TEST-RETEST RELIABILITY OF CEREBROVASCULAR MEASURES DURING THE ORAL CONTRACEPTIVE CYCLE

Nathan T. Adams

A thesis submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Master of Arts in the Department of Exercise and Sport Science (Exercise Physiology) in the College of Arts & Sciences.

Chapel Hill
2022

Approved by:

Adam Kiefer

Anthony C. Hackney

Michelle L. Meyer

Lee Stoner

ABSTRACT

Nathan T. Adams: Test-retest Reliability of Cerebrovascular Measures during the Menstrual Cycle in Women Using Oral Contraceptives
(Under the direction of Lee Stoner)

In vascular testing, women who use oral contraceptives are tested in the placebo phase to control for potential confounding variables. This methodological decision may not be necessary if cerebrovascular measurements are reliable between days in women using oral contraceptives. We measured the reliability of middle cerebral artery blood flow velocity, heart-middle cerebral artery pulse wave velocity, and two cognitive tasks in 13 women, and 5 men on three days within an oral contraceptive cycle (21.49 ± 3.21 years, 72.2% female, 23.78 ± 2.53 kg/m^2). Women had better reliability for main outcomes of middle cerebral artery blood flow velocity (ICC: 0.718 v. 0.575) and heart-middle cerebral artery pulse wave velocity (ICC: 0.929 v. 0.844). These results suggest that women using oral contraceptives have similar reliability to men and they could be tested in any cycle phase during future vascular studies.

ACKNOWLEDGEMENTS

Thank you to Dr. Lee Stoner for allowing me to make mistakes, anticipating support, and allowing me to take the biggest step in my entire life and career. Thank you for allowing a small kid from a small school to come do big science. Thank you as well for my UNC committee and faculty, and my family from Pacific Lutheran University who have helped me prepare for this project before I knew it was coming.

To the CML team, thank you for so quickly taking me in and mentoring me in almost every way possible. Gabe, Patricia, Lauren, and Alex and now Simon and Craig; you all are mentors to me in more ways that I know and I am so happy to have followed you and soaked up your hearts for people and health.

Thank you to Kyle, Amanda, and the cohort team. Activities, buds, bumpin' around in the back office and beating undergrads in flag football. We may have to move away from each other, but we will always have those IM highlights. Kyle, I've never had a true roommate until these two years. Thank you for pushing me physically, sending me tweets even if I've already seen them and being one of the kindest and most patient people I've met. I value our friendship from these two years incredibly highly and I wish the best for you always.

Lastly, thank you to my family. Every car conversation on the way back from baseball practices, music lessons, letting me fail and instilling me a personal work ethic. This is a project 23 years in the making. Thank you for your guidance and the persistence that I learned at home.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AFT	Action Fluency Test
BP	Blood Pressure
BRS	Baroreflex Sensitivity
CA	Cerebral Autoregulation
CPP	Cerebral Perfusion Pressure
CVR	Cerebrovascular Resistance
CVRi	Cerebrovascular Resistance Index
DBP	Diastolic Blood Pressure
DCCS	Dimensional Card Change Sort Test
ECG	Electrocardiogram
ET	Eye-tracking
$ETCO_2$	End-Tidal Carbon Dioxide
fNIRS	Function Near-Infrared Spectroscopy
FT	Flanker Task
Hb	Hemoglobin
hmPWV	Heart-Middle Cerebral Artery Pulse Wave Velocity
HR	Heart Rate
HRT	Hormone Replacement Therapy
HUTT	Head Up Tilt Test
ICA	Internal Carotid Artery
ICC	Intra-class Correlation
LBNP	Lower Body Negative Pressure

MAP	Mean Arterial Pressure
MCA	Middle Cerebral Artery
MCA_V	Middle Cerebral Artery Blood Flow Velocity
MDC	Minimal Detectable Change
MRI	Magnetic Resonance Imaging
NIRS	Near-Infrared Spectroscopy
NVC	Neurovascular Coupling
OC	Oral Contraceptive
PET	Positron Emission Tomography
PWV	Pulse Wave Velocity
SBP	Systolic Blood Pressure
SEM	Standard Error of Measurement
SNS	Sympathetic Nervous System
TCD	Transcranial Doppler
TMT	Trail Making Test
VFT	Verbal Fluency Test
ΔHb	Change in Hemoglobin

CHAPTER 1: OVERVIEW

THESIS STRUCTURE

This thesis includes 6 chapters. **Chapter 1** provides general background and a rationale for this thesis. **Chapter 2** is a literature review which discusses the significance of the project and background of all measurements. **Chapter 3** is an additional literature review discussing each aspect of study design. **Chapter 4** includes the study methodology. **Chapter 5** includes the results, and **Chapter 6** is a discussion of the results including a comparison to prior literature and important implications of the findings from this study.

KEY TERMINOLOGY

Table 1. Key terminology important for understanding this study.

Term	Definition
Blood Flow Velocity	The velocity at which blood moves through a section of an artery. Within this study, the artery is most commonly the Middle Cerebral Artery. Measured in units cm/s.
Cerebral Blood Flow	General term relating to the delivery of blood flow to the brain and it's associated regulating mechanisms. The most important cerebral blood flow measurement in this study is Middle Cerebral Artery Blood Flow Velocity.
Cognitive Function	The ability to focus, shift, and sustain attention in the organization of top-down reasoning in the action of completing a goal-oriented task.
Exogeneous Hormones	Whereas endogenous hormones represent the naturally cycling amounts of substances produced from within a body, exogenous hormones represent similar substances purposefully administered to a person for some pharmacological purpose. In this study, the exogenous hormones and methods of replicating estrogen and progesterone.
Oral Contraceptives	Pharmacological intervention used as a contraceptive, or as a method of regulating the menstrual cycle and bleeding period if women's health.
Oral Contraceptive Cycle	A full cycle of a course of oral contraceptives (inactive, early active, and late active phases). In this protocol, this cycle consists of 28 days with 21 active days and 7 inactive days.
Reliability	The degree to which measurements provide similar results when measured in standardized resting conditions.

THESIS RATIONALE

Early studies measuring cerebrovascular health either excluded women or biological sex was not mentioned.[1–3] In current studies, women using oral contraceptives (OC) are tested only in the placebo phases in order to limit the potential variability of vascular outcomes caused by exogenous ovarian hormones. This methodological decision is made based on the assumption that estrogen receptors in the brain could promote vasodilation and impact the reliability of cerebrovascular measurements such as middle cerebral artery blood flow velocity (MCA_V) and heart – middle cerebral artery pulse wave velocity (hmPWV), however there is conflicting literature on the reliability and changes in cerebrovascular measurements between days in women taking OCs.[4–6] Testing women only during the inactive phase limits our understanding of what may be occurring in women using OCs, and if women have similar reliability of cerebrovascular measurements to men, this process may not be necessary.

OVERALL OBJECTIVE AND APPROACH

The overall goal of this research was to confirm or challenge the assumption that exists behind the decision to test women taking OCs in the inactive phase in vascular research. To test this assumption, a randomized, two-group repeated measure reliability study design was conducted with ratings measured by one rater. A cohort of 20 men, and 20 women using OCs were recruited and tested on three occasions corresponding with OC cycle phase timelines. Women were tested during their inactive pill phase, and twice during the active pill phase. While men do not have fluctuation of ovarian hormones or take oral contraceptives, they were also tested on three occasions in a similar time frame. The intraclass correlation coefficient (ICC) for each outcome was compared between sex groups, with good reliability set as a value greater than 0.750.[7] **Aim 1** established the between-day reliability of cerebrovascular measurements. MCA_V

and heart-MCA pulse wave velocity (hmPWV) were observed in three resting postures during each experimental visit, and the overall averages of each day were used to calculate reliability. **Aim 2** evaluated the reliability of cognitive function tasks. Cerebral blood flow increases during with increased cognitive demand and MCA_V has been connected to cognitive diseases such as Alzheimer's and related dementias.[8–12] At the end of each experimental visit, two cognitive test were administered: the Flanker Task (FT) and the Trail-Making Test type B (TMT). To evaluate cognitive load, task activated changes in MCA_V known as neurovascular coupling (NVC) and pupil diameter were evaluated using eye-tracking (ET) during these two tests. Completing these aims may inform if women can be tested at any OC cycle phase in future studies.

INNOVATION AND SIGNIFICANCE

While exogenous estrogen likely varied in women between the two visits in the active phase and the placebo phase, acceptable reliability of MCA_V and hmPWV in both sex groups (ICC > 0.75) was expected. MCA_V does not have excellent reliability (ICC > 0.900) in most populations and the mechanisms for between day reliability and successful repeated measurements are not totally understood.[3,13] The young, healthy population in this study would be expected to have healthy arteries leading to consistent MCA_V and hmPWV between days. Additionally, cognitive tasks were expected to be reliable between visits as any noted variability would more attributable to learning effects than acute changes in exogenous ovarian hormones. If reliability of these measures is comparable between the group of women using OCs and the group of men, this would challenge the assumption that is given for testing women in the inactive phase of their OC cycle. Establishing reliability will improve the precision of measurements so that the physiology of women can be better understood, and future research can precisely determine when and how women can be included in future studies.

ASSUMPTIONS, DELIMITATIONS & LIMITATIONS

ASSUMPTIONS

1. Subjects followed pre-assessment guidelines as confirmed at the beginning of each study visit.
2. Pre-assessment guidelines were sufficient for controlling baseline changes in outcomes.
3. All subjects answered medical history questionnaire truthfully.
4. Female participants were in the cycle phase of their OC that they attested to in assessment screening and scheduling.

DELIMITATIONS

1. Randomization of the OC cycle phase for first visit was sufficient for counteracting the potential learning effect of the cognitive function tests.
2. Reliability evaluated in multiple postures has greater application to future study protocols compared to if MCA_V was only measured in the supine posture.

LIMITATIONS

1. There may have been a learning effect for tests of cognitive function between visits.
2. While blood samples were collected with the plan of testing for estrogen in each participant, an ELIZA kit was not purchased in time and therefore the hormonal profile and OC cycle phase of each participant was only confirmed by participant responses to screening and scheduling questionnaires.
3. Some measurements were inherently linked by multi-collinearities and therefore they may have trended towards or away from acceptable reliability in tandem.

CHAPTER 2: LITERATURE REVIEW - SIGNIFICANCE

INTRODUCTION TO TOPIC AND SIGNIFICANCE

Since the introduction of OCs in the 1960s, as many as 200 million women have been estimated to have used OCs at some point which makes them one of the most prevalent forms of pharmaceuticals taken globally.[14] OCs include exogenous ovarian hormones—progestins and synthetic estrogens—that may cause small vascular changes such has vasodilation, however the extent of these changes and the reliability of physiological outcomes in women using OCs has not been fully explored.[4] This is especially true in cerebrovascular measurements. There is limited literature evaluating cerebral health and function related to OCs, and between-phase reliability has not been measured. Cerebral health and blood flow to the brain is one of the most important physiological processes as disruptions in this process can result in events such as ischemic stroke, where brain tissue is lost rapidly and irreversibly,[15] or more drastic outcomes such as mortality (Figure 1). Therefore, mechanisms that maintain cerebral blood flow are very important to

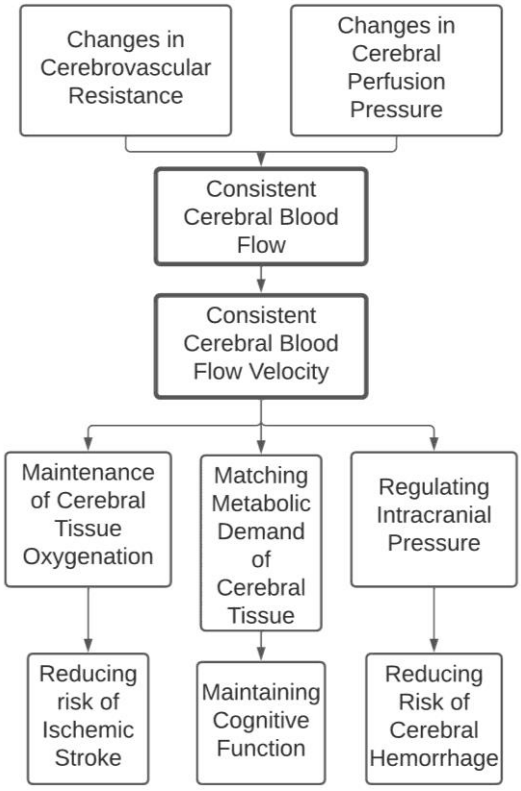

Figure 1. A conceptual model highlighting the important outcomes of maintaining middle cerebral artery blood flow reliability, one of the primary outcomes of this study.

understand. This project was proposed to establish the reliability of cerebral health and function measurements between OC phases.

