). Given the variability demonstrated in the current study, and lack of measurement sensitivity in previous reports, it is paramount plasma oestrogen and progesterone are measured to confirm the menstrual cycle phase and also if ovulation has occurred. The present study was able to determine the correct phase of the menstrual cycle that the participant was in during the time of the experimental trial, thus enabling a 'true' response to the cerebrovascular challenges.

5.2 Cerebrovascular Regulation during the Valsalva manoeuvre

The results of the present study found that MCAv was impacted by the different concentrations of sex hormone levels, as indicated during phase IIb of the VM. The results did in part agree with the hypothesis of the study, which stated that cerebrovascular regulation would have a diminishing response. There were no significant differences in autoregulatory index values, however, the results for phase IV was trending towards significance (*P = 0.084)*. Similarly, there was a trend for differences in MAP in our data. Given the large interaction effect size of MAP (Partial η^2 =0.233) it is likely that the study is underpowered (see limitations). Albeit non-significant, the MAP during ML phase IIb is likely to contribute to the elevated MCAv, due to the relationship between the two variables (see Section 3.9.2). As VM mouth pressures were standardised, and completed in the seated position, the increase in intracranial pressure (ICP) would be expected to be similar. Thus, an increase in MAP during the VM would raise cerebral perfusion pressure (CPP, see Section 2.1). AI is seen to counteract changes in phase II better than phase IV, due to the slower and lower magnitude change in MAP. CA does not work instantaneously (Aaslid et al., 1989; Tiecks et al., 1995) and that there is a ~5 s lag before a vascular response (Zhang et al., 1998). CA is a mechanism that interplays with a multitude of factors; therefore, it is difficult to isolate the exact factor that is altering CA response. Furthermore, Labrecque et al. (2017) stated that a multiple indices approach of assessing dCA is required to improve physiological understanding of dCA and to indicate modified function.

Our study showed a differential MCAv response during the VM, which indicates that cerebrovascular regulation may be altered by ovarian hormones concentrations. Few studies have investigated the menstrual cycle and cerebrovascular regulation. Favre and Serrador (2019) and Abidi et al. (2017), concluded that the menstrual cycle does not alter cerebrovascular regulation, which is a contrast to the results we obtained, which shows that the menstrual cycle does modify the response to BP challenges. Our study showed that during ML, when both oestrogen and progesterone was high, there was an increase in phase IIb MCAv. This finding is difficult to interpret given it is the only phase where post hoc test revealed significance and AI was unchanged. Furthermore, our results may differ from Favre and Serrador (2017) and Abidi et al. (2017) due to our smaller sample size. Analysis of other VM phases, whilst only trending toward significance, LF phase III and IV $MCAv_{mean}$ remain lower than both EF and ML. Furthermore, MAP was ~10 mm Hg lower during phase III in LF and aligns with lower $MCAv_{mean}$ values at this time. As similar post strain $P_{ET}CO_2$ values were observed it is possible that a larger sample size may reveal a significant difference in the MAP response across a number of phases in the VM during LF. Additionally, ovarian hormones may also vasodilate the basal arteries of the brain (Krejza et al., 2001; Krejza et al., 2004; Krejza et al., 2003) and artificially lower cerebral flow velocities (see limitations Section).

5.3 Cerebrovascular Regulation: Comparisons with existing data

The present study, however, challenges previous notions, as the study has found that fluctuating hormone concentrations may affect cerebrovascular response to known challenges. Favre and Serrador (2019) investigated the effects of blood pressure changes using the sit-to-stand manoeuvre and the repeated squat-to-stand manoeuvres across the menstrual cycle. The results from Favre and Serrador (2019) showed that there were no significant differences in MCAv between EF, LF, and ML (P = 0.116). Furthermore, as mentioned in Section 5.1, Favre and Serrador (2019) did not identify any changes in sex hormone concentrations in saliva, while the current study plasma oestrogen and progesterone concentrations corroborate the tracking data and confirmed the position of the participants within their cycle (Table 2).

Abidi et al. (2017) reported that the menstrual cycle did not have a significant effect on cerebral autoregulation during the VM and sit-to-stand. However, the authors only

investigated the low hormone (EF) and high hormone (ML) phases of the menstrual cycle. Similar to our study, Abidi et al. (2017) used the VM to induce blood pressure changes in their participants, however, only phase II was investigated. Investigating all phases would have allowed for the comparison of the different stages as well as examination of the decreases in blood pressure. Moreover, Brassard, Ferland-Dutil, et al. (2017) identified that the brain is superior at counteracting hypertensive challenges to CA. Therefore, a more pronounced effect of female sex hormone on CA could have been identified in phase IIa and phase III of the VM. However, this opportunity was missed as Abidi et al. (2017) decided to exclude analysis of the other phases of the VM.

5.4 Future Considerations and Limitations

Future studies investigating the effects of female ovarian hormones on cerebral regulation should consider examining both the MCA and PCA to identify if regional variation in regulation exist in the brain, as indicated for CO_2 (Sato et al., 2012). Furthermore, understanding how CA reacts to spontaneous fluctuations and forced oscillations in ABP. It is possible that "stressed function" may be altered whilst resting absolute values remain unchanged. Moreover, as mentioned by Labrecque et al. (2017) there is a need for multiple assessments in order to identify any functional changes that occur to dCA. As mentioned in Section 2.7, there is no gold standard when it comes to measuring cerebral autoregulation. Some argue that forced oscillations allows for more robust results to be analysed and it reflects the strains of everyday activities (Claassen et al., 2009; Simpson & Claassen, 2018; Tan, 2012). However, others say spontaneous fluctuations should be the main method as CA would be most effective in this range and forced oscillations would induce an autonomic response (Tzeng & Panerai, 2018; Zhang et al., 1998). Therefore, the incorporation of both forced oscillations and spontaneous fluctuations in research is needed. Multiple methods of perturbing MAP (sit-to-stand and thigh cuff occlusion) were employed in this study as part of a broader research question, however, only the forced oscillation induced by the VM are reported in this thesis. The significant effects of VM phase reported in the two-way ANOVA, indicate that the intervention was successful in producing forced oscillations in MAP. Moreover, this study showed the importance of obtaining sex hormone concentrations through blood sampling. The use of this technique will ensure more reliable results which is pivotal for timing in research looking at the effects of female ovarian hormones.

We acknowledge that there are some limitations in the study that should be addressed. We used transcranial Doppler ultrasound to measure cerebral blood velocity in our participants. The assumption with using the TCD is that the diameter of the vessel of interest remains unchanged throughout. However, it has been reported that there is a <4% change in vessel diameter during little changes in MAP (30 ± 16 mm Hg) (Giller, Bowman, Dyer, Mootz, & Krippner, 1993). Furthermore, using high resolution magnetic resonance imaging (MRI), Verbree et al. (2014) found that MCA diameter increases in response to hypercapnia. Additionally, it has been suggested that sympathetic activation during isometric hand grip exercise is responsible for a reduction in MCA diameter (Verbree et al., 2017). Therefore, the results from this study will need to be validated through other cerebral blood flow techniques. Moreover, as previously mentioned in Section 5.2, changing ovarian sex hormone concentrations cause dilation of basal arteries, further emphasising the need for other methods of measuring CBF. Furthermore, a limitation of this study includes the difficulties of accurately measuring arterial PCO_2 levels as the VM consists of performing a breath hold. $P_{ET}CO_2$ cannot be used as an accurate measure of arterial CO_2 as a VM correlates to a significant reduction in cardiac output and therefore, a decreased washout of CO_2 in the tissue (Meyer, Gotoh, Takagi, & Kakimi, 1966). Moreover, there is a delay of ~6 s for a response in $MCAv_{mean}$ to be observed (Pott et al., 2000). However, as mentioned above in Section 2.14, a decrease in $PaCO_2$ contributes to the 10 – 15% decrease in MCAv during a 15 s VM (Pott et al., 2000), therefore, accurate measures of $PaCO_2$ is of great importance in identifying any significant changes in MCAv seen.

As alluded to in the preface, conducting a study looking at the effects of ovarian sex hormones on cerebrovascular regulation posed a significant challenge. Recruitment of participants was difficult as most female university students were using oral contraception or an intra-uterine device. The study initially had 10 participants. However, due to participant withdrawal, equipment availability and the COVID-19 pandemic, participant numbers were reduced to seven for the analysis. The reduction in numbers for analysis underpowered some aspects of the study, as the interaction effect of VM phase and trial for MAP showed a trend. Individual variability in cycle length, and frequency of ovulation, meant on several occasions trials had to be repeated on the next cycle, despite a rigorous tracking schedule. Furthermore, hormone concentrations were not available to the researchers until following the

experimental trial. Phlebotomy and blood analysis were outsourced to SCL Wellington. The laboratory has limited opening hours which hampered data collection outside the working week.

Chapter Six: Conclusion

6.1 Conclusion

The results from this study show that there are small differences in the cerebrovascular response to the VM across the menstrual cycle. The method of challenging cerebrovascular regulation through the VM produced both hypertensive and hypotensive changes to blood pressure. We suggest that future studies investigate additional methods of challenging CA as the VM does not allow for the measurement of CO_2 which is a known potent regulator of CA. Furthermore, including data from PCA to identify if the response is heterogeneous throughout the brain is warranted. Moreover, this study only looked at forced oscillations in ABP when spontaneous fluctuations in ABP also occurs. Future studies should include both forced and spontaneous oscillations when investigating CA, to further understand the mechanisms that contribute to CA and the responses to different types of blood pressure challenges.