This initial literature review sought to identify knowledge gaps and hypothesize the mechanisms that may influence the reliability of cerebrovascular measurements in women taking OCs (Figure 2). We planned to observe women who had changes in exogenous ovarian hormones due to their usage of OCs. If MCA_V were to be found unreliable, we expected that this would be caused by changes in blood pressure and arterial stiffness when estrogen reacts with the estrogen receptors within the vasculature of any individual person. However, we expected that in young, healthy adults that the regulatory homeostatic mechanisms which stabilize vascular parameters would be able to respond to changes in estrogen. Therefore, we expect acceptable reliability of cerebrovascular and cognitive function measurements.

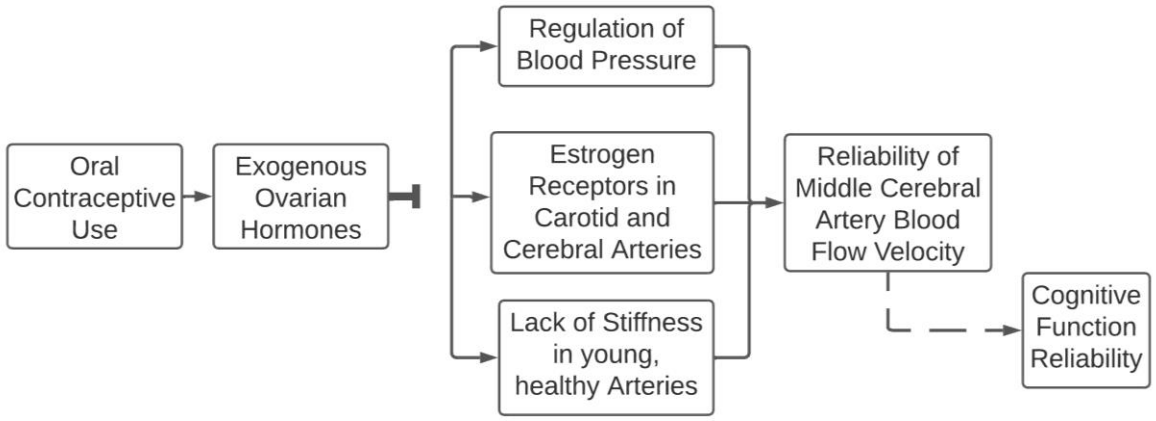

Figure 2. A proposed mechanism for the impacts of oral contraceptives on middle cerebral artery blood flow velocity and cognitive function reliability between cycle phases.

To understand the decisions made for this protocol and comprehend why study decisions were made, certain background information must be introduced. This literature review describes how endogenous hormones have been found to impact cardiovascular and cerebrovascular

measurements. Those outcomes will then be discussed with the effects of exogenous hormones. The following table of contents is a succinct description of the included topics (Table 2).

Table 2. Literature Review Contents.

Consideration	Why Important	Page
Definitions	Define key concepts relevant to this proposal	7-8
Review of Oral Contraceptives	Understanding ovarian hormones and the developments of oral contraceptives	8-11
Oral Contraceptives and Cerebrovascular Function	Connecting oral contraceptives to measurements of cerebrovascular function	11-13
Ovarian Hormones and Cognitive Function	Connecting oral contraceptives to measurements of cognitive function	13-16
Summary	Summary of why this study is needed, what is known, what is not known, and what gap was identified for this study to fill.	16-17

DEFINITIONS

The first definition crucial to understanding this protocol is the construct of reliability. Reliability on a broad scale has been defined as the ability of a measurement to be replicated, and it is a combination of consistency and agreement.[7] Reliability in this study referred to the similarity in measurements in individual participants between the three experimental visits. For example, acceptable overall agreement would suggest that each participant had similar measurement values during each study visit, regardless of their OC cycle phase.

A second clarification is the difference between OC and the natural menstrual cycle. As has been discussed in previous reviews, the menstrual cycle and OC cycle are separate physiological processes and language between those two processes should not be interchangeably used.[1] Therefore, within this project the only terms used were OC cycle (instead of menstrual cycle), and placebo phase, and active phase. While cisgender men lack a menstrual cycle and do not take OCs, a simulated cycle was used to schedule men at time points in a similar manner to women. This was done to ensure men were measured with similar number of

days between visits, and their randomization resulted in similar scheduling goal dates for each of the three visits.

The primary measurement of cerebral health and function in this study was MCA_V. This measurement was characterized by the velocity of blood traveling through the MCA reported in cm/s, and this was measured with a transcranial Doppler (TCD). The MCA is an artery within the Circle of Willis, and it is responsible for supporting most cerebral tissue oxygenation as most cerebral blood flow. Increases in MCA_V have been associated with a higher metabolic demand of cerebral tissue which will be measured in attempt to observe the NVC coupling phenomenon, and MCA_V changes to moderate intracranial pressure.

Cognitive function is regularly defined by a broad range of domains related to executing goal-oriented activities, maintaining attention, switching between points of focus, and responding quickly to visual or other types of stimuli. There is not one measurement and unit available for comparing overall cognitive function between cognitive domains or cognitive tests. Within this study, cognitive tests were selected for their utility and use as evaluated by scientific organizations (NIH) and novelty of tests with their connection to latent measurements of cognitive function like ET.

THE MENSTRUAL CYCLE AND THE ORAL CONTRACEPTIVE CYCLE

With the increased inclusion of women in physiological research, there is greater understanding of the biphasic response in physiological systems caused by changes in ovarian hormones. This can cause variability between sexes and limit reliability, however there are not consistently robust studies available to confirm or challenge this assumption in every measurement. In eumenorrheic women, the two naturally cycling types of hormones are estrogens (17β-estradiol and estrone) and progesterone which normally repeat a fluctuation

known as the menstrual cycle. These hormones can be replicated in exogenous form and some of the same physiological responses caused by endogenous ovarian hormones can be observed in women taking OCs.

Recent estimates show that at one time, 12.6% of American women of reproductive age are using OCs, and 85% of women in the United States have used an OC at some point for a significant amount of time.[14,16] Modern hormonal contraceptives are either just the hormone progestin alone, or combine a progestin with estrogens which is considered a combined OC. Combined OCs with both ovarian hormones present are more commonly used and investigated in scientific literature.[17,18] This study only included women using combined OCs. As replacement for endogenous estrogens appearing in eumenorrheic women, most OCs include either ethinyl estradiol or estradiol, and recently natural estrogens such as estradiol valerate are being introduced in some OCs.[19] There is a wide variety of progestins that have been introduced as developments of androgenic or antiandrogenic activity have been advanced.[19] Progestins are classified by generation, in order of when they have been made available to be used in OCs.

In addition to the content and dosage, OC prescriptions vary in length and frequency of when estrogen and progestins are taken and when an inactive tablet is taken. As OCs were developed, the initial regimen was a 21 day active phase with a 7 day inactive phase because the manufacturers would only develop OCs similar to the natural patterns of menstruation.[20] Since then, extended-cycle regimes have been introduced and regularly used including an 84/7 or a 120/4 ratio of active pill days to inactive days.[19,21] As it is the most common and generalizable prescription, women using a 28-day OC cycle were included in this study.

The most conventional form of OC is the monophasic OC, wherein there is a static composition of hormones across the active phase of the pill series, and there are other

prescriptions such as multiphasic and triphasic.[22] Multiphasic OCs include mostly combined tablets, as well as a few estrogen only tablets, then the inactive pills. Triphasic OCs contain a static estrogen dosage, and the progestins dosage increases every week over the 21 active days. This study primarily included women who were taking monophasic OCs which allowed for the most robust comparison and strong generalizability. Many studies discussing OCs and vascular changes focus primarily on monophasic prescriptions.[23]

ENDOGENOUS OVARIAN HORMONES AND CEREBRAL BLOOD FLOW

While this project was interested in the effects of exogenous hormones on cerebrovascular measurements, understanding natural ovarian hormones and cerebral health can be helpful in predicting what may be observed within this study (Tabl**e 3).**

Table 3. Previous findings of cerebrovascular reliability as impacted by endogenous ovarian hormones.

Author and year	Research Question and Aim	Study Design	Findings
Peltonen et al. 2016[24]	Determine cerebrovascular responses in different parts of the menstrual cycle.	Young, healthy women (n = 11) tested during days 1-5 and 12-16 of the cycle.	Greater cerebral vasodilation was observed in the late follicular (high hormone) phase.
Krejza et al. 2013[25]	Comparison of cerebrovascular reactivity across phases of the natural menstrual cycle in response to ACE injection.	Young, healthy, eumenorrheic women (n = 19) were examined on days 5, 13, and 26 of the menstrual cycle.	Using common carotid artery blood flow as a proxy for cerebral vasodilation, increases in common carotid artery blood flow were positively associated with estrogen and progesterone.
Krejza et al. 2001[26]	Comparison of common carotid artery blood flow velocity and cross-sectional area in different phases of the menstrual cycle.	Young, healthy, nulliparous women (n = 14) were observed at least 11 times during a single menstrual cycle.	Common carotid artery blood flow velocity significantly changes during the menstrual cycle, with the highest velocities matching high levels of estrogen on day 14 of the cycle (end of follicular phase).
Krejza et al. 2003[27]	Resistance index of cerebrovascular arteries was compared at multiple time points of the menstrual cycle.	Young, healthy, eumenorrheic women (n = 17) were tested on at least 12 days during a single menstrual cycle.	Resistance of cerebrovasculature is likely negatively associated with estrogen, which results in a decreased cerebrovascular impedance and resistance index.

These studies evaluated cerebral health in similar sample populations and with similar measurements to the current study. Endogenous hormones and exogenous hormones from OCs or hormone replacement therapy (HRT) are not consistently interchangeable, however the results of studies evaluating the menstrual cycle are a good first step to understanding how OCs may impact cerebral health. Generally, all these included studies observed changes in cerebrovascular measurements that could be connected to different menstrual cycle phases or differences in the endogenous ovarian hormone profile.

EXOGENOUS OVARIAN HORMONES AND CARDIOVASCULAR MEASUREMENTS

Before exploring cerebrovascular mechanisms, the general effects of OCs on the general cardiovascular system must be understood. As the cerebral arteries are inseparably connected to the central cardiovascular system, cardiovascular changes can be connected to cerebrovascular changes.

Key Message

Changes in exogenous hormones impact peripheral vasculature and may change cerebral blood flow and cerebrovascular resistance during oral contraceptive phases.

Endogenous estrogen binds to cellular estrogen receptors and has numerous effects including lowering blood pressure (BP),[28] increasing vascular smooth muscle remodeling,[29] protecting against endothelial damage,[30] and increasing synthesis of nitric oxide.[31,32] Compared to age-matched men, pre-menopausal women have lower prevalence of cardiovascular disease, an advantage that significantly decreases upon menopause which suggests that endogenous ovarian hormones have cardioprotective effects.[31,33–35] It is well accepted that changes of estrogen and progesterone within a single menstrual cycle as well as over a lifetime have associated changes in cardiovascular function.