References

Aaslid, R., Lindegaard, K. F., Sorteberg, W., & Nornes, H. (1989). Cerebral autoregulation dynamics in humans. *Stroke, 20*(1), 45-52. 10.1161/01.str.20.1.45

Aaslid, R., Markwalder, T. M., & Nornes, H. (1982). Noninvasive transcranial Doppler ultrasound recording of flow velocity in basal cerebral arteries. *J Neurosurg, 57*(6), 769-774. 10.3171/jns.1982.57.6.0769

Abidi, S., Nili, M., Serna, S., Kim, S., Hazlett, C., & Edgell, H. (2017). Influence of sex, menstrual cycle, and oral contraceptives on cerebrovascular resistance and cardiorespiratory function during Valsalva or standing. *J Appl Physiol (1985), 123*(2), 375-386. 10.1152/japplphysiol.00035.2017

Ainslie, P. N., Ashmead, J. C., Ide, K., Morgan, B. J., & Poulin, M. J. (2005). Differential responses to CO2 and sympathetic stimulation in the cerebral and femoral circulations in humans. *J Physiol, 566*(Pt 2), 613-624. 10.1113/jphysiol.2005.087320

Ainslie, P. N., & Duffin, J. (2009). Integration of cerebrovascular CO2 reactivity and chemoreflex control of breathing: mechanisms of regulation, measurement, and interpretation. *Am J Physiol Regul Integr Comp Physiol, 296*(5), R1473-1495. 10.1152/ajpregu.91008.2008

Ainslie, P. N., Ogoh, S., Burgess, K., Celi, L., McGrattan, K., Peebles, K., . . . Burgess, K. R. (2008). Differential effects of acute hypoxia and high altitude on cerebral blood flow velocity and dynamic cerebral autoregulation: alterations with hyperoxia. *J Appl Physiol (1985), 104*(2), 490-498. 10.1152/japplphysiol.00778.2007

Ainslie, P. N., & Poulin, M. J. (2004). Ventilatory, cerebrovascular, and cardiovascular interactions in acute hypoxia: regulation by carbon dioxide. *J Appl Physiol (1985), 97*(1), 149-159. 10.1152/japplphysiol.01385.2003

Ali, Y. S., Daamen, N., Jacob, G., Jordan, J., Shannon, J. R., Biaggioni, I., & Robertson, D. (2000). Orthostatic intolerance: a disorder of young women. *Obstet Gynecol Surv, 55*(4), 251-259.

Andresen, M., Hadi, A., Petersen, L. G., & Juhler, M. (2015). Effect of postural changes on ICP in healthy and ill subjects. *Acta Neurochir (Wien), 157*(1), 109-113. 10.1007/s00701-014-2250-2

Attwell, D., Buchan, A. M., Charpak, S., Lauritzen, M., Macvicar, B. A., & Newman, E. A. (2010). Glial and neuronal control of brain blood flow. *Nature, 468*(7321), 232-243. 10.1038/nature09613

Bain, C. A., Lees, K. R., Lumsden, M. A., & Walters, M. R. (2005). Effect of a gonadotropin releasing hormone analog on cerebral hemodynamics in premenopausal women. *Climacteric, 8*(2), 193-197. 10.1080/13697130500118068

Bathala, L., Mehndiratta, M. M., & Sharma, V. K. (2013). Transcranial doppler: Technique and common findings (Part 1). Annals of Indian Academy of Neurology, 16(2), 174–179. https://doi.org/10.4103/0972-2327.112460

Battisti-Charbonney, A., Fisher, J., & Duffin, J. (2011). The cerebrovascular response to carbon dioxide in humans. *J Physiol, 589*(Pt 12), 3039-3048. 10.1113/jphysiol.2011.206052

Becker, H. F., Polo, O., McNamara, S. G., Berthon-Jones, M., & Sullivan, C. E. (1996). Effect of different levels of hyperoxia on breathing in healthy subjects. *J Appl Physiol (1985), 81*(4), 1683-1690. 10.1152/jappl.1996.81.4.1683

Behre, H. M., Kuhlage, J., Gassner, C., Sonntag, B., Schem, C., Schneider, H. P., & Nieschlag, E. (2000). Prediction of ovulation by urinary hormone measurements with the home use

ClearPlan Fertility Monitor: comparison with transvaginal ultrasound scans and serum hormone measurements. *Hum Reprod, 15*(12), 2478-2482. 10.1093/humrep/15.12.2478

Belanger, M., Allaman, I., & Magistretti, P. J. (2011). Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation. *Cell Metab, 14*(6), 724-738. 10.1016/j.cmet.2011.08.016

Berglund, E. S., Hirschberg, A. L., & Scherwitzl, R. (2015). Identification and prediction of the fertile window using NaturalCycles. *Eur J Contracept Reprod Health Care, 20*(5), 403-408. 10.3109/13625187.2014.988210

Bevan, J. A., & Hwa, J. J. (1985). Myogenic tone and cerebral vascular autoregulation: the role of a stretch-dependent mechanism. *Ann Biomed Eng, 13*(3-4), 281-286.

Birch, A. A., Neil-Dwyer, G., & Murrills, A. J. (2001). The repeatability of cerebral autoregulation assessment using sinusoidal lower body negative pressure. *Physiological measurement, 23*(1), 73.

Bowton, D. L., Haddon, W. S., Prough, D. S., Adair, N., Alford, P. T., & Stump, D. A. (1988). Theophylline effect on the cerebral blood flow response to hypoxemia. *Chest, 94*(2), 371-375.

Brackley, K. J., Ramsay, M. M., Broughton Pipkin, F., & Rubin, P. C. (1999). The effect of the menstrual cycle on human cerebral blood flow: studies using Doppler ultrasound. *Ultrasound Obstet Gynecol, 14*(1), 52-57. 10.1046/j.1469-0705.1999.14010052.x

Brackley, K. J., Ramsay, M. M., Pipkin, F. B., & Rubin, P. C. (1998). A longitudinal study of maternal bloodflow in normal pregnancy and the puerperium: analysis of Doppler waveforms using Laplace transform techniques. *British Journal of Obstetrics and Gynaecology, 105*(1), 68-77. DOI 10.1111/j.1471-0528.1998.tb09353.x

Brassard, P., Ferland-Dutil, H., Smirl, J. D., Paquette, M., Le Blanc, O., Malenfant, S., & Ainslie, P. N. (2017). Evidence for hysteresis in the cerebral pressure-flow relationship in healthy men. *Am J Physiol Heart Circ Physiol, 312*(4), H701-H704. 10.1152/ajpheart.00790.2016

Brassard, P., Tymko, M. M., & Ainslie, P. N. (2017). Sympathetic control of the brain circulation: Appreciating the complexities to better understand the controversy. *Auton Neurosci, 207*, 37-47. 10.1016/j.autneu.2017.05.003

Brown, A. M., & Ransom, B. R. (2007). Astrocyte glycogen and brain energy metabolism. *Glia, 55*(12), 1263-1271. 10.1002/glia.20557

Carmignoto, G., & Gomez-Gonzalo, M. (2010). The contribution of astrocyte signalling to neurovascular coupling. *Brain Res Rev, 63*(1-2), 138-148. 10.1016/j.brainresrev.2009.11.007

Ceric, F., Silva, D., & Vigil, P. (2005). Ultrastructure of the human periovulatory cervical mucus. *J Electron Microsc (Tokyo), 54*(5), 479-484. 10.1093/jmicro/dfh106

Chandra, A., Li, W. A., Stone, C. R., Geng, X., & Ding, Y. (2017). The cerebral circulation and cerebrovascular disease I: Anatomy. *Brain Circ, 3*(2), 45-56. 10.4103/bc.bc_10_17

Cheng, Y. C., Vyas, A., Hymen, E., & Perlmuter, L. C. (2011). Gender differences in orthostatic hypotension. *Am J Med Sci, 342*(3), 221-225. 10.1097/MAJ.0b013e318208752b

Chow, B. W., Nunez, V., Kaplan, L., Granger, A. J., Bistrong, K., Zucker, H. L., . . . Gu, C. (2020). Caveolae in CNS arterioles mediate neurovascular coupling. *Nature, 579*(7797), 106-110. 10.1038/s41586-020-2026-1

Chrissobolis, S., Budzyn, K., Marley, P. D., & Sobey, C. G. (2004). Evidence that estrogen suppresses rho-kinase function in the cerebral circulation in vivo. *Stroke, 35*(9), 2200-2205. 10.1161/01.STR.0000136951.85586.c8

Chrissobolis, S., & Sobey, C. G. (2004). Influence of gender on K+-induced cerebral vasodilatation. *Stroke, 35*(3), 747-752. 10.1161/01.STR.0000116867.28589.3A

Claassen, J. A., Levine, B. D., & Zhang, R. (2009). Dynamic cerebral autoregulation during repeated squat-stand maneuvers. *J Appl Physiol (1985), 106*(1), 153-160. 10.1152/japplphysiol.90822.2008

Cohen, J. (2013). *Statistical power analysis for the behavioral sciences*: Routledge.

Czosnyka, M., Harris, N. G., Pickard, J. D., & Piechnik, S. (1993). CO 2 cerebrovascular reactivity as a function of perfusion pressure—a modelling study. *Acta neurochirurgica, 121*(3-4), 159-165.

Dabbs Jr, J. M. (1991). Salivary testosterone measurements: collecting, storing, and mailing saliva samples. *Physiology & Behavior, 49*(4), 815-817.

Davis, M. J., & Hill, M. A. (1999). Signaling mechanisms underlying the vascular myogenic response. *Physiol Rev, 79*(2), 387-423. 10.1152/physrev.1999.79.2.387

Diomedi, M., Cupini, L. M., Rizzato, B., Ferrante, F., Giacomini, P., & Silvestrini, M. (2001). Influence of physiologic oscillation of estrogens on cerebral hemodynamics. *J Neurol Sci, 185*(1), 49-53. https://doi.org/10.1016/S0022-510X(01)00462-2

Dunn, L. T. (2002). Raised intracranial pressure. *J Neurol Neurosurg Psychiatry, 73 Suppl 1*(suppl 1), i23-27. 10.1136/jnnp.73.suppl_1.i23

Edvinsson, L. (1975). Neurogenic mechanisms in the cerebrovascular bed. Autonomic nerves, amine receptors and their effects on cerebral blood flow. *Acta physiologica Scandinavica. Supplementum, 427*, 1-35.

Fantini, S., Sassaroli, A., Tgavalekos, K. T., & Kornbluth, J. (2016). Cerebral blood flow and autoregulation: current measurement techniques and prospects for noninvasive optical methods. *Neurophotonics, 3*(3), 031411. 10.1117/1.NPh.3.3.031411

Favre, M. E., & Serrador, J. M. (2019). Sex differences in cerebral autoregulation are unaffected by menstrual cycle phase in young, healthy women. *Am J Physiol Heart Circ Physiol, 316*(4), H920-H933. 10.1152/ajpheart.00474.2018

Fehring, R. J. (2002). Accuracy of the peak day of cervical mucus as a biological marker of fertility. *Contraception, 66*(4), 231-235. Pii S0010-7824(02)00355-4

Doi 10.1016/S0010-7824(02)00355-4

Filosa, J. A., & Blanco, V. M. (2007). Neurovascular coupling in the mammalian brain. *Exp Physiol, 92*(4), 641-646. 10.1113/expphysiol.2006.036368

Fog, M. (1939a). Cerebral circulation I. Reaction of pial arteries to epinephrine by direct application and by intravenous injection. *Archives of Neurology and Psychiatry, 41*(1), 109-118. DOI 10.1001/archneurpsyc.1939.02270130119007

Fog, M. (1939b). Cerebral circulation II. Reaction of pial arteries to increase in blood pressure. *Archives of Neurology and Psychiatry, 41*(2), 260-268. DOI 10.1001/archneurpsyc.1939.02270140046003

Fortune, J. B., Bock, D., Kupinski, A. M., Stratton, H. H., Shah, D. M., & Feustel, P. J. (1992). Human Cerebrovascular Response to Oxygen and Carbon-Dioxide as Determined by Internal Carotid-Artery Duplex Scanning. *Journal of Trauma-Injury Infection and Critical Care, 32*(5), 618-628. 10.1097/00005373-199205000-00014

Geary, G. G., Krause, D. N., & Duckles, S. P. (1998). Estrogen reduces myogenic tone through a nitric oxide-dependent mechanism in rat cerebral arteries. *American Journal of Physiology-Heart and Circulatory Physiology, 275*(1), H292-H300.

Geary, G. G., Krause, D. N., & Duckles, S. P. (2000a). Estrogen reduces mouse cerebral artery tone through endothelial NOS- and cyclooxygenase-dependent mechanisms. *Am J Physiol Heart Circ Physiol, 279*(2), H511-519. 10.1152/ajpheart.2000.279.2.H511

Geary, G. G., Krause, D. N., & Duckles, S. P. (2000b). Gonadal hormones affect diameter of male rat cerebral arteries through endothelium-dependent mechanisms. *Am J Physiol Heart Circ Physiol, 279*(2), H610-618. 10.1152/ajpheart.2000.279.2.H610

Giller, C. A., Bowman, G., Dyer, H., Mootz, L., & Krippner, W. (1993). Cerebral arterial diameters during changes in blood pressure and carbon dioxide during craniotomy. *Neurosurgery, 32*(5), 737-741; discussion 741-732. 10.1227/00006123-199305000-00006

Gonzales, R. J., Ghaffari, A. A., Duckles, S. P., & Krause, D. N. (2005). Testosterone treatment increases thromboxane function in rat cerebral arteries. *Am J Physiol Heart Circ Physiol, 289*(2), H578-585. 10.1152/ajpheart.00958.2004

Gonzales, R. J., Krause, D. N., & Duckles, S. P. (2004). Testosterone suppresses endothelium-dependent dilation of rat middle cerebral arteries. *Am J Physiol Heart Circ Physiol, 286*(2), H552-560. 10.1152/ajpheart.00663.2003

Gordon, G. R., Choi, H. B., Rungta, R. L., Ellis-Davies, G. C., & MacVicar, B. A. (2008). Brain metabolism dictates the polarity of astrocyte control over arterioles. *Nature, 456*(7223), 745-749. 10.1038/nature07525

Gosling, R. G., & King, D. H. (1974). The role of measurement in peripheral vascular surgery: arterial assessment by Doppler-shift ultrasound. In: SAGE Publications.

Greenfield, J. C., Jr., Rembert, J. C., & Tindall, G. T. (1984). Transient changes in cerebral vascular resistance during the Valsalva maneuver in man. *Stroke, 15*(1), 76-79. 10.1161/01.str.15.1.76

Gulbenkian, S., Uddman, R., & Edvinsson, L. (2001). Neuronal messengers in the human cerebral circulation. *Peptides, 22*(6), 995-1007. https://doi.org/10.1016/S0196-9781(01)00408-9

Hamel, E. (2004). Cholinergic modulation of the cortical microvascular bed. *Prog Brain Res, 145*, 171-178. 10.1016/S0079-6123(03)45012-7

Hamner, J. W., Ishibashi, K., & Tan, C. O. (2019). Revisiting human cerebral blood flow responses to augmented blood pressure oscillations. *J Physiol, 597*(6), 1553-1564. 10.1113/JP277321

Hamner, J. W., & Tan, C. O. (2014). Relative contributions of sympathetic, cholinergic, and myogenic mechanisms to cerebral autoregulation. *Stroke, 45*(6), 1771-1777. 10.1161/STROKEAHA.114.005293

Hamner, J. W., Tan, C. O., Lee, K., Cohen, M. A., & Taylor, J. A. (2010). Sympathetic control of the cerebral vasculature in humans. *Stroke, 41*(1), 102-109. 10.1161/STROKEAHA.109.557132

Hamner, J. W., Tan, C. O., Tzeng, Y. C., & Taylor, J. A. (2012). Cholinergic control of the cerebral vasculature in humans. *J Physiol, 590*(24), 6343-6352. 10.1113/jphysiol.2012.245100

Hatcher, R. A., Breedlove, B., Judy, B., & Martin, N. (1988). *Contraceptive Technology, 1988-1989*: Irvington Publishers.

Haykowsky, M. J., Eves, N. D., Warburton, D. E. R., & Findlay, M. J. (2003). Resistance exercise, the Valsalva maneuver, and cerebrovascular transmural pressure. *Med Sci Sports Exerc, 35*(1), 65-68. 10.1249/01.MSS.0000043449.65647.C9

Heistad, D. D., & Kontos, H. A. (1983). Cerebral circulation. In *Handbook of Physiology. The Cardiovascular System. Peripheral Circulation and Organ Blood Flow* (Vol. 3, pp. 137-182): Am. Physiol. Soc. Bethesda, MD.

Heistad, D. D., & Marcus, M. L. (1978). Evidence that neural mechanisms do not have important effects on cerebral blood flow. *Circulation research, 42*(3), 295-302.

Heistad, D. D., Marcus, M. L., Said, S. I., & Gross, P. M. (1980). Effect of acetylcholine and vasoactive intestinal peptide on cerebral blood flow. *Am J Physiol, 239*(1), H73-80. 10.1152/ajpheart.1980.239.1.H73

Hill, M. A., Zou, H., Potocnik, S. J., Meininger, G. A., & Davis, M. J. (2001). Signal transduction in smooth muscle - Invited review: Arteriolar smooth muscle mechanotransduction: Ca2+ signaling pathways underlying myogenic reactivity. *Journal of Applied Physiology, 91*(2), 973-983.