Surprisingly, exogenous hormones do not have the same protective effects. Two large studies evaluating the effects of HRT in post-menopausal women found that estrogens did not mitigate risk of cardiovascular diseases.[36,37] Of relevance to this project are the effects of OC on cardiovascular measurements within one OC cycle phase. In groups similar to our desired sample population, the active phase of OC cycles were found to have lower sympathetic baroreflex sensitivity (BRS), lower mean arterial pressure (MAP), lower diastolic blood pressure (DBP),[38] and a higher resting heart rate (HR).[39] Arterial stiffness in multiple studies was found to be unchanged between active and inactive phases of OC cycles, however this may not apply to the carotid and cerebral stiffness measurements in this study.[23,39] Estrogen receptors are known to suppress activation of the sympathetic nervous system (SNS) and reduce BP, and these cardiovascular changes were assessed and further evaluated in a parallel project (JP).

EXOGENOUS OVARIAN HORMONES AND CEREBROVASCULAR MEASUREMENTS

While cerebral health and function measurement reliability has been explored in eumenorrheic women, this study is interested in the changes to cerebral reliability and specifically MCA_V due to changes in *exogenous* ovarian hormones. While not the same as OCs, ovarian stimulation during in vitro fertilization has been connected to increased blood flow to the brain through both the internal carotid artery (ICA) and MCA during high hormone phases.[40] Another study evaluated regional blood flow in the cortical region of the brain after showing two groups of women faces, an action that activates the cortical region and greater increases in cerebral blood flow were observed during high hormone phases.[41] While different outcomes and variables were observed, high exogenous ovarian hormones were associated with increased cerebral blood flow in these studies.

To our knowledge there have been two previous studies that have evaluated changes in MCA_V during different phases of the OC cycle, and between women and men (Table 4).[5, 6] These two studies were performed by the same group and included two interventions for measuring change in MCA_V, the Valsalva maneuver and deep breathing. The construct of reliability was not calculated in these studies, however inferences can be made from the results of these studies. These two studies had conflicting results about the impact of OC cycle phase within the female participants, but both found sex differences in the response to stimuli. This suggests that there are differences in MCA_V between women using OCs and men, but it is unclear if MCA_V has reliability in women using OCs.

Table 4. Previous findings of MCAV changes in women taking OCs.

Author and year	Research Question and Aim	Study Design	Findings
Abidi et al. 2017[5]	Comparing cerebrovascular resistance and cerebral blood flow in response to the Valsalva maneuver or standing posture in men and in different phases of women taking OCs.	Men (n = 13), women not taking OCs (n = 12), and women taking OCs (n = 14) with the two groups of women each being measured during their respective low- and high-hormone phases.	There were sex differences in the MCA_V response to the Valsalva maneuver. Placebo phase OC users had consistently higher MCA_V in all postures.
Nili et al. 2017[6]	Evaluating between sex and between OC phase cerebrovascular responses to deep breathing.	Men (n = 13), women not taking OCs (n = 12), and women taking OCs (n = 14) with the two groups of women each being measured during their respective low- and high-hormone phases.	There were sex differences in MCA_V response to deep breathing. There was no effect of OC cycle on MCA_V.

GAP IN KNOWLEDGE: RELIABILITY OF CEREBRAL HEALTH BETWEEN ORAL CONTRACEPTIVE CYCLE PHASES

There are very few studies evaluating reliability of MCA_V, and no current studies evaluating reliability within women using OCs. Of the two studies that measured cerebral blood flow, there were conflicting findings about the reliability of MCA_V across OC cycle phases. It is still unknown if there is acceptable reliability of cerebral health outcomes between OC cycle phases.

OVARIAN HORMONES AND COGNITIVE FUNCTION

In multiple studies, cognitive function has been directly related to cerebral blood flow as there is an increased demand for oxygen in cerebral tissue during cognitive activation.[8–10] This response known as "neurovascular coupling" can be measured by administering cognitive function assessments and measuring changes in MCA_V.[42] Cognitive function is important to observe as general declines in cognitive function during life have been related to risk of dementia and Alzheimer's disease.[43] There have been significant sex differences observed between men and women in the risk for dementia,[44–47] Alzheimer's disease,[45,46,48] as well as in baseline cognitive function and cognitive decline over time. All of these long term outcomes highlight the importance of exploring the mechanisms of sex differences in cognition.[49] Despite the wide use of OC over recent history and within the western world, there is little known about the effects of OC on various domains of cognitive function. Cognitive reliability between OC phases has not been established. Cognitive function is an outcome proposed to fluctuate with changes in cerebral health and cerebral blood flow as outlined in the proposed mechanism (Figure 2).

Cognitive function can be evaluated using tests representing different domains. Previous studies have evaluated menstrual cycle phase and a range of cognitive domains and found only significant differences in memory, but not in verbal fluency, visuospatial competency, and attention.[50] While repeating the attention domain, the current study sought to establish reliability of the domains of processing speed, switching, and inhibition of cognitive function using the FT and TMT. These specific cognitive tests have not been explored in women taking OCs, however they have previously been evaluated in eumenorrheic women (Table 5).

The cognitive domains of processing speed,[54] attention,[55] switching,[56] and inhibition[56] are important to measure as they all have been correlated with cognitive decline. In the selected

young, healthy population, it is unlikely that large significant differences would be observed in cognitive domains like those associated with significant cognitive decline. However, small differences between sexes and OC phases could suggest development of cognitive diseases with long term exposure to OC usage.

Table 5. Previous findings of cognitive tests in women of different cycle phases of hormone profiles.

Author and year	Research Question and Aim	Study Design	Findings
Flanker Task			
Wang et al. 2020[51]	Menstrual cycle reliability of attentional tasks of cognitive function.	Young, healthy women (n = 34) completed a modified Flanker Task and the Attentional network test during menses, the follicular phase, and the luteal phase.	Response times of the two cognitive tests were slower but more accurate in the luteal phase. High progesterone is associated with greater allocation of attention.
Upadhayay et al. 2014[52]	Pilot comparison of cognitive tests in men on one occasion, and women during the preovulatory and postovulatory phases.	Young, healthy, men (n = 21) and eumenorrheic women (n = 21) were examined given the traditional Flanker Task.	Women tested during the luteal phase of the menstrual cycle performed better on tests of attention as well as in other cognitive domains.
Trail-Making Test Type B			
Lokken et al. 2006[53]	Cognitive comparison of young women, post-menopausal women, and post-menopausal women having received HRT.	Four groups of women (n = 48) completed a battery of cognitive tests including the Trail-Making Test part B.	In post-menopausal women, Trail-Making Test part B results were significantly faster in women who had received HRT.

A modified version of the FT using faces has been explored in women during different phases of the natural menstrual cycle.[51] This study had many limitations in application to the current protocol. The modified FT evaluated faces instead of arrows, and therefore the emotional attention domain was more responsible for outcomes rather than the processing speed domain of the traditional FT. However, significant differences in attention were found with improved performance being linked to high levels of progesterone as sampled in saliva. This would suggest that higher ovarian hormone levels may be connected to improved attention. Another study evaluated the traditional FT in men and in women during the follicular and luteal phases of their

natural menstrual cycle.[52] Women in the follicular phase and men had similar cognitive results for the FT, however women in the post-ovulatory or luteal phase had improved results on the attentional task. This is a second piece of evidence connecting the high ovarian hormone phase to improved cognitive function, however it is still unclear if exogenous ovarian hormones impact FT performance.

The TMT was evaluated in four groups of women with various menstrual characteristics.[53] The four groups were young (18-22) and older (35-48) pre-menopausal women, and then post-menopausal women who had not taken exogenous replacement estrogen, and those who did in the form of HRT. The younger groups both completed the TMT during the follicular phase. The post-menopausal groups can be relatively compared to younger women who are and who are not taking OCs. Within those two groups, the women who had been receiving HRT performed significantly better at the TMT. This suggest that there may be cognitive (processing speed, switching) benefits of exogenous ovarian hormones.

GAP IN KNOWLEDGE: RELIABILITY OF COGNITIVE FUNCTION BETWEEN ORAL CONTRACEPTIVE CYCLE PHASES

It is not known how OCs affect most domains of cognitive function. This study was the first to explore these cognitive tests across the OC cycle, as well as using eye-tracking (ET) and measuring NVC.

SUMMARY

A review of the contents of the literature section can be found in **Table 6**.

Table 6. Literature Review Summary.

Consideration	Summary
Definitions	Key concepts were described at the beginning of this proposal.
Review of Oral Contraceptives	Oral contraceptives have known effect on vascular profile and could impact the reliability of cerebrovascular measurements.
Oral Contraceptives and Cerebrovascular Function	While our proposed mechanism addresses the potential for changes in resting cerebrovascular reliability to exist, this mechanism has not yet been measured or tested. This section reviewed the two studies to date that have measured reliability of cerebrovascular measurements in some form across the oral contraceptive cycle.
Oral Contraceptives and Cognitive Function	Some studies have measured between cycle changes in the response to cognitive tests in eumenorrheic women and in women taking oral contraceptives, although this is the first study to measure these domains of cognitive function in women taking oral contraceptives.
Measurement Considerations	Considerations of all measurements were described in this section.

WHY IS THIS STUDY NEEDED?

With OCs not having been part of previous studies, reliability of cerebral health and function measurements is unknown. There is a need to establish the reliability of cerebrovascular measurements across the OC cycle to determine the if future studies should test women only in the placebo phase.

WHAT IS KNOWN

It is known that ovarian sex hormones impact vascular function and standard fluctuation during the OC cycle causes central and peripheral changes in cardiovascular measurements. It is known that in healthy adults, cerebral health and proper cerebral blood flow is crucial for maintaining consciousness, cognitive function, and brain health. Changes in central cardiovascular health are also known to be moderate changes in cerebrovascular tone to maintain consistent blood flow to the brain at rest, and increased oxygenation representing blood flow is delivered to active regions of the brain during cognitive activation.

WHAT IS NOT KNOWN

The reliability of cerebral health and overall cognitive function reliability in women using OCs are unknown. There are few studies evaluating reliability of these measurements across the cycle in women using OCs.

CRITICAL NEED

This study is needed to determine the reliability and changes of cerebral health and function parameters during different phases of the OC cycle. To our knowledge, this was the first study assessing the reliability of these cerebrovascular measurements in women taking OCs. The findings of this study will challenge or confirm the methodological need to control for the OC cycle.

CHAPTER 3: LITERATURE REVIEW – RATIONALE FOR APPROACH

METHODOLOGICAL AND RIGOR CONSIDERATIONS

To clearly identify how phases of the menstrual cycle affect cerebrovascular health, internal and external validity need to be considered. The goal of this section of the literature review is to discuss the rationale for each decision made in designing this study. All cerebral and cognitive measurements will be discussed including their importance and connection to the proposed mechanism. The final parts of this review will be the methodological considerations including key study design peices, statistical decisions, and other decisions to improve scientific rigor.

STUDY DESIGN CONSIDERATIONS

To best detect the changes that may be present in cerebrovascular measurements between OC cycle phases, a reliability study design was prepared. Specifically, a two-group repeated measures observational study. Two-groups were necessary to be able to compare the reliability between the group of men and the group of women using OCs. One outcome may not reach good reliability, but if the reliability is similar between the sex groups this would suggest comparable reliability. This design aimed to control conditions between visits except for the changes in OC cycle phase to increase the likelihood that changes in measurement reliability can be associated with changes in levels of exogenous hormones from the presence of OCs. A full list of study design considerations can be found in **Table 7.**

While a double-blind randomized study design may have been the strongest scientific procedure, there are ethical drawbacks to blinding women to their reproductive health situation.

Therefore, the choice was made to include women who were previously taking OC as the primary experimental cohort. A control group of men was selected to be able to attribute the differences in cerebrovascular measurement reliability to changes in exogenous ovarian hormones or the lack thereof. Men were also chosen because the research question was based on challenging the assumption that men and women are more similar in the placebo phase. Reliability may not be excellent in women, but if it is comparable to men and women have similar reliability then it could be argued that women could be tested in any OC cycle phase reliably.

Table 7. Explanation of study design considerations.

Consideration	Choices	Selection	Explanation
Study design	Randomized Parallel Randomized Crossover Cohort Crossover Case-Control	Observational Cohort Study	Where asking participants to begin or cease taking an OC may be invasive and add unnecessary burden to participants, it is more ethical to observe a cohort of participants previously and independently using OC.
Types of reliability	Inter-rater/Inter-tester Overall Repeated measures Parallel-Forms Internal Consistency	Multiple Measures Overall ICC (2, k)	To analyze similar individuals using similar measurements at different time-points, a reliability method is used. This allowed for the detection of potential changes between similar testing conditions outside of change OC cycle phase. Multiple measures were used in three different postures, and the construct of multiple measures from other reliability studies was used. Repeated-measures reliability can also be analyzed with the study design.
Exogeneous hormones	Oral Contraceptives Hormonal replacement therapy	Oral contraceptives	When evaluating the mechanisms of exogenous hormones on cerebrovascular reliability, the two forms of hormones include very different population groups. Using OCs allows for the recruitment of the desired young, healthy population.
Control Group	Eumenorrheic women Post-menopausal women Women taking varying OC doses Men	Men	Previous studies have compared women taking OC to eumenorrheic women and men in the same study. Comparing to men directly pointed to the research question, and also offered the strongest ability to detect and identify differences due to variation in exogenous ovarian hormones.
Analysis of Oral Contraceptive Cycle Phase	Self-Report Survey Saliva Blood Analysis	Self-Report Survey Blood Analysis	When the presence of exogenous hormones is a key part of the proposed mechanism, it is crucial to determine the presence of exogenous hormones accurately and precisely in a participant which is best determined by blood assay analysis.
Randomization	Randomized Visit Sequence Randomized Postures Unrandomized	Randomized First Visit	To minimize participant anxiety impacting measurements, a familiarization visit was conducted for all participants to gain comfort with the measurements and then the first visit was randomized to OC cycle phase. Postural change was not randomized to standardize the postural change stimulus that participants experienced during experimental visits.
Blinding	Double-Blind Single-Blind Unblinded	Unblinded	Performing a double-blind experiment where participants did not know their treatment of reproductive health pharmacology would be unethical, however the research team can be blinded to reduce the risk of bias in favor of a certain outcome. There was not a reliable method available for blinding the research team to participant cycle dosage and phase, therefore this study did not have blinding included.

PRE-VISIT EXPERIMENTAL CONTROL

To ensure subjects report for each visit under standardized conditions, pre-participation guidelines were required before experimental visits (Table 8). Reminder emails including the following list were sent 48-72 hours before every visit, and participants were asked to confirm their adherence to pre-testing behaviors at the beginning of every visit (Appendix C). Experimental visits were rescheduled if pre-participation guidelines are not followed. All of the following participation guidelines have been cited as important considerations for controlling potential confounding variables in research including women taking OCs.[1]

Table 8. Pre-participation guidelines.

Consideration	Explanation	Control Procedure
No vigorous exercise 48 hours before testing.	Limitation of the impact of physical activity on cardiovascular and cerebrovascular measurements between days.	Participants were reminded via email of visit schedule and pre-participation guidelines 72 hours before their scheduled familiarization and experimental visits.
No moderate exercise 24 hours before testing.		
Abstinence from alcohol 12 hours before testing.	Limitation of the impact of physical activity on cardiovascular and cerebrovascular measurements between days.	Participants were reminded via email or text of visit schedule and pre-participation guidelines 72 hours before their scheduled familiarization and experimental visits.
Abstinence from caffeine 12 hours before testing.		
Fasted 8 hours before testing.	Limits the potential of physiological variation to be caused by certain types of food (ex. High glycemic content, extreme caloric differences) and recent feeding.	Participants were asked to fast, which was confirmed at the beginning of each experimental visit.

INTERNAL VALIDITY

Certain internal validity considerations were necessary to ensure that our sex-stratified groups could be appropriately compared and changes in outcomes could be attributed to variations in exogenous hormones. Considerations made included recruiting participants who are similar in age, and free of diseases that could affect vascular measurements (Table 9).

Table 9. Internal Validity Considerations.

Consideration	Explanation	Control Procedure
Repeated time of experimental visits.	Measurements have been found to have repeated variability that can be attributed to diurnal variations.[1,57]	All experimental visits for any participant were conducted at a similar of day.
Screening procedures	Eliminates potential variability of sample population, increases likelihood of homogeneity between groups.	During recruitment, participants were required to provide their age and medical history. Women were asked to provide the name of their oral contraceptive and specify for how long they have been taking that specific medication.

POPULATION/SAMPLING

For the most robust comparison between OC phases and between sexes, a homogenous group of men and women were recruited. A sample of young, healthy individuals was a strength of this study in that confounding effects of age, cardiovascular diseases, or other health concerns can be limited. There were strict inclusion criteria for recruitment in this study that ensure such a sample could be attained (Table 10).

Table 10. Inclusion Criteria.

Criteria	Method	Rationale
Age: 18-35 y.	Initial email contact screening, familiarization visit screening	Younger individuals have less risk of cardiometabolic disease, and the variability do to cardiometabolic disease risk can be limited.
Recreationally active; 1-10 h / week	Initial email contact screening, familiarization visit screening	Highly trained individuals have differences in cardiovascular health outside of the variables wish to be observed in this study.
Women taking combined OC for at least 6 months prior to participation	Initial email contact screening, familiarization visit screening	Testing women within the first 6 months of beginning an OC prescription may have resulted in variability caused by the body acutely adjusting to the new OC. Women using OCs for 6 months are expected to have a regular variability in response to their OC.
Free from cardiovascular, renal, or metabolic disease	Initial email contact screening, familiarization visit screening	Diseases which impact the central vasculature could have potentially introduced unaccounted variability to the desired measurements.

For greater application and external validity, common forms and dosages of combined OCs were evaluated. Within a 2012 survey, women in the United States were found to most commonly use OCs with more than 30 mcg of ethinyl estradiol, and third generation

progestins.[58] This study initially aimed to only recruit women taking combined, monophasic OCs with at least 0.030 mg of ethinyl estradiol. After the beginning of the study, recruitment was broadened to include women who had been taking any combined OC for at least 6 months prior to participation.

Participants were also screened for exclusion criteria so that a homogenous sample could be analyzed (Table 11). The homogenous sample allowed for differences to be applied to the desired controlled variables (sex, OC cycle phase) and limited the variability caused by extraneous participant characteristics.

Table 11. Exclusion Criteria.

Criteria	Method	Rationale
Known cardiovascular, renal, or metabolic Diseases	Initial email contact screening, familiarization visit screening	Diseases which impact the central vasculature could have introduced unaccounted variability to the desired measurements.
Cognitive impairment	Familiarization visit screening	Known cognitive impairment would likely have impacted the reliability and generalizability of cognitive evaluation.
Age > 36 y.	Initial email contact screening, familiarization visit screening	Vascular changes and potential risk of cardiovascular disease increases with age.
Women pregnant, planning to become pregnant, or who have experienced complications in a previous pregnancy.	Initial email contact screening, familiarization visit screening	An active pregnancy has significant associated changes to hormonal and vascular health and has been shown to impact vascular measurements. Women with pregnancy would likely have unexplained variability in vascular measurements and outcomes.
Current smoker; cigarette, marijuana, or electronic vape	Initial email contact screening, familiarization visit screening	Smoking is known to negatively impact cardiovascular health. Including these individuals would have limited the ability of this study to analyze a homogenous, healthy population.[59]
Use of medication known to impact cardiovascular function	Initial email contact screening, familiarization visit screening	Medication known to impact cardiovascular function would not allow for the measurement of true cardiovascular and cerebrovascular function. **Examples:** beta-blockers, ACE inhibitors, statins
Reported substance abuse	Initial email contact screening, familiarization visit screening	Long term substance abuse can significantly impact vascular measurements and would have limited the ability of a homogenous group to be analyzed.[60,61]

EXTERNAL VALIDITY/GENERALIZABILITY

Participants were excluded with considerations for important cardiovascular, cerebrovascular, and cognitive measurements to include a homogenous sample. Strong exclusion criteria can limit the generalizability of the results and increase the likelihood that changes in outcomes may be the result of changes in the explanatory, mechanistic variables (exogenous estrogen). Excluding a broad range of individuals limits the ability of this study to represent overall changes in cardiovascular measurements due to changes in sex hormones, but a more tightly controlled population strengthened the ability to observe cerebrovascular changes in response to OC cycle phase.

A broad range of women taking any kind of OC for at least 6 months was included in this study. As opposed to a more restricted inclusion of women taking specific kind of OCs, these results can represent variation caused by most known types of OCs.

SEX AS A BIOLOGICAL FACTOR

Sex was a key consideration of participant grouping in this study, and therefore both women taking OCs and men were included. Important to note is the limited inclusion of cisgender men and women only. When referring to men and women, cisgender individuals are being discussed. Further studies are necessary to determine reference values and establish reliability for transgender individuals, and those undergoing transition intended HRT.

ETHNICITY & RACE

People of color face numerous health disparities, including higher cardiovascular disease mortality rates. This study was not adequately powered to determine whether there are race/ethnicity risk interactions with cerebrovascular measurements, and the initial sample did not

include a sufficiently sized group to be able to perform subgroup analysis by ethnicity and race. The sample was recruited from the population of Orange County, North Carolina.

STATISTICAL CONSIDERATIONS

For this study, a reliability design was required with associated reliability statistics. Reliability was assessed using ICC, standard error of measurement (SEM), minimal detectable change (MDC), and MDC%.

ICC values allowed for the evaluation of agreement between OC phases. ICC values range from 0.00 to 1.00 and therefore reliability of any group or outcome can be compared to another. This allowed for the comparison of reliability with different outcomes, and also between different outcomes enabling us to determine if certain cerebrovascular measurements were more reliable than others. SEM analysis offered information on the variability of a measurement, which was used to determine the limits of agreement based on the standard deviations of repeated measures. MDC is a form of analysis that determined the smallest about of statistical change in a measurement that can be attributed to real change in a physiological construct and not due to error of the measurement or measurement techniques. MDC% is based on MDC, and allows for a percentage quantification of how precise a measurement is.

The specific calculation of agreement is important to consider, and study design can dictate which calculation of agreement is best. Following guidelines reported in previous papers discussing the reliability statistic, a two-way mixed effects, absolute agreement, multiple measurements calculation was selected.[7] Two-way mixed effects was chosen because there was only one rater who measured and averaged multiple measurements.

MEASUREMENT CONSIDERATIONS

As this study relied on determining reliability of measurements, constructs of our chosen physiological measurements, the specific measurements, and the rationale for their inclusion are important to understand and consider (Table 12). The following table discusses the constructs necessary for evaluating the components of the proposed mechanism (Figure 2).

Table 12. The chosen measurement techniques and explanations for measurement constructs from the proposed mechanism.

Construct	Choices	Selection	Explanation
Cerebral Blood Flow Velocity	MRI PET TCD NIRS fNIRS	TCD	TCD is a simple measurement that can be measured and collected simultaneously with other measurements in the given lab space. TCD does not measure whole cerebral blood flow, but the MCA is the largest artery of the Circle of Willis and therefore is a good estimate of blood flow velocity to the whole brain.
Ovarian Hormone Profile	Saliva Phlebotomy Self-Reported Survey	Self-Reported Survey Phlebotomy	While most invasive, collecting blood samples for the analysis of ovarian hormone profile is the best way to ensure an accurate and precise measurement of estrogen and progesterone can be measured. Participants also responded to a survey of their cycle phase which was used for primary visit scheduling.
Cognitive Function	TMT VFT AFT FT Stroop Test DCCS Working Memory Picture Vocabulary Pattern Comparison	TMT FT	The TMT and FT were selected as cognitive function measurements that have not previously been explored in women taking OCs. The TMT has previously been measured by our lab group, however there are no published studies that have used the TMT and FT with eye-tracking and NVC.