Ho, K. J., & Liao, J. K. (2002). Nonnuclear actions of estrogen. *Arterioscler Thromb Vasc Biol, 22*(12), 1952-1961. 10.1161/01.Atv.0000041200.85946.4a

Iadecola, C., & Nedergaard, M. (2007). Glial regulation of the cerebral microvasculature. *Nat Neurosci, 10*(11), 1369-1376. 10.1038/nn2003

Ibrahim, J., McGee, A., Graham, D., McGrath, J. C., & Dominiczak, A. F. (2006). Sex-specific differences in cerebral arterial myogenic tone in hypertensive and normotensive rats. *Am J Physiol Heart Circ Physiol, 290*(3), H1081-1089. 10.1152/ajpheart.00752.2005

Ide, K., Eliasziw, M., & Poulin, M. J. (2003). Relationship between middle cerebral artery blood velocity and end-tidal PCO2 in the hypocapnic-hypercapnic range in humans. *J Appl Physiol (1985), 95*(1), 129-137. 10.1152/japplphysiol.01186.2002

Iqbal, S. (2013). A comprehensive study of the anatomical variations of the circle of willis in adult human brains. *J Clin Diagn Res, 7*(11), 2423-2427. 10.7860/JCDR/2013/6580.3563

Jansen, G. F., Krins, A., Basnyat, B., Odoom, J. A., & Ince, C. (2007). Role of the altitude level on cerebral autoregulation in residents at high altitude. *J Appl Physiol (1985), 103*(2), 518-523. 10.1152/japplphysiol.01429.2006

Junqueira, L. F., Jr. (2008). Teaching cardiac autonomic function dynamics employing the Valsalva (Valsalva-Weber) maneuver. *Adv Physiol Educ, 32*(1), 100-106. 10.1152/advan.00057.2007

Kety, S. S., & Schmidt, C. F. (1945). The Determination of Cerebral Blood Flow in Man by the Use of Nitrous Oxide in Low Concentrations. *American Journal of Physiology, 143*(1), 53-66.

Kety, S. S., & Schmidt, C. F. (1946). The Effects of Active and Passive Hyperventilation on Cerebral Blood Flow, Cerebral Oxygen Consumption, Cardiac Output, and Blood Pressure of Normal Young Men. *J Clin Invest, 25*(1), 107-119. 10.1172/JCI101680

Kety, S. S., & 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. *J Clin Invest, 27*(4), 484-492. 10.1172/JCI101995

Kitazono, T., Faraci, F. M., Taguchi, H., & Heistad, D. D. (1995). Role of potassium channels in cerebral blood vessels. *Stroke, 26*(9), 1713-1723. 10.1161/01.STR.26.9.1713

Kontos, H. A., Wei, E. P., Navari, R. M., Levasseur, J. E., Rosenblum, W. I., & Patterson, J. L., Jr. (1978). Responses of cerebral arteries and arterioles to acute hypotension and hypertension. *Am J Physiol, 234*(4), H371-383. 10.1152/ajpheart.1978.234.4.H371

Krause, D. N., Duckles, S. P., & Pelligrino, D. A. (2006). Influence of sex steroid hormones on cerebrovascular function. *J Appl Physiol (1985), 101*(4), 1252-1261. 10.1152/japplphysiol.01095.2005

Krejza, J., Mariak, Z., Huba, M., Wolczynski, S., & Lewko, J. (2001). Effect of endogenous estrogen on blood flow through carotid arteries. *Stroke, 32*(1), 30-36. 10.1161/01.str.32.1.30

Krejza, J., Mariak, Z., Nowacka, A., Melhem, E. R., & Babikian, V. L. (2004). Influence of 17-beta-estradiol on cerebrovascular impedance during menstrual cycle in women. *J Neurol Sci, 221*(1-2), 61-67. 10.1016/j.jns.2004.03.028

Krejza, J., Siemkowicz, J., Sawicka, M., Szylak, A., Kochanowicz, J., Mariak, Z., . . . Bert, R. (2003). Oscillations of cerebrovascular resistance throughout the menstrual cycle in healthy women. *Ultrasound Obstet Gynecol, 22*(6), 627-632. 10.1002/uog.907

Kulik, T., Kusano, Y., Aronhime, S., Sandler, A. L., & Winn, H. R. (2008). Regulation of cerebral vasculature in normal and ischemic brain. *Neuropharmacology, 55*(3), 281-288. 10.1016/j.neuropharm.2008.04.017

Labrecque, L., Rahimaly, K., Imhoff, S., Paquette, M., Le Blanc, O., Malenfant, S., . . . Brassard, P. (2017). Diminished dynamic cerebral autoregulatory capacity with forced oscillations in mean arterial pressure with elevated cardiorespiratory fitness. *Physiol Rep, 5*(21), e13486. 10.14814/phy2.13486

Lassen, N. A. (1959). Cerebral blood flow and oxygen consumption in man. *Physiol Rev, 39*(2), 183-238. 10.1152/physrev.1959.39.2.183

Lee, J. M., Grabb, M. C., Zipfel, G. J., & Choi, D. W. (2000). Brain tissue responses to ischemia. *J Clin Invest, 106*(6), 723-731. 10.1172/JCI11003

Lipson, S. F., & Ellison, P. T. (1989). Development of protocols for the application of salivary steroid analysis to field conditions. *Am J Hum Biol, 1*(3), 249-255. 10.1002/ajhb.1310010304

Lisabeth, L., & Bushnell, C. (2012). Stroke risk in women: the role of menopause and hormone therapy. *Lancet Neurol, 11*(1), 82-91. 10.1016/S1474-4422(11)70269-1

Lucas, S. J., Tzeng, Y. C., Galvin, S. D., Thomas, K. N., Ogoh, S., & Ainslie, P. N. (2010). Influence of changes in blood pressure on cerebral perfusion and oxygenation. *Hypertension, 55*(3), 698-705. 10.1161/HYPERTENSIONAHA.109.146290

Makowicz, G., Poniatowska, R., & Lusawa, M. (2013). Variants of cerebral arteries - anterior circulation. *Pol J Radiol, 78*(3), 42-47. 10.12659/PJR.889403

Marieb, E. N., & Hoehn, K. (2018). *Human Anatomy & Physiology, Global Edition*. Harlow, United Kingdom, UNITED KINGDOM: Pearson Education Limited.

McCulloch, T. J., Boesel, T. W., & Lam, A. M. (2005). The effect of hypocapnia on the autoregulation of cerebral blood flow during administration of isoflurane. *Anesth Analg, 100*(5), 1463-1467, table of contents. 10.1213/01.ANE.0000148623.06596.7E

McCulloch, T. J., Visco, E., & Lam, A. M. (2000). Graded hypercapnia and cerebral autoregulation during sevoflurane or propofol anesthesia. *Anesthesiology, 93*(5), 1205-1209.

Mendelsohn, M. E., & Karas, R. H. (1999). The protective effects of estrogen on the cardiovascular system. *N Engl J Med, 340*(23), 1801-1811. 10.1056/NEJM199906103402306

Meng, L., & Gelb, A. W. (2015). Regulation of cerebral autoregulation by carbon dioxide. *Anesthesiology, 122*(1), 196-205. 10.1097/ALN.0000000000000506

Meno, J. R., Ngai, A. C., & Winn, H. R. (1993). Changes in pial arteriolar diameter and CSF adenosine concentrations during hypoxia. *J Cereb Blood Flow Metab, 13*(2), 214-220. 10.1038/jcbfm.1993.26

Meyer, J. S., Gotoh, F., Takagi, Y., & Kakimi, R. (1966). Cerebral hemodynamics, blood gases, and electrolytes during breath-holding and the Valsalva maneuver. *Circulation, 33*(5 Suppl), II35-48. 10.1161/01.cir.33.5s2.ii-35

Miller, V. M., & Vanhoutte, P. M. (1991). Progesterone and modulation of endothelium-dependent responses in canine coronary arteries. *Am J Physiol, 261*(4 Pt 2), R1022-1027. 10.1152/ajpregu.1991.261.4.R1022

Muppa, P., Sheldon, R. S., McRae, M., Keller, N. R., Ritchie, D., Krahn, A. D., . . . Raj, S. R. (2013). Gynecological and menstrual disorders in women with vasovagal syncope. *Clin Auton Res, 23*(3), 117-122. 10.1007/s10286-013-0190-1

Nelson, M. T., & Quayle, J. M. (1995). Physiological roles and properties of potassium channels in arterial smooth muscle. *Am J Physiol, 268*(4 Pt 1), C799-822. 10.1152/ajpcell.1995.268.4.C799

Nishimura, N., Iwasaki, K., Ogawa, Y., & Aoki, K. (2010). Decreased steady-state cerebral blood flow velocity and altered dynamic cerebral autoregulation during 5-h sustained 15% O2 hypoxia. *J Appl Physiol (1985), 108*(5), 1154-1161. 10.1152/japplphysiol.00656.2009

Nishimura, N., Iwasaki, K., Ogawa, Y., & Shibata, S. (2007). Oxygen administration, cerebral blood flow velocity, and dynamic cerebral autoregulation. *Aviat Space Environ Med, 78*(12), 1121-1127.