Abbreviations: AFT; Action Fluency Test, DCCS; Dimensional Card Change Sort Test, ECG; Electrocardiogram, fNIRS; Functional Near-Infrared Spectroscopy, FT; Flanker Task, HR; Heart Rate, MRI; Magnetic Resonance Imaging, NVC; Neurovascular Coupling, OC; Oral Contraceptive, PET; Positron Emission Tomography, TCD; Transcranial Doppler, TMT; Trail-Making Test, VFT; Verbal Fluency Test

PRIMARY OUTCOME: MIDDLE CEREBRAL ARTERY BLOOD FLOW VELOCITY

The primary construct of interest for reliability is cerebral health, measured by MCA_V. For measuring brain blood flow, Magnetic resonance imaging (MRI) is the most accurate and established technique which can measure velocity and volumetric flow, however this comes at great cost financially, and it limits the ability to simultaneously collect other measurements. An alternative is the use of TCD ultrasound which is a valid, reliable, non-invasive, and continuous

measurement of MCA_V.[2] TCD is measured using one or two small external probes, which allow for the simultaneous measurement of MCA_V and other measurements. MCA_V is a useful measurement as changes in MCA_V have been associated with increased cardiovascular disease risk,[62,63] cognitive decline,[64–66] and the risk of Alzheimer's disease and related dementias.[11,12] This measurement was the primary outcome as it is a common and generalizable measurement of cerebral blood flow. While maintaining reliability between phases, MCA_V was expected to change slightly as previous reviews have discussed the mechanistic impact of ovarian hormones on estrogen receptors in the cerebrovasculature (Figure 2).[67]

There are multiple arteries within the cerebrovasculature that could be isonated for measurement, however the MCA has been found to have the highest velocities and is most consistently and repeatably measured compared to others attainable with sonography within the cerebrovasculature (Anterior and Posterior Cerebral Arteries).[2] The MCA also receives the greatest blood flow of all cerebral arteries.[68] MCA_V has a strong ability to resist dilation in response to BP changing stimuli, which suggests that changes in MCA_V can be associated with changes in general cerebral blood flow.[69] Reference values for MCA_V have been established by sex and age with total averages of 71 cm/s and 52.8 cm/s being reported in two different large population studies.[70,71] In both of these studies, women had a slightly higher MCA_V compared to men in the same age groups. In participants with subarachnoid hemorrhage, a 20% MCA_V reduction was observed suggesting that ~10 cm/s reduction in MCA_V observed in any

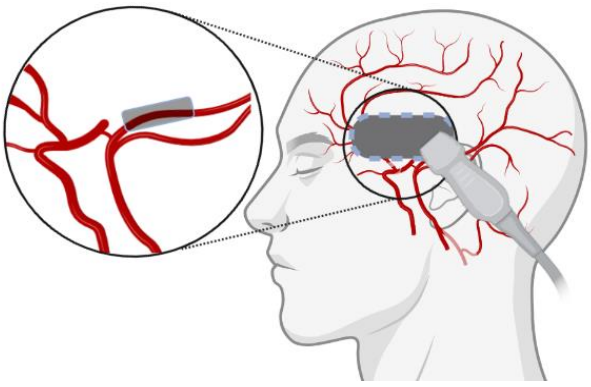

Figure 3. Location of the transcranial doppler measurement area and the section of the middle cerebral artery to be measured.

individual would be clinically significant.[72,73] A crucial consideration in measurement of MCA_V using ultrasound is precise placement of the ultrasound probe. Following the initial study describing the measurement of cerebral blood flow, the probe was placed just cranially to the zygomatic arch, 1-5 cm anterior to the ear, this done as the temporal bone and temporal window are thin enough for ultrasound waves to penetrate and reach the MCA (Figure 3).[2] Additional considerations are the settings for proper isonation, which include a frequency of 2 mHz, a depth of 45 – 55 mm, and gated filters from 3.4 Hz to 100 Hz.

TCD measurement of MCA_V was attained continuously during all three experimental visits. Specific time points were selected at rest in all postures with no other measurement being actively taken at the same time (such as oscillometric BP) to maintain cerebral homeostasis, or during the postural change and cognitive tests. For analysis of MCA_V, 10 cardiac cycles of TCD measurements were selected in each the supine, semi-recumbent, and seated postures. In previous studies, inter-rater reliability has been found to have acceptable reliability in both the seated posture (ICC = 0.82) and in the supine posture (ICC = 0.734).[3] This measurement is technically challenging and requires training, therefore the measurement technician (NA) completed 25 training measurements with quality data signal before formal data collection occurred and a reliability study was conducted. All TCD measurements were conducted by the same member of the research team (NA), who was found to have acceptable reliability for measuring MCA_V in the supine posture (ICC = 0.888) and in all postures (ICC = 0.971).

HEART-MIDDLE CEREBRAL ARTERY PULSE WAVE VELOCITY

hmPWV is a measurement of cerebral and carotid artery stiffness that was analyzed for reliability. hmPWV was measured using the TCD and electrocardiogram (ECG). Our group previously measured BrainPWV using simultaneous ultrasound at the carotid artery and the

MCA.[74,75] This method is challenging as it requires simultaneous isonation of both the carotid and cerebral arteries simultaneously. An alternative method is hmPWV which measures pulse arrival time between the ECG and the TCD signal. The hmPWV measurement includes all of the carotid to cerebral segment as well as some of the ascending aorta.

PWV is a measure of arterial stiffness, and hmPWV is a developing method of arterial stiffness in the carotid and cerebral arteries. Stiffness in the central arteries has been connected to impaired cognition,[76] and increased cerebral perfusion may cause damage end-organ damage to the small arteries of the brain.[77,78] Microvascular damage in the brain potentially caused by stiffness induced end-organ damage has been connected to various diseases including dementia, therefore it is important to be able to measure upper aortic and cerebral stiffness.[79] In the initial study that introduced carotid-cerebral PWV, the mean value was of carotid-cerebral PWV was 499.3 ± 78.6 cm/s.[75] Since hmPWV also includes the aorta which is the largest arterial segment in the body, it is expected that there would be a slightly higher velocity between these two similar measurements. An initial reliability study was conducted with the measurement technician who conducted this study (NA) and acceptable reliability was found in the supine posture (ICC = 0.809) and in all postures (ICC = 0.794). This study measured hmPWV as there are potentially sex differences in vascular tone and the development of arterial stiffness due to the presence of estrogen and the action of estrogen receptors.[67] In studies evaluating aortic arterial stiffness, an increase of 1 m/s has been considered clinically meaningful with an increased risk of cardiovascular events and mortality.[80]

SECONDARY OUTCOME: COGNITIVE FUNCTION

Cognitive function encompasses a wide range of domains related to cognitive-modulation of goal-directed activity, and is associated with cerebral blood flow.[81] As cerebral blood flow

was a primary outcome of this protocol and the two constructs are connected, this protocol attempted to connect OC phase, to cerebral health reliability, and furthermore to cognitive function reliability.

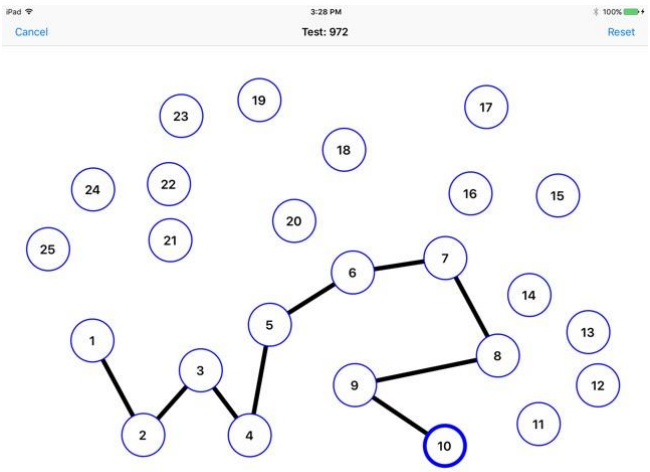

Figure 4. Example of the Trail-Making Test type B screen from the INPL software.

When asked to rank the most important domains of cognitive function, cognitive experts responded; executive function and episodic memory, language, processing speed, and attention, leaving many options to be selected for this project.[81] The NIH-toolbox battery of cognitive assessments measures many of these domains and includes the Eriksen FT, Dimensional Change Card Sort Test, List Sorting Working Memory Test, Picture Sequence Memory Test, Oral Reading Recognition Test, Picture Vocabulary Test, and the Pattern Comparison Processing Speed test.[81] Of these options and for this protocol, the FT was selected for analysis of the executive function and attention domains of cognition, and the TMT was selected for the evaluation of processing speed, switching, and inhibition domains of cognitive function. These cognitive tests have not been assessed in women taking OCs. The cognitive battery was measured at the end of every experimental visit. While not part of the NIH toolbox, the TMT was selected for its novel connection with ET.

The TMT consists of 26 sequential dots that participants select in order as fast as possible with the total time from start to completion being the outcome (Figure 4). With electronic administration of this outcome, participants responded by tapping dots on a screen with their

fingers. The standard FT consists of 5 arrows pointing left or right either congruently (the same direction) or incongruently (the middle arrow points opposite the four others). The participant is tasked with identifying the direction of the middle arrow. In this project, a modified version of the FT was administered using letters instead of arrows. The letters X, C, V, and B were part of the visual stimulus with the X and C letters corresponding to the "left" response and the V and B letters responding to the "right" response. As the letters presented a more difficult challenge of recalling which letter corresponds with left and right, the letters were printed near the screen where the stimulus was presented for the participant to refer to during the trial. Participants responded to the FT using a custom handheld controller. Response time of congruent and incongruent trials were recorded. The difference in correct congruent and correct incongruent response times was reported as the flanker effect, as well as the accuracy rate.

Cognition could be affected by unreliable cerebral blood flow during OC cycle phases and there are inconsistent findings about cognitive responses in different domains and in response to different OC hormonal profiles. In research reviewed above, eumenorrheic women performed better on cognitive tests in the high hormone phases of their menstrual cycle.[51,52] Cognitive function is important to measure as it is an indicator of cognitive disease risk, and diseases such as Alzheimer's and dementia have sex differences in prevalence as well as incidence rates.[44,46–49,82] Therefore, determining cognitive reliability and sex differences that can be accounted to OC are important as there may be misunderstood pathophysiological differences.

EYE-TRACKING

The performance of the cognitive tests can also be compared to the latent physiological responses of ET to determine if effort and cognitive load changes similarly to the results of performance on the cognitive tests. This is important to measure as ET is involuntary

physiological response of cognitive load that may have less variation due to cognitive performance than the results of the cognitive tests. Cognitive load may not be able to increase without changes in the MCA_V response which is where ET as a cognitive measurement fits in as a dependent outcome within the proposed mechanistic model (Figure 2).

Pupil diameter was the primary outcome of the ET measurements, with increased pupil diameter having previously been found to be a direct measurement of mental activity and correlating with the difficulty of given tasks.[83] Similar to the measurement of NVC, ET measures the change from baseline as determination of cognitive load. To our knowledge, this was the first study using these novel ET measurements paired with the TMT and FT.

NEUROVASCULAR COUPLING

There is a notable temporal and spatial connection between metabolic demand of the brain via activation of cerebral neurons and regional cerebral blood flow, known as NVC which can be observed during nearly all cerebral activation.[84] Therefore, measuring blood flow can be a surrogate for measuring regional synaptic and neural activation. This measurement is important because it offers information about cognitive load beyond the results of performance on the cognitive tests. This measurement is also the direct connection between the outcomes of cerebral blood flow and cognitive function at the end of the proposed mechanism. MCA_V was continuously measured, and changes were measured for the duration of the cognitive tasks in all experimental visits. MCA_V during task activation was averaged during the two cognitive tests, as well as independently compared to MCA_V at rest.

EXOGNEOUS OVARIAN HORMONES

In a study that primarily investigates the impact of exogenous hormones on physiological results, it is important to have an accurate measurement of those ovarian hormones. Multiple

methods of measuring OC cycle or menstrual cycle phase have been used in past studies including self-reported survey, saliva measurements of hormones, or measurement of hormones in blood using plasma or serum samples. It is crucial to determine the specific ovarian hormone profile in this study as the differences in ovarian hormones are part of the mechanistic model for causing potential sex differences in the outcomes of cerebral health and cognitive function reliability. Blood samples were collected in this study, however due to the full sample size not being reached for the defense of this thesis, blood was not analyzed for exogenous estrogen. This study relied on survey response for OC cycle phase confirmation, and blood will be analyzed for publication when the full sample size has been collected.

Measurement of estrogens and progestins in blood requires the collection of blood, which were executed by venipuncture at the end of each experimental visit to limit disturbing other measures for the participant. Venipuncture is a technique requiring skill and training, and for participant comfort, one member of the research team will be trained prior to the beginning of data collection and collected all plasma and serum samples (NA).

POTENTIAL CHALLENGES & ALTERNATIVE STRATEGIES

ATTRITION

Due to the nature of a protocol with multiple visits, participant drop out was possible. According to sample size calculations of 36, our recruitment goal of 40 was chosen to offset a potential 10% attrition rate. Due to time constraints and limitations from testing only in the mornings, this sample size was not achieved for the initial analysis.

MEASUREMENT TIMING

Some outcomes of this study have been reported as difficult to obtain without a skilled technician, specifically the TCD measurement. As the skull architecture and cerebral vessels of

all participants are likely to be different, isonation of the MCA can be difficult and result in a long period of time for finding the MCA_V signal. Therefore, the main operator (NA) was required to identify the TCD signal within 20 minutes of the initial supine resting period. In addition, the technician trained to be able to obtain quality data in 25 participants prior to data collection and an interindividual reliability study was conducted to determine effective reliability within MCA_V and hmPWV.

UNMET RECRUITMENT TARGETS

Due to inclusion criteria and the available population, the desired sample size was not reached limiting the ability of conclusions to be made from the results. Scheduling availability of the lab space and equipment was considered and discussed, however the current project did not fully recruit the total sample. Multiple lab spaces for connected projects were explored to ensure to the completion of all studies, and the total population will be recruited following defense of this study. Resources for recruitment available to University of North Carolina at Chapel Hill projects such as ResearchForMe were used to recruit participants.

COVID-19

The ongoing COVID-19 pandemic and associated public health precautions were a challenge for the duration of this study. Health and safety recommendations made by the state and local governments, as well as by the host institution and Department of Exercise and Sport Science were followed during the duration of the study. A majority of participants wore a face covering for all of their visits, and the research team wore face coverings to ensure participant safety and comfort. For some participants, wearing a face covering may have induced small amounts of discomfort or psychological stress. The continuation of robust safety precautions and

cleaning processes which were in place before this study began continued and were revised as necessary with the developments of the pandemic.

CARRY-OVER EFFECTS

The necessity of multiple study visits had associated carry-over and learning effects, especially in the administration of cognitive tests. For this purpose, a familiarization visit was used to ensure comfort of the participant during the first experimental visit and an opportunity to first be exposed to the cognitive tasks. In addition, the first visit that participants visit the lab was randomized which minimized the learning effect impacting any one OC phase more than the others.

TIMELINE

As this project included many moving parts, many steps, and only one hard deadline, it was crucial to establish a timeline for the tracking of progress. Therefore, included was the proposed timeline for all major milestones in this project (Table 13).

Table 13. Project Timeline & Milestones.

Activities	Start Date	End Date
Pilot Testing	8/1/2021	11/1/2021
Equipment SOP's current	6/1/2021	8/22/2021
Protocol/IRB	2/12/2021	10/1/2021
Staff training	8/1/2021	11/1/2021
Study forms/ database	2/12/2021	1/9/2022
Set-up filling system (OneDrive)	8/1/2021	12/1/2021
Recruitment	1/1/2022	3/29/2022
Data collection	1/16/2022	3/29/2022
Aim 1 analysis	3/1/2022	4/15/2022
Aim 2 analysis	3/1/2022	4/15/2022
Hand Document to Committee	**4/26/2022**	
Defend	**5/3/2022**	
Respond to defense changes	5/4/2022	5/15/2022
Submit thesis to graduate school	**6/1/2022**	
Authorship order agreement	5/6/2022	5/15/2022
Prepare Manuscript	6/1/2022	7/15/2022
Milestones		
50% recruitment	1/30/2022	
100% recruitment	2/28/2022	
100% data collection	3/29/2022	
Final database lock	3/29/2022	
100% data analysis	4/5/2022	
Submit Manuscript	5/16/2022	

CHAPTER 4: METHODOLOGY

This study was reported in accordance with GRRAS (Guidelines for Reporting Reliability and Agreement Studies).[85] Ethical approval was obtained from the University of North Carolina at Chapel Hill institutional review board (IRB #21-2465), and all participants provided written informed consent prior to participating in the study.

PARTICIPANTS

A homogenous group of young, healthy adults was selected for this study to minimize the confounding influence of age and cardiometabolic abnormalities. A convenience sample of young (18-35 years), healthy, and recreationally active men and women were recruited from the population of a large state university between December 2021 and April 2022. While a total sample of 40 participants with 20 men and 20 women was attempted to be recruited, a total of 18 participants were included, 13 women and 5 men. Women were included if they had been taking a combined OC pill for at least 6 months. Exclusion criteria included pregnancy, current smoker, any known cardio-metabolic disorders, or use of medications known to affect cardiovascular function.

RECRUITMENT

Participants were recruited via email lists, class announcements, paper flyers, social media posts, and word of mouth. Flyers were posted around university common areas including academic buildings, student union spaces, campus recreation facilities, and outside where accessible. Email recruitment was sent to student clubs from the university clubs and organizations page, specifically clubs that may have an interest in health and wellness such as

sport clubs. Recruitment materials included information about the inclusion criteria (OC users including dosage and common brand names), time commitment, measurements, and contact information of the research team (NA & JP). Prospective participants filled out a survey for screening and confirmation of inclusion criteria, then eligible participants were contacted by the research team for the scheduling of visits.

GROUP ALLOCATION

Participants were allocated to two groups based on biological sex. Inclusion as a participant was dependent on participant characteristics, namely for women if they have been taking hormonal OCs.

RANDOMIZATION

Sequence of visits for female participants was scheduled to match with the respective hormonal OC phases. While men do not have a hormonal cycle or take OCs, they were scheduled as such with a simulated cycle on three occasions to ensure that men and women had similar amounts of days between visits. Visits were scheduled within one OC cycle with visits no greater than 14 days apart. The cycle phase of the first visit for all participants was randomized. For all participants, a random number list from 1-3 was generated from a computer with the numbers determining the OC cycle phase of the first visit with the following number allocation to the following first visit phase; 1 – placebo phase, 2 – early active phase, 3 – late active phase.

SAMPLE SIZE

While this was the first study to establish reliability in the given populations, multiple previous studies analyzing the reliability of cerebral blood flow have used sample sizes of 10.[3,86,87] With an a priori alpha level of Pearson's Correlation test set at $\alpha = 0.05$ and a power of 0.80, an effect size of 0.923 can be achieved with a total sample size of 36. (Appendix F)

Accounting for dropout, we planned to recruit 40 total participants: 20 into each group men, and women taking oral contraceptives.

STUDY DESIGN

This reliability study was designed to investigate the reliability of cerebrovascular measurements during different OC menstrual cycle phases. All participants visited the lab for a familiarization visit, and then three experimental visits. Participants participated in three experimental visits within a four-week period. The timing of visits for women aligned with the three phases of the OC cycle, and the men's visits were scheduled at matched time points. The phase of the first experimental visit was randomized by a set of computer-generated numbers. The experimental visit measurement process is outlined in **Figure 5.**

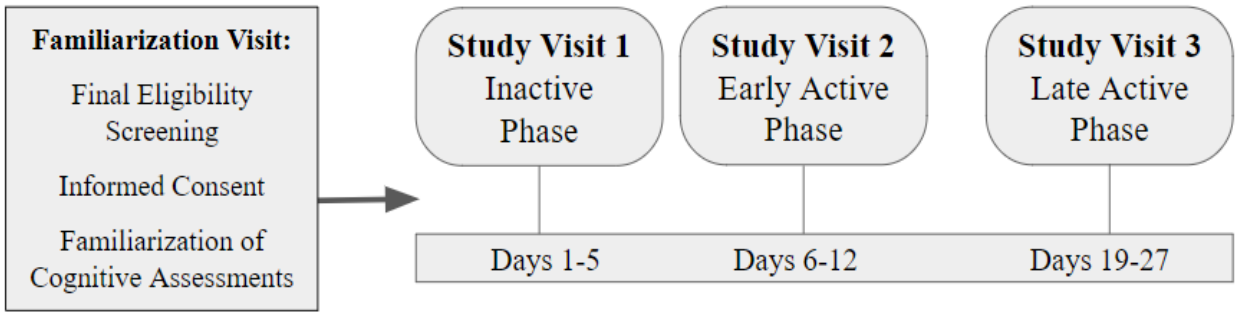

Figure 5. The experimental visit timeline for participants. The three visit occasions are listed in one order, however the first experimental visit were randomized for all participants. In the listed example, this would represent the complete experimental timeline for a participant who was randomized to "1", and began their first visit in the inactive phase.

All experimental visits were identical for the purpose of obtaining reliability data between cycle phase points. All participants visited the lab for a familiarization visit, and then three experimental visits. The first visit occurred within 1-7 days of the familiarization, and then the three experimental visits occurred during the different phases of the OC cycle for women or during the simulated cycle phases for men. The first OC pill phase for women or simulated cycle

phase for men was randomized and then the following visits were planned at least two days apart, in accordance with the following OC cycle phases.

Familiarization visits included collection of informed consent and fitting of the Equivital ECG vest. The cognitive tests were also introduced with a familiarization trial of the TMT, and the FT. Time of familiarization visits was approximately 30 minutes.

DATA COLLECTION

All familiarization and experimental visits were primarily completed by the two PIs (NA & JP), with assistance from trained undergraduate research assistants. All data was collected within the same lab space within the Cardiometabolic Lab on the University of North Carolina at Chapel Hill campus. Ambient environmental information (temperature, humidity, barometric pressure) was collected at the beginning of every visit to monitor potential extreme abnormalities in environmental conditions that could impact vascular measurements.[88,89] To limit the impact of diurnal variation on measurements, all visits for a single participant occurred at a similar time of day.[57] Due to the requirement of fasting, most visits took place in the mornings.

EXPERIMENTAL MEASURES

All three experimental visits were identical, and the experimental visit protocol is shown in **Figure 6.** Following pre-testing guidelines, participants visited the lab where resting vitals, anthropometric measurements, and environmental conditions were recorded. Descriptive statistics, and general participants characteristics were collected. Participants were fitted to all lab equipment, and then directed to rest in the supine position for 10 minutes. After being passively moved to the subsequent postures, participants rested for five minutes, and cerebrovascular measurements were repeated. This process was repeated for the two following postures, the semi-recumbent and seated positions. Immediately following the cerebrovascular

testing measurements, the participant was passively moved to the supine position where the cognitive tests were measured. Finally, the participant was removed of all experimental equipment and then plasma and serum samples were collected. Experimental visits lasted approximately 60-90 minutes.