Ogoh, S., Brothers, R. M., Eubank, W. L., & Raven, P. B. (2008). Autonomic neural control of the cerebral vasculature: acute hypotension. *Stroke, 39*(7), 1979-1987. 10.1161/STROKEAHA.107.510008

Ogoh, S., Nakahara, H., Ainslie, P. N., & Miyamoto, T. (2010). The effect of oxygen on dynamic cerebral autoregulation: critical role of hypocapnia. *J Appl Physiol (1985), 108*(3), 538-543. 10.1152/japplphysiol.01235.2009

Ogoh, S., Nakahara, H., Ueda, S., Okazaki, K., Shibasaki, M., Subudhi, A. W., & Miyamoto, T. (2014). Effects of acute hypoxia on cerebrovascular responses to carbon dioxide. *Exp Physiol, 99*(6), 849-858. 10.1113/expphysiol.2013.076802

Ogoh, S., Sato, K., Nakahara, H., Okazaki, K., Subudhi, A. W., & Miyamoto, T. (2013). Effect of acute hypoxia on blood flow in vertebral and internal carotid arteries. *Exp Physiol, 98*(3), 692-698. 10.1113/expphysiol.2012.068015

Ohkura, T., Teshima, Y., Isse, K., Matsuda, H., Inoue, T., Sakai, Y., . . . Yaoi, Y. (1995). Estrogen Increases Cerebral and Cerebellar Blood Flows in Postmenopausal Women. *Menopause-the Journal of the North American Menopause Society, 2*(1), 13-18.

Orshal, J. M., & Khalil, R. A. (2004). Gender, sex hormones, and vascular tone. *Am J Physiol Regul Integr Comp Physiol, 286*(2), R233-249. 10.1152/ajpregu.00338.2003

Osol, G., & Halpern, W. (1985). Myogenic properties of cerebral blood vessels from normotensive and hypertensive rats. *Am J Physiol, 249*(5 Pt 2), H914-921. 10.1152/ajpheart.1985.249.5.H914

Ospina, J. A., Duckles, S. P., & Krause, D. N. (2003). 17beta-estradiol decreases vascular tone in cerebral arteries by shifting COX-dependent vasoconstriction to vasodilation. *Am J Physiol Heart Circ Physiol, 285*(1), H241-250. 10.1152/ajpheart.00018.2003

Ospina, J. A., Krause, D. N., & Duckles, S. P. (2002). 17β-Estradiol increases rat cerebrovascular prostacyclin synthesis by elevating cyclooxygenase-1 and prostacyclin synthase. *Stroke, 33*(2), 600-605.

Panerai, R. B. (2008). Cerebral autoregulation: from models to clinical applications. *Cardiovasc Eng, 8*(1), 42-59. 10.1007/s10558-007-9044-6

Pearce, W. J. (1995). Mechanisms of hypoxic cerebral vasodilatation. *Pharmacol Ther, 65*(1), 75-91.

Peggs, K. J., Nguyen, H., Enayat, D., Keller, N. R., Al-Hendy, A., & Raj, S. R. (2012). Gynecologic disorders and menstrual cycle lightheadedness in postural tachycardia syndrome. *Int J Gynaecol Obstet, 118*(3), 242-246. 10.1016/j.ijgo.2012.04.014

Pelligrino, D. A., & Galea, E. (2001). Estrogen and cerebrovascular physiology and pathophysiology. *Jpn J Pharmacol, 86*(2), 137-158. 10.1254/jjp.86.137

Perry, B. G., Cotter, J. D., Mejuto, G., Mundel, T., & Lucas, S. J. (2014). Cerebral hemodynamics during graded Valsalva maneuvers. *Front Physiol, 5*, 349. 10.3389/fphys.2014.00349

Peterson, E. C., Wang, Z., & Britz, G. (2011). Regulation of cerebral blood flow. *Int J Vasc Med, 2011*, 823525. 10.1155/2011/823525

Pott, F., van Lieshout, J. J., Ide, K., Madsen, P., & Secher, N. H. (2000). Middle cerebral artery blood velocity during a valsalva maneuver in the standing position. *J Appl Physiol (1985), 88*(5), 1545-1550. 10.1152/jappl.2000.88.5.1545

Querido, J. S., Ainslie, P. N., Foster, G. E., Henderson, W. R., Halliwill, J. R., Ayas, N. T., & Sheel, A. W. (2013). Dynamic cerebral autoregulation during and following acute hypoxia: role of carbon dioxide. *J Appl Physiol (1985), 114*(9), 1183-1190. 10.1152/japplphysiol.00024.2013

Remmen, J. J., Aengevaeren, W. R., Verheugt, F. W., & Jansen, R. W. (2005). Normal values of pulmonary capillary wedge pressure and the blood pressure response to the Valsalva manoeuvre in healthy elderly subjects. *Clin Physiol Funct Imaging, 25*(6), 318-326. 10.1111/j.1475-097X.2005.00630.x

Rivera-Lara, L., Zorrilla-Vaca, A., Geocadin, R. G., Healy, R. J., Ziai, W., & Mirski, M. A. (2017). Cerebral Autoregulation-oriented Therapy at the Bedside: A Comprehensive Review. *Anesthesiology, 126*(6), 1187-1199. 10.1097/ALN.0000000000001625

Robison, L. S., Gannon, O. J., Salinero, A. E., & Zuloaga, K. L. (2019). Contributions of sex to cerebrovascular function and pathology. *Brain Res, 1710*, 43-60. 10.1016/j.brainres.2018.12.030

Rosengarten, B., Spiller, A., Aldinger, C., & Kaps, M. (2003). Control system analysis of visually evoked blood flow regulation in humans under normocapnia and hypercapnia. *Eur J Ultrasound, 16*(3), 169-175. https://doi.org/10.1016/S0929-8266(02)00070-8

Sato, A., Sato, Y., & Uchida, S. (2001). Regulation of regional cerebral blood flow by cholinergic fibers originating in the basal forebrain. *Int J Dev Neurosci, 19*(3), 327-337. https://doi.org/10.1016/S0736-5748(01)00017-X

Sato, K., Fisher, J. P., Seifert, T., Overgaard, M., Secher, N. H., & Ogoh, S. (2012). Blood flow in internal carotid and vertebral arteries during orthostatic stress. *Exp Physiol, 97*(12), 1272-1280. 10.1113/expphysiol.2012.064774

Schneider, G. H., Helden, A., Franke, R., Lanksch, W. R., & Unterberg, A. (1993). Influence of Body Position on Jugular Venous Oxygen Saturation, Intracranial Pressure and Cerebral Perfusion Pressure. In A. W. Unterberg, G.-H. Schneider, & W. R. Lanksch (Eds.), *Monitoring of Cerebral Blood Flow and Metabolism in Intensive Care* (pp. 107-112). Vienna: Springer Vienna. 10.1007/978-3-7091-9302-0_19

Schubert, R., & Brayden, J. E. (2005). Stretch-activated Cation Channels and the Myogenic Response of Small Arteries. In A. Kamkin & I. Kiseleva (Eds.), *Mechanosensitivity in Cells and Tissues*. Moscow: Academia.

Schubert, R., & Mulvany, M. J. (1999). The myogenic response: established facts and attractive hypotheses. *Clin Sci (Lond), 96*(4), 313-326.

Simard, M., Arcuino, G., Takano, T., Liu, Q. S., & Nedergaard, M. (2003). Signaling at the gliovascular interface. *Journal of Neuroscience, 23*(27), 9254-9262. 10.1523/JNEUROSCI.23-27-09254.2003

Simpson, D., & Claassen, J. (2018). CrossTalk opposing view: dynamic cerebral autoregulation should be quantified using induced (rather than spontaneous) blood pressure fluctuations. *J Physiol, 596*(1), 7-9. 10.1113/JP273900

Simpson, E., Rubin, G., Clyne, C., Robertson, K., O'Donnell, L., Jones, M., & Davis, S. (2000). The role of local estrogen biosynthesis in males and females. *Trends Endocrinol Metab, 11*(5), 184-188. 10.1016/s1043-2760(00)00254-x

Skarsgard, P., van Breemen, C., & Laher, I. (1997). Estrogen regulates myogenic tone in pressurized cerebral arteries by enhanced basal release of nitric oxide. *Am J Physiol, 273*(5 Pt 2), H2248-2256.

Skow, R. J., MacKay, C. M., Tymko, M. M., Willie, C. K., Smith, K. J., Ainslie, P. N., & Day, T. A. (2013). Differential cerebrovascular CO(2) reactivity in anterior and posterior cerebral circulations. *Respir Physiol Neurobiol, 189*(1), 76-86. 10.1016/j.resp.2013.05.036

Smirl, J. D., Hoffman, K., Tzeng, Y. C., Hansen, A., & Ainslie, P. N. (2015). Methodological comparison of active- and passive-driven oscillations in blood pressure; implications for the assessment of cerebral pressure-flow relationships. *J Appl Physiol (1985), 119*(5), 487-501. 10.1152/japplphysiol.00264.2015

Smith, B. A., Clayton, E. W., & Robertson, D. (2011). Experimental arrest of cerebral blood flow in human subjects: the red wing studies revisited. *Perspect Biol Med, 54*(2), 121-131. 10.1353/pbm.2011.0018

Stirone, C., Boroujerdi, A., Duckles, S. P., & Krause, D. N. (2005). Estrogen receptor activation of phosphoinositide-3 kinase, akt, and nitric oxide signaling in cerebral blood vessels: rapid and long-term effects. *Mol Pharmacol, 67*(1), 105-113. 10.1124/mol.104.004465

Strandgaard, S., & Sigurdsson, S. T. (2008). Point:Counterpoint: Sympathetic activity does/does not influence cerebral blood flow. Counterpoint: Sympathetic nerve activity does not influence cerebral blood flow. *J Appl Physiol (1985), 105*(4), 1366-1367; discussion 1367-1368. 10.1152/japplphysiol.90597.2008a

Su, H. I., & Freeman, E. W. (2009). Hormone changes associated with the menopausal transition. *Minerva Ginecol, 61*(6), 483-489.

Su, H. W., Yi, Y. C., Wei, T. Y., Chang, T. C., & Cheng, C. M. (2017). Detection of ovulation, a review of currently available methods. *Bioengineering & translational medicine, 2*(3), 238-246. 10.1002/btm2.10058

Subudhi, A. W., Panerai, R. B., & Roach, R. C. (2009). Acute hypoxia impairs dynamic cerebral autoregulation: results from two independent techniques. *J Appl Physiol (1985), 107*(4), 1165-1171. 10.1152/japplphysiol.00498.2009

Subudhi, A. W., Panerai, R. B., & Roach, R. C. (2010). Effects of hypobaric hypoxia on cerebral autoregulation. *Stroke, 41*(4), 641-646. 10.1161/STROKEAHA.109.574749

Tameem, A., & Krovvidi, H. (2013). Cerebral physiology. *Continuing Education in Anaesthesia Critical Care & Pain, 13*(4), 113-118. 10.1093/bjaceaccp/mkt001

Tan, C. O. (2012). Defining the characteristic relationship between arterial pressure and cerebral flow. *J Appl Physiol (1985), 113*(8), 1194-1200. 10.1152/japplphysiol.00783.2012

Tan, C. O., & Taylor, J. A. (2014). Integrative physiological and computational approaches to understand autonomic control of cerebral autoregulation. *Exp Physiol, 99*(1), 3-15. 10.1113/expphysiol.2013.072355

Tanabe, K., Susumu, N., Hand, K., Nishii, K., Ishikawa, I., & Nozawa, S. (2001). Prediction of the potentially fertile period by urinary hormone measurements using a new home-use monitor: comparison with laboratory hormone analyses. *Hum Reprod, 16*(8), 1619-1624. 10.1093/humrep/16.8.1619

Thomas, J. R., Nelson, J. K., & Silverman, S. J. (2015). *Research methods in physical activity*. Human kinetics.

Tiecks, F. P., Lam, A. M., Aaslid, R., & Newell, D. W. (1995). Comparison of static and dynamic cerebral autoregulation measurements. Stroke, 26(6), 1014-1019.

Tiecks, F. P., Lam, A. M., Matta, B. F., Strebel, S., Douville, C., & Newell, D. W. (1995). Effects of the Valsalva Maneuver on Cerebral Circulation in Healthy Adults. *Stroke, 26*(8), 1386-1392. 10.1161/01.Str.26.8.1386

Tzeng, Y. C., & Ainslie, P. N. (2014). Blood pressure regulation IX: cerebral autoregulation under blood pressure challenges. *Eur J Appl Physiol, 114*(3), 545-559. 10.1007/s00421-013-2667-y

Tzeng, Y. C., Chan, G. S., Willie, C. K., & Ainslie, P. N. (2011). Determinants of human cerebral pressure-flow velocity relationships: new insights from vascular modelling and Ca(2)(+) channel blockade. *J Physiol, 589*(Pt 13), 3263-3274. 10.1113/jphysiol.2011.206953

Tzeng, Y. C., Lucas, S. J., Atkinson, G., Willie, C. K., & Ainslie, P. N. (2010). Fundamental relationships between arterial baroreflex sensitivity and dynamic cerebral autoregulation in humans. *J Appl Physiol (1985), 108*(5), 1162-1168. 10.1152/japplphysiol.01390.2009

Tzeng, Y. C., MacRae, B. A., Ainslie, P. N., & Chan, G. S. (2014). Fundamental relationships between blood pressure and cerebral blood flow in humans. *J Appl Physiol (1985), 117*(9), 1037-1048. 10.1152/japplphysiol.00366.2014

Tzeng, Y. C., & Panerai, R. B. (2018). CrossTalk proposal: dynamic cerebral autoregulation should be quantified using spontaneous blood pressure fluctuations. *J Physiol, 596*(1), 3-5. 10.1113/JP273899

van Beek, A. H., Claassen, J. A., Rikkert, M. G., & Jansen, R. W. (2008). Cerebral autoregulation: an overview of current concepts and methodology with special focus on the elderly. *J Cereb Blood Flow Metab, 28*(6), 1071-1085. 10.1038/jcbfm.2008.13

Van Lieshout, J. J., Wieling, W., Karemaker, J. M., & Secher, N. H. (2003). Syncope, cerebral perfusion, and oxygenation. *J Appl Physiol (1985), 94*(3), 833-848. 10.1152/japplphysiol.00260.2002

van Mook, W. N., Rennenberg, R. J., Schurink, G. W., van Oostenbrugge, R. J., Mess, W. H., Hofman, P. A., & de Leeuw, P. W. (2005). Cerebral hyperperfusion syndrome. *Lancet Neurol, 4*(12), 877-888. 10.1016/S1474-4422(05)70251-9

Verbree, J., Bronzwaer, A., van Buchem, M. A., Daemen, M., van Lieshout, J. J., & van Osch, M. (2017). Middle cerebral artery diameter changes during rhythmic handgrip exercise in humans. *J Cereb Blood Flow Metab, 37*(8), 2921-2927. 10.1177/0271678X16679419

Verbree, J., Bronzwaer, A. S., Ghariq, E., Versluis, M. J., Daemen, M. J., van Buchem, M. A., . . . van Osch, M. J. (2014). Assessment of middle cerebral artery diameter during hypocapnia and hypercapnia in humans using ultra-high-field MRI. *J Appl Physiol (1985), 117*(10), 1084-1089. 10.1152/japplphysiol.00651.2014

Vrselja, Z., Brkic, H., Mrdenovic, S., Radic, R., & Curic, G. (2014). Function of circle of Willis. *J Cereb Blood Flow Metab, 34*(4), 578-584. 10.1038/jcbfm.2014.7

Wasserman, A. J., & Patterson, J. L., Jr. (1961). The cerebral vascular response to reduction in arterial carbon dioxide tension. *J Clin Invest, 40*(7), 1297-1303. 10.1172/JCI104359

Whittaker, J. R., Bright, M. G., Driver, I. D., Babic, A., Khot, S., & Murphy, K. (2017). Changes in arterial cerebral blood volume during lower body negative pressure measured with MRI. *NeuroImage* 10.1016/j.neuroimage.2017.06.041

Willekes, C., Hoogland, H. J., Keizer, H. A., Hoeks, A. P. G., & Reneman, R. S. (1999). Three months use of third-generation oral contraceptives does not affect artery wall properties. *Ultrasound in medicine & biology, 25*(5), 723-728. 10.1016/s0301-5629(99)00020-4

Williams, B. (1981). Simultaneous cerebral and spinal fluid pressure recordings. *Acta neurochirurgica, 59*(1-2), 123-142.

Willie, C. K., Colino, F. L., Bailey, D. M., Tzeng, Y. C., Binsted, G., Jones, L. W., . . . Ainslie, P. N. (2011). Utility of transcranial Doppler ultrasound for the integrative assessment of cerebrovascular function. *J Neurosci Methods, 196*(2), 221-237. 10.1016/j.jneumeth.2011.01.011

Willie, C. K., Macleod, D. B., Shaw, A. D., Smith, K. J., Tzeng, Y. C., Eves, N. D., . . . Ainslie, P. N. (2012). Regional brain blood flow in man during acute changes in arterial blood gases. *J Physiol, 590*(14), 3261-3275. 10.1113/jphysiol.2012.228551

Willie, C. K., Tzeng, Y. C., Fisher, J. A., & Ainslie, P. N. (2014). Integrative regulation of human brain blood flow. *J Physiol, 592*(5), 841-859. 10.1113/jphysiol.2013.268953

Zhang, R., Behbehani, K., & Levine, B. D. (2009). Dynamic pressure-flow relationship of the cerebral circulation during acute increase in arterial pressure. *J Physiol, 587*(Pt 11), 2567-2577. 10.1113/jphysiol.2008.168302

Zhang, R., Crandall, C. G., & Levine, B. D. (2004). Cerebral hemodynamics during the Valsalva maneuver: insights from ganglionic blockade. *Stroke, 35*(4), 843-847. 10.1161/01.STR.0000120309.84666.AE

Zhang, R., Zuckerman, J. H., Giller, C. A., & Levine, B. D. (1998). Transfer function analysis of dynamic cerebral autoregulation in humans. *Am J Physiol, 274*(1 Pt 2), H233-241. 10.1152/ajpheart.1998.274.1.H233

Zhang, R., Zuckerman, J. H., Iwasaki, K., Wilson, T. E., Crandall, C. G., & Levine, B. D. (2002). Autonomic neural control of dynamic cerebral autoregulation in humans. *Circulation, 106*(14), 1814-1820. 10.1161/01.CIR.0000031798.07790.FE

Zonta, M., & Carmignoto, G. (2002). Calcium oscillations encoding neuron-to-astrocyte communication. *J Physiol Paris, 96*(3-4), 193-198. https://doi.org/10.1016/S0928-4257(02)00006-2

Appendix

Appendix A: Ethics Approval Letter

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

Date: 11 February 2019

Dear Stephanie Korad

Re: Ethics Notification - **SOA 18/77** - **The effect of estrogen on cerebrovascular regulation in eumenorrheic women.**

Thank you for the above application that was considered by the Massey University Human Ethics Committee: **Human Ethics Southern A Committee** at their meeting held on **Monday, 11 February,**

Approval is for three years. If this project has not been completed within three years from the date of this letter, reapproval must be requested.