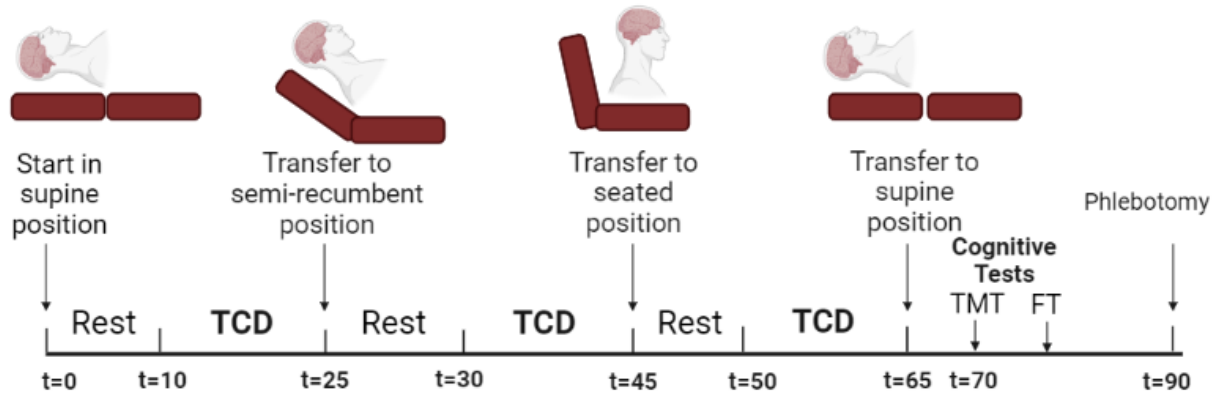

Figure 6. The experimental visit protocol.

PRIMARY OUTCOMES: CEREBRAL HEMODYNAMICS

The primary outcome of the proposed mechanism is the reliability of cerebral health and function reliability, and the two measurements of MCA_V and hmPWV were included in that construct. As part of the secondary outcome, cognitive tasks (TMT, FT) were assessed and cognitive load (NVC, ET) was measured.

Middle Cerebral Artery Blood Flow Velocity

MCA_V was chosen as the primary outcome as it is a crucial measurement of cerebrovascular blood flow and because previous work has shown responsiveness to exercise,[90–93] cognitive tests,[94–96] and other clinical measures of cardiovascular health.[97–99] MCA_V was measured using TCD (Doppler-BoxX, DWL, Germany). Ultrasound measurement was obtained through the temporal window of the skull with a 45-55 mm depth and a 2-MHz probe. The data was acquired continuously during the entirety of the experimental visits using an analog-to-

digital converter (PowerLab 35 Series, ADInstruments, Australia). As long as a good signal was available, bilateral MCA_V was measured continuously during the postural portions of the experimental protocol. One probe was removed during the cognitive tests so that the ET device could be appropriately fit to the participant. TCD Ultrasound is a valid and reliable non-invasive technique for measuring MCA_V.[2] In a two-visit reliability trial mirroring the experimental visit design of this study, acceptable inter-rater reliability was previously found by the technician who measured MCA_V in this study. A total of six participants similar to the population recruited in this study completed two visits, and MCA_V was reliable in the supine posture (ICC = 0.888) and in all postures (ICC = 0.971).

Heart-Middle Cerebral Artery Pulse Wave Velocity

Another measure conducted using the TCD is hmPWV. ECG measures were collected using a vest (eq02+ Life Monitor, Equivital, United Kingdom). Velocity was calculated as distance divided by pulse arrival time divided, where the distance is the measured between the suprasternal notch for the heart and the temple to estimate the Circle of Willis. Pulse arrival time was calculated as the delay between the peak of the QRS complex of the ECG and the upstroke of the brain blood flow wave form (Figure 7). These data were both collected via within the LabChart software (LabChart 8, ADInstruments, Australia) and analyzed

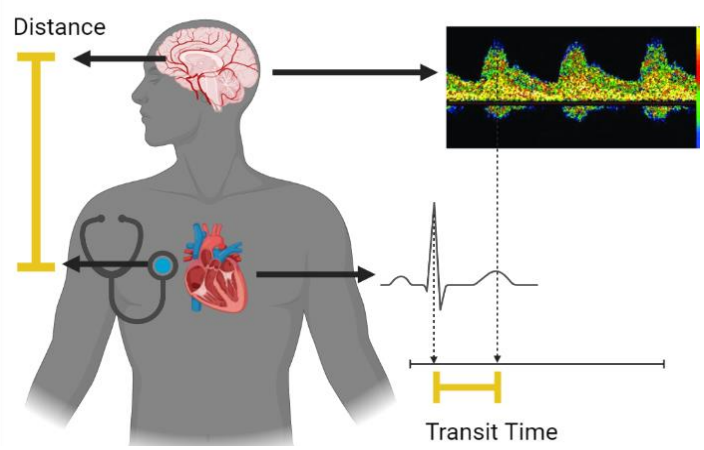

Figure 7. Details for the measurement of hmPWV. Transit time was determined by the delay between the R Wave of the ECG and the upstroke of the TCD flow waveform. Distance was measured between the suprasternal notch and the temple area of TCD measurement.

using macros developed for precisely analyzing transit times between two sites. Three consecutive cardiac cycles were evaluated from time points with high quality data, and the closest two were averaged for analysis.

SECONDARY OUTCOME: COGNITIVE FUNCTION

Cognitive Tests: Trail-Making Test type B

The TMT was administered electronically to participants at the end of every experimental visit. The participant was transferred to the supine posture for the cognitive assessments. An iPad tablet (iPad, Apple, California) with the application INPL TMT (Motus Design Group, British Columbia) administered the test and automatically recorded the time to completion. Standardized instructions were given before every test trial (Appendix E) and the outcome of time to completion was recorded which was the time between the countdown and connecting all of the 26 points (1, A, 2, B, 3, etc.). With electronic administration, the participant connected the dots by tapping the screen with their finger as opposed to tracing between dots with a pencil as has been done in studies where the task is given on paper. The trail paths were automatically randomized between trials to minimize the learning effects, and the timer was hidden for the duration of the test as not to disturb or induce stress for the participant. The participant was also fitted with eye-tracking glasses for the duration of the test and MCA_V was continuously measured.

Cognitive Tests: Flanker Task

To explore the agreement of executive function tests between OC phases, the FT was administered in the supine position at the end of the experimental visits and after the TMT. The FT is a validated and established measurement of inhibition control and attention in adults as

included in The National Institutes of Health Toolbox Cognitive Battery.[81] The results of reaction time and accuracy for the tests was recorded.

A modified version of the FT was administered to increase the difficulty of the stimulus for participants. Instead of arrows as introduced in the traditional FT, the letters X, C, V, and B were used. The letters X and C were associated with the "left" input for the participant and the letters V and B were matched with the participant responding with the "right" input. Incongruent and congruent stimuli were determined by the difference or similarity of the central and flanking letters. For example, while a stimulus of XXCXX has different letters in the relevant central letter and flanking stimuli, all of the letters in this string are associated with the "left" response therefore this stimulus is considered "congruent." The FT was administered electronically, with the visual stimuli being delivered on an iPad over the participant. A total of 50 trials were included within a single task. Participant responses were recorded using a custom-made research device that allowed participants to select the "right" or "left" response with their index fingers. ET and MCA_V were recorded during this task in the same manner as during the TMT.

Eye-Tracking

ET is a novel and developing method of determining cognitive load and other cognitive parameters. The Pupil Core (Pupil Core, Pupil Labs, Germany) device includes three cameras, one for each of the eyes, and a world camera which faces outwards. All cameras were positioned to record the appropriate field of view, and the tested for calibration. Recording of ET and the cognitive assessments did not begin until appropriate calibration had been reached. Recordings from all three cameras was taken during the durations of the cognitive tests and Pupil Labs detection software was used to evaluate pupil dilation. Pupil diameter was the primary outcome as the changes in the size of pupils has been reported as an indicator of cognitive load. Blink rate

has also been reported during longer tests of cognitive function and attention, however it is not applicable for short tests such as the TMT where participants use short-term scanning patterns. Sections of the recordings were selected to begin when the task began and end as the tasks were completed, and that data was exported. The 2d++ estimation was used to estimate diameter. For the exported time points, mean pupil diameter and pupil confidence was reported.

Neurovascular Coupling

NVC is an indirect, latent method of determining cognitive load. Using TCD, MCA_V was measured during the cognitive battery. Due to the increased metabolic demand of the frontal lobe tissue of the brain, it would be expected that cerebral blood flow would increase compared to the resting supine measurements. Between the transfer from the seated posture to supine often reduced the signal quality of the TCD and the technician adjusted the measurement as best as possible before administering the cognitive tasks. If quality MCA_V was not attainable, the data was not included in the final analysis. NVC was reported as the percent change of MCA_V between the supine posture and the MCA_V measured during each of the cognitive tasks.

MECHANISTIC OUTCOMES

Electrocardiogram

ECG was recorded for measurement of HR and as the first part of the measurement of hmPWV. ECG was continuously measured using Equivital Vest. The data was collected onto the LabChart software.

Mean Arterial Pressure

MAP was measured using an automatic oscillometric brachial cuff (Vicorder, SMT Medical, Germany). For PWV measurements including hmPWV, BP is a known confounder of the measurement.[100] With this consideration, MAP was collectied to be used as a covariate to

ensure that changes in hmPWV can be attributed to changes in arterial stiffness and not arterial pressure.

Hormonal Profile: Blood Estrogen

Blood samples (plasma and serum) were taken at the conclusion of each experimental visit. As has been discussed, studies evaluating mechanisms that include hormones should use an appropriate and reliable method of confirming the hormonal profile for participants. Assays were selected for their appropriate ability to detect low-levels of synthetic estrogens (Ultra-Sensitive, Thermofisher, Massachusetts). Assays were not able to be purchased and run on an incomplete portion of the total sample. Plasma and serum were collected for all experimental visits, were stored in an appropriate freezer, and can be analyzed following the collection of all experimental data.

QUALITY CONTROL

For a given outcome all measurement and analysis were conducted by a single observer. The current data set was checked by an independent observer.

DATA MANAGEMENT

All data was stored securely within Excel Workbook, (Microsoft, Washington) saved within password protected cloud-based data services (OneDrive, Microsoft, Washington) and also kept on transportable USB drives kept by members of the research team (JP & NA). Participants were recorded with depersonalized code of OCXX with XX referring to the participant number (ex., Participant 1 = OC01, 2 = OC02).

STATISTICAL ANALYSIS

Statistical analyses were performed using Jamovi (Version 1.6), and IBM SPSS Statistics (Version 27.0.0.0).[101,102] Raw data and summary statistics were presented as mean [standard

deviation (SD)] and reliability data were presented as ICC [(ICC); 95% confidence interval (95%CI)]. The α-level was set a priori at 0.05 for all statistical procedures.

Both the group of men and women were evaluated for test-retest reliability. Reliability measures both correlation and agreement, however there are a number of methods which have been previously used to quantify reliability depending on the specific study design, the desired model, the type of agreement, and the definition all determined by the research question.[7] Due to the repeated-measures study design with reliability being evaluated by one rater, a 2-way mixed-effects, absolute agreement model was appropriate known as ICC(2, k) using the Shrout and Fleiss convention.[103] Within the reliability construct, variance between the participants was calculated as the mean squares between the averages of each visit, and the variance within the participant was calculated as the mean squares within the three days for each participant. ICC was calculated as mean square for rows – mean square for error / (mean square for rows + [mean square for columns – mean square for error / n]) with 0 indicating no reliability between the phases (columns) and 1 indicating perfect reliability. While ICC values do not have standardized values for comparison, the following was used to qualitatively score ICC values: values less than 0.50 are indicative of poor agreement, values between 0.50 and 0.75 indicate moderate agreement, values between 0.75 and 0.90 indicate good agreement, and values greater than 0.90 indicate excellent agreement.[7] Reliability statistics were reported following suggestions put forth in previous reviews of the ICC statistic.

Reliability statistics were calculated for MCA_V, hmPWV, TMT, FT, Pupil Diameter, and NVC, with test-restest occasions occurring in the placebo, early active, and late active phases. In addition to ICC, reliability statistics included SEM, MDC, and MDC%. The SEM was calculated as the SD between participants*√(1-ICC). MDC was calculated as 1.96*SEM*√2, and MDC% was calculated as (MDC/mean)*100, where the mean is the mean score of all trials.

For the NVC outcome, differences in MCA_V are reported as the % change from the supine measurement to the measurements of MCA_V during the TMT and FT. To determine if there was a significant NVC response to the cognitive tests, a one-way ANOVA was conducted between the supine measurement of MCA_V and the measurement of MCA_V during the TMT and FT. ANOVA results are reported as [F(df1, df2) = F-value, p-value].