If the nature, content, location, procedures or personnel of your approved application change, please advise the Secretary of the Committee.

Yours sincerely

Professor Craig Johnson
Chair, Human Ethics Chairs' Committee and Director (Research Ethics)

Research Ethics Office, Research and Enterprise
Massey University, Private Bag 11 222, Palmerston North, 4442, New Zealand **T** 06 350 5573; 06 350 5575 **F** 06 355 7973
E humanethics@massey.ac.nz **W** http://humanethics.massey.ac.nz

Appendix B: Consent Form

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

PARTICIPANT CONSENT FORM

The effect of female sex hormones on cerebrovascular regulation in eumenorrheic women

I have read the participant information sheet for the above experiment and had the procedures, and potential risks explained to me by the researchers. I am satisfied that my concerns and questions have been addressed fully.

I understand that I have the right to withdraw my consent for being a participant at any time without giving reasons and without penalty.

I have read the information sheet describing this project, and I have no known medical or other condition which would exclude me from being a participant in this experiment.

I have been given one week to consider my involvement in the project.

☐ I agree to participate as an experimental participant.

☐ I understand that thereafter I can withdraw at any time without reason and without penalty.

☐ I would like to take the blood that is not used for the study

Declaration by Participant:

I _____ hereby consent to take part in this study.

Signature: _____ Date: _____

Te Kunenga ki Pūrehuroa
Massey University – School of Health Sciences College of Health
PO Box 756, Wellington 6140, New Zealand T 0800 MASSEY www.massey.ac.nz

Appendix C: Health Screening Questionnaire

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Health Screening Questionnaire Code:

Name: _____

Address: _____

Phone: _____

Age: _____

Please read the following questions carefully. If you have any difficulty, please advise the exercise specialist who is conducting the exercise test.

Please answer all of the following questions by ticking only **one** box for each question:

1. **Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?**
   Yes ☐ No ☐
2. **Do you feel pain in your chest when you do physical activity?**
   Yes ☐ No ☐
3. **In the past month, have you had chest pain when you were not doing physical activity?**
   Yes ☐ No ☐
4. **Do you lose your balance because of dizziness or do you ever lose consciousness?**
   Yes ☐ No ☐
5. **Do you have bone or joint problems (for example, back, knee, or hip) that could be made worse by a change in your physical activity?**
   Yes ☐ No ☐
6. **Are you currently taking any medication, prescription or over the counter?**
   Yes ☐ No ☐

If yes, please state which medication _____

7. **Do you have a history of cerebrovascular disease or injury (i.e. stroke, Transient ischaemic attack (TIA), Arterio-venous malformations (AVM))?**
   Yes ☐ No ☐

8. **Do you have any personal history of heart disease (coronary or atherosclerotic disease)?**

   Yes ☐ No ☐

9. **Have any immediate family members had heart problems prior to the age of 50?**

   Yes ☐ No ☐

10. **Are you a regular smoker or were you a regular smoker?**

    Yes ☐ No ☐

If you were a former smoker, when did you stop _____

11. **Are you diabetic? (Type 1 or Type 2)**

    Yes ☐ No ☐

12. **Have you been recently diagnosed with hypertension?**

    Yes ☐ No ☐

13. **Have you recently been diagnosed with vascular disease (i.e. atherosclerosis or peripheral arterial disease)?**

    Yes ☐ No ☐

14. **Have you recently been diagnosed with a heart murmur or arrhythmia?**

    Yes ☐ No ☐

15. **Have you been hospitalised recently?**

    Yes ☐ No ☐

16. **Are you or could you be pregnant?**

    Yes ☐ No ☐

17. **Do you use the oral contraceptive pill or the implant or intra uterine device?**

    Yes ☐ No ☐

18. **Have you experienced endometriosis in the past year?**

    Yes ☐ No ☐

19. **Have you been diagnosed with Polycystic Ovary Syndrome (PCOS)?**

    Yes ☐ No ☐

20. **Do you have HIV or Hepatitis B?**

    Yes ☐ No ☐

21. Do you suffer from Raynaud's disease?

Yes ☐ No ☐

This questionnaire has been designed to recognise the small population of individuals aged 16 - 69 where physical activity may not be appropriate. The questions included in this questionnaire are derived from the Physical Activity Readiness Questionnaire (British Columbia Dept. of Health (Canada)) revised by Thomas et al. (1992) and the American College of Sports Medicine (ACSM) health history questionnaire (ACSM, 2013).

The information provided by you on this form will be treated with the strictest confidentiality.

I have read, understood and completed this questionnaire accurately and completely.

Signature: _____ Date: _____

References

American College of Sports Medicine. (2013). *ACSM's guideline for exercise testing and prescription*. Lippincott Williams & Wilkins.

Thomas S, Reading J and Shephard RJ. Revision of the Physical Activity Readiness Questionnaire (PAR-Q). *Can J Sport Sci* 17(4): 338-345.

Appendix D: Information Sheet

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

COLLEGE OF HEALTH
TE KURA HAUORA TANGATA

Participant Information Sheet

Project Title: The effect of oestrogen on cerebrovascular regulation in eumenorrheic women

Researchers:

Stephanie Korad
Phone: [redacted]
Email: Stephanie.Korad.1@uni.massey.ac.nz

Sally Lark
Phone: (04) 801 5799 extn 63497
Email: S.Lark@Massey.ac.nz

Blake Perry
Phone: (04) 801 5799 extn 63492
Email: B.G.Perry@Massey.ac.nz

Toby Mundel
Phone: 06 356 9099 extn 84538
Email: T.Mundel@massey.ac.nz

You have been invited to participate in a study investigating the effect of female sex hormones on brain blood flow regulation in eumenorrheic women. You will be asked to visit the laboratory on four occasions and collectively will take ~8 hours of your time. We are seeking healthy women between 18 and 40 years of age.

The requirements are described below;

- Be a non-smoking female
- Be between 18 and 40 years of age
- Be free from cardiovascular (including vascular), neurological, respiratory and metabolic disease
- Be willing to complete 2 familiarisations and 3 experimental trials which will equate to approximately 8 hours total time
- Be healthy and have a regular menstrual cycle
- Not taking the oral contraceptive pill (if the participant has stopped taking the pill for 6+ months and has a regular menstrual cycle, then they may participate in the study) or the implant or intra uterine device.

Why are we doing this study?

Throughout the menstrual cycle, there are fluxes in female sex hormone levels and in response to the changing hormone levels of the menstrual cycle, vascular functions change, including vascular functions in the brain. Elevated female sex hormone levels are associated with a reduction in brain blood flow impedance and resistance of the cerebral vessels and therefore is linked to an increase in brain blood flow velocity. During day 1 – 5 of the menstrual cycle (early follicular phase) both oestrogen and progesterone hormone levels are at its lowest, during day 12 -16 (late follicular phase) oestrogen is at its peak whilst progesterone stays low, while both hormones are at their highest during days 20 – 25 (mid-luteal phase). During the high hormone phase of the menstrual cycle there was a greater increase in blood pressure (BP) during the Valsalva manoeuvre (breath hold), suggesting that there was a greater peripheral brain blood flow stimulation.

Currently, there have not been a lot of studies done looking at the effects of low and high women sex hormone levels on brain blood flow responses during dynamic blood pressure changes. Cycling female

sex hormones can influence brain blood flow, however, the isolated effects of female sex hormone on brain responses has yet to be demonstrated.

What is the aim of this study?

This study aims to investigate the potential regulatory role of female sex hormone in brain blood flow function by comparing responses during low and high internal hormone concentrations.

If I agree to take part, what will I be asked to do?

You will be asked to come into the laboratory four times, with an accumulated time cost of ~8 hours. Both sessions will take place at the Massey University, Wellington. Direction to the facility will be provided should you agree to participate. The first visit will serve as a familiarisation and will take ~1-hour long. During this time, you will be screened, informed of all procedures and familiarised with the equipment used for data collection. All potential risks will be explained to you. You will have had this Information sheet for at least one week, and we will answer any questions you may have about any of the experimental procedures. Prior to the first session, we will ask for your informed, written, consent. Once consent is given you will receive an ovulation kit, which you will use to collect information about your period and track it for two months. The next visit will be the second familiarisation session and during this session the information you collected will be examined and what phase of the menstrual cycle you are currently in will be noted. Once we know what phase you are in, appointments will be made for a day when you are in your early follicular stage, when you are in your late follicular stage, and finally when you are in your mid-luteal phase. This session will take ~2 hours, details of this session are explained below.

Outline

Upon arrival to the laboratory you will be seated in a chair and instrumented (see experimental measurements below). The first protocol you will do is the sit-to-stand manuouvre. This will require you to sit normally for 1 minute, then stand up for 1 minute. You will repeat this 3 times then have a rest before the next protocol. The next protocol is the Valsalva manoeuvre (breath hold), which requires you to blow into a tube attached to a pressure gauge and maintain a pressure of 40 mmHg for approximately 15s. This will only be done once for each experimental session. Once the Valsalva is completed, you will rest for 10 minutes to ensure that the physiological effects of the Valsalva will not affect the next procedure. The next procedure to be performed is the thigh cuff occlusion test. This test requires you to have your thighs occluded for 5 minutes, then the cuff will be released. The cuff will be inflated above resting blood pressure and may cause mild discomfort.. All three test induce changes in blood pressure and cerebral blood flow in different ways. How well these two variables track each other provides an indication of brain blood flow regulation. Once all the protocols have been completed, all equipment will be removed from you.

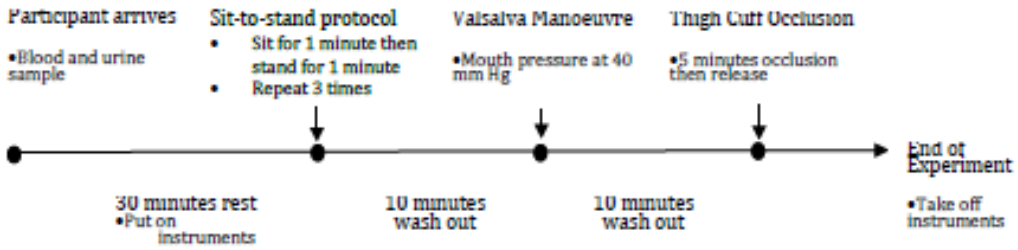

Figure 1 Protocol overview

Experimental Measurements

- *Blood pressure and heart rate* - All cardiovascular measures will be continuously recorded both during rest and during the trial. Heart rate will be measured using a three lead ECG with electrodes placed on each side of the collarbone and on the ribs on the left side. Blood pressure and cardiac output will be measure using a blood pressure cuff placed on the tip of the finger. This device gives accurate blood pressure and cardiac output information that will be constantly monitored during the trial.
-
- *Middle and posterior cerebral artery blood flow (velocity)* - The measurement of two cerebral arteries blood flow will be also continuously recorded throughout the experimental trial. This will involve the use of Doppler imaging which is a **non-invasive and non-transmitting** method of measuring blood flow. This equipment uses a small ultrasound wave that measures how fast the blood within a specific blood vessel is travelling. The Doppler probe will be fixed to the left side of the head using a head band which makes sure that blood flow from the same artery is measured. This technique allows dynamic changes in blood velocity to be tracked.

- *Expired gas composition* - The measurement of expired gas composition (concentration of oxygen and carbon dioxide) will be recorded continuously. This will simply require breathing through a mouthpiece. An online system will be used which will enable the analysis of each breath in real time.

- *Sex hormone levels* – The measurement of female sex hormones will be collected at the beginning of the session by doing a blood test, therefore blood samples will need to be collected. This will be done by a trained supervisor. The sample will be centrifuged to separate the serum from the blood, where the serum will provide information about estrogen and progesterone levels. The measurement of both sex hormones gives information about the phase on the menstrual cycle you are in.

- Ovulation Kit – An ovulation kit will be given to participants at the first familiarisation session to help the researchers identify what phase of the menstrual cycle that the participant is in, by measuring the luteinizing hormone that is in the urine. The ovulation kit used in this study will require participants to urinate on the stick provided. Colour bands will appear on the stick indicating what stage of menstruation the participant is currently in. It is best that the kit is taken around the same time each day to ensure accuracy. The day after the period ends will be day one of the cycle. The person should begin the ovulation kit on day 10 as the luteinizing hormone will begin to rise at that time.

What are the risks?

Fainting

It is unlikely, yet possible, that syncope may occur during standing (you could faint). Blood pressure and CBF will be continuously monitored throughout and after all sets. Signs of fainting (e.g., nausea, pallor, sudden decrease in heart rate and/or blood pressure, or a constant decrease in blood pressure) will be constantly monitored for an acute drop in blood pressure or if requested the trial will be terminated. If any symptoms are present the subject will be aided to a supine position until blood pressure and CBF have recovered. The symptoms of fainting and the likelihood of fainting should subside upon assuming a supine position. This will be achieved by the aid of the researchers.

Pain

The thigh cuff experiment may induce some pain when performed. You can request the procedure to stop and discontinued if the pain is too great to be tolerated. Researchers will be present and have the appropriate protocol in place in case the pain gets too much for you. You will be consistently monitored throughout the experiment and the researchers are highly knowledgeable about normal and abnormal values of the measurements taken, and therefore know when to stop experimental procedures. All researchers are trained in comprehensive first aid.

What are the benefits?

Your participation may possibly further increase the knowledge of the effects of female sex hormones on brain blood flow responses and provide a greater insight in the role that female sex hormones may play in brain health in older women.

What are my rights?

- You can ask questions on any aspect of the project at any time, and we will do our best to answer them to your satisfaction.
- As a participant in the study you will provide information on the understanding that your name will not be used unless you give permission to the researcher.
- You have the right to view your own data at any stage and have it explained to you.
- You will also be given access to a summary of the project findings when it is concluded.
- **You can withdraw from the project at any time, without giving any reason and without penalty.**

What about compensation for injury?

If physical injury results from your participation in this study, you should visit a treatment provider to make a claim to ACC as soon as possible. ACC cover and entitlements are not automatic and your claim will be assessed by ACC in accordance with the *Accident Compensation Act 2001*. If your claim is accepted, ACC must inform you of your entitlements, and must help you access those entitlements. Entitlements may include, but not be limited to, treatment costs, travel costs for rehabilitation, loss of earnings, and/or lump sum for permanent impairment. Compensation for mental trauma may also be included, but only if this is incurred as a result of physical injury. If your ACC claim is not accepted you should immediately contact Blake Perry. Blake Perry will initiate processes to ensure you receive compensation equivalent to that to which you would have been entitled had ACC accepted your claim.

Am I eligible?

Although voluntary, your participation will also be confirmed on criteria relating to health and safety. We are looking for healthy participants, 18-40 years of age.

- *Be Female with a regular menstrual cycle*
- *Not be on the oral contraceptive pill or any contraceptive methods*
- *Non-smoker*
- *Be free from cardiovascular (including vascular), neurological, respiratory and metabolic disease*
- *Be willing to complete 2 familiarisations and 3 experimental trials which will equate to approximately 8 hours total*
- *Have several non-invasive physiological measures taken*
- *Be willing to have blood collection taken*

For health/safety reasons, you should **not** participate if any of the following apply to you:

- *You have any known heart, cardiovascular (including vascular disease such as atherosclerosis of peripheral vascular disease) or cerebrovascular (i.e. stroke) condition or if a member of your family died below the age of fifty (50) as a result of a heart condition.*

- *You have a metabolic disease (i.e diabetes)*
- *You have a musculoskeletal injury that is aggravated by physical exertion*
- *You have ever had an injury or any medical condition that you think may affect your ability to sense pain or discomfort.*
- *You are taking prescribed medication including the oral contraception pill.*
- *You are a regular smoker*
- *You have cultural or religious sensitivities about human body measurements.*
- *You have any other reason to consider that you are not in good health.*
- *You are hyper- or hypotensive – including postural hypotension and have fainted recently*
- *You have sensitive/irritable skin or a skin disease (infectious or non-infectious).*
- *You are currently taking, or within the past week have taken, any over-the-counter drugs or nutrition supplements containing ephedrine, synephrine or other sympathomimetic compounds.*
- *You have, or have had within the last 2 weeks, a viral or bacterial illness.*
- *You have history of endometriosis and poly cystic ovary syndrome (PCOS)*

Anything else I need to know?

You will be asked to wear suitable clothing that you feel comfortable in. Showers are available should you need them. Also prior to participation you need to;

- Refrain from alcohol for 24 hours prior to all trials
- Complete trials in a fasted state. No food for 6 hours prior to the trail.
- No alcohol or caffeine for 12 hours prior to all trials

All data obtained from this study will be kept strictly confidential. Data will be identified as a code only. Results will be made available to you at the completion of the study. In the event that an abnormality is identified in the data you will be referred to your general practitioner with the relevant information.

If you are interested in taking part:

Contact: Stephanie Korad
Postgraduate Student
Massey University, Wellington Campus
Wellington, New Zealand
Email: Stephanie.Korad.1@uni.massey.ac.nz
Phone : [redacted]

This project has been reviewed and approved by the Massey University Human Ethics Committee: Southern A, Application SOA 18/77. If you have any concerns about the conduct of this research, please contact Dr Lesley Batten, Chair, Massey University Human Ethics Committee: Southern A, telephone +64 63569099 x 85094, email humanethicsoutha@massey.ac.nz .