CHAPTER 5: RESULTS

A total of 23 participants were recruited and randomized between January 2022 and April 2022 (Figure 8). Five participants opted out of participation before experimental visits were conducted (n = 5). Eighteen participants completed at least one experimental visit (21.49 ± 3.21 years, 72.2% female, 23.78 ± 2.53 kg/m^2, Table 14). One participant did not complete testing due to schedule conflicts, (n = 1) and one participant was not able to be analyzed for a full data set due to difficulty obtaining a quality signal with the TCD (n = 1). Complete data was available for 15 participants. Timing of the first visit in accordance with the participant's timing in the oral contraceptive cycle or simulated oral contraceptive cycle was randomized evenly between the participants (first visit: placebo = 6, early active = 6, late active = 6).

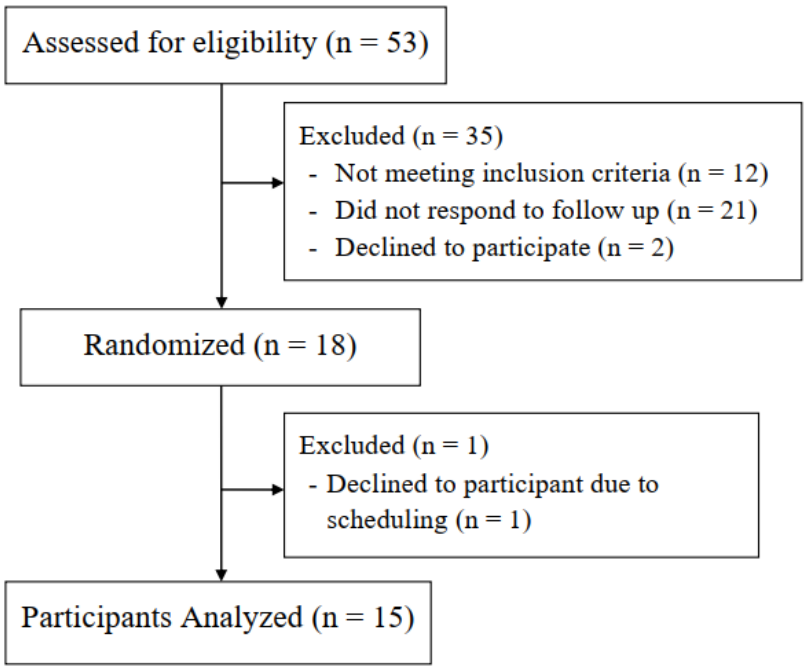

Figure 8. Diagram of included participants and reasons for exclusion.

Table 14. Participant Characteristics

	Female	Male	Total
N	13	5	18
Age (yrs)	21.5 (3.52)	21.4 (2.55)	21.5 (2.53)
Height (cm)	168 (6.71)	71.6 (2.16)	171 (8.12)
Weight (kg)	68.3 (8.89)	71.6 (2.16)	69.2 (7.70)
BMI (kg/m^2)	24.3 (2.75)	22.4 (1.00)	23.8 (2.53)

Data reported as mean (standard deviation). Abbreviations: yrs, years; cm, centimeters; kg, kilograms; m, meters.

The OC information for each female participant was collected during recruitment as a requirement for participation in the study (Table 15). Most participants were taking a 21/7 monophasic prescription (n = 9), however there were a few participants taking triphasic OC (n = 2), multiphasic (n = 1), and one monophasic prescription with an extended 24/4 cycle (n = 1). Most participants had 1st generation (n = 7) and 3rd generation (n = 5) progestins, with one participant taking an OC with a 2nd generation progestin.

Table 15. The oral contraceptive information for the women who completed at least one experimental visit. Phase is noted as the number of active pill days then the inactive days. When prescriptions included a 75 mg ferrous fumarate supplement as the inactive pill series, that was noted with a Y.

Brand Label	n	Phase	Progestin Dosage (mg)	Progestin Generation	Ethinyl Estrogen Dosage (mg)	Ferrous Fumarate?
Alyacen 1/35	1	21/7	1.0	1st	0.035	N
Blisovi Fe	2	21/7	1.0	1st	0.020	Y
Estarylla 0.25/0.035	1	21/7	0.25	3rd	0.035	N
Junel 1/20	2	21/7	1.0	1st	0.020	Y
Larissia	1	21/7	0.10	2nd	0.020	N
Loestrin 1/10	1	24/2/2	1.0	1st	0.010	Y
Ortho Tri-Cyclen	1	7/7/7/7	0.18, 0.215, 0.25	3rd	0.035	N
Sprintec	2	21/7	0.25	3rd	0.035	N
Taytulla 1/20	1	24/4	1.0	1st	0.020	Y
Tri-Estarylla	1	7/7/7/7	0.18, 0.215, 0.25	3rd	0.035	N

Abbreviations: n, participants; mg, milligrams

Of the 18 participants who completed at least one experimental visit included in the analysis, 13 participants were female and 5 were male. Environmental conditions for all visits were similar across all visits. (Temperature; 22.0 °C [0.354], Humidity; 22.0% [13.9%], Barometric Pressure; 754 mmHg [4.35]).

HEMODYNAMIC

MIDDLE CEREBRAL ARTERY BLOOD FLOW VELOCITY

A box plot of the mean MCA_V for all participants during each phase is shown below (Figure 9). For all participants, the average MCA_V was 69.34 cm/s (11.06 cm/s). For women, the mean MCA_V was 71.65 cm/s (11.27 cm/s) and for men the mean MCA_V was 64.01 cm/s (8.50 cm/s). The reliability of MCA_V overall, for women, and for men can be found in **Table 16.** The full battery of reliability statistics for each outcome and group can be found in **Appendix A**. The overall agreement of MCA_V for women was moderate with an ICC of 0.718 (n = 13, 95% CI;

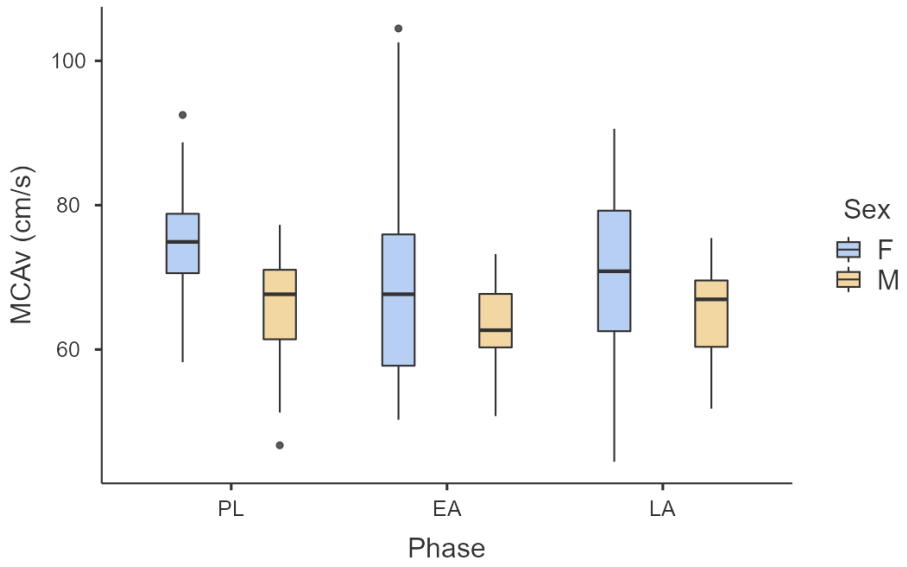

Figure 9. Box plot of the MCAV values separated by phase the participant was in during each visit and by sex.

0.553 - 0.828). The overall agreement of MCA_V for men was moderate with an ICC of 0.575 (n = 5, 95% CI; 0.131 - 0.816).

Table 16. Intraclass Correlation Coefficients of MCAV separated by sex. Effect of subjects was random and measures effects were fixed. ICC was evaluated based on a mean-rating (k = 3), with absolute-agreement, using a 2-way mixed-effects model.

		95% Confidence Interval		F Test with True Value 0			
	Intraclass Correlation	Lower Bound	Upper Bound	Value	df1	df2	Sig
Female Reliability	0.718	0.553	0.828	2.379	29	58	.003
Male Reliability	0.575	0.131	0.816	1.338	14	28	.247

HEART – MIDDLE CEREBRAL ARTERY PULSE WAVE VELOCITY

The hmPWV averages for all participants are shown in a box plot separated by sex and phase of visit (Figure 10). The grand mean of hmPWV for all participants across all visits and all

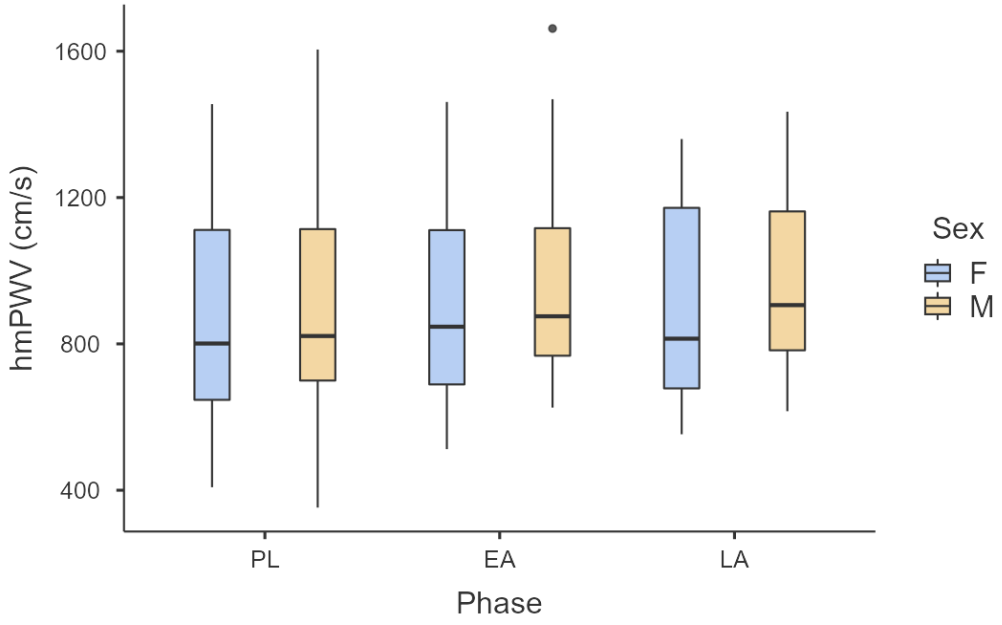

Figure 10. Box plot of the hmPWV values separated by phase the participant was in during each visit and by sex.

postures was 908.9 cm/s (282.7 cm/s). For women, the mean hmPWV was 892.4 cm/s (267.8 cm/s) and for men the mean hmPWV was 951.7 cm/s (313.1 cm/s).

The reliability statistics for the hmPWV measurement can be found in **Table 17.** For women, the hmPWV measurement was found to have excellent reliability with an ICC of 0.929 (95% CI; 0.889 - 0.956). Measurement of hmPWV for men was found to have acceptable reliability with an ICC of 0.844 (95% CI; 0.681 - 0.932).

Table 17. Intraclass Correlation Coefficients of the heart-middle cerebral artery pulse wave velocity measurements separated by sex. Coefficient values were calculated using absolute agreement assuming an absent interaction effect. Effect of subjects was random and measures effects are fixed. ICC was evaluated based on a mean-rating (k = 3), absolute-agreement, 2-way mixed-effects model.

		95% Confidence Interval		F Test with True Value 0			
	Intraclass Correlation	Lower Bound	Upper Bound	Value	df1	df2	Sig
Female Reliability	0.929	0.889	0.956	14.462	29	58	.000
Male Reliability	0.844	0.681	0.932	6.180	14	28	.000

COGNITIVE

TRAIL-MAKING TEST

The TMT Type-B was completed by participants in an average of 26.98 s (8.33 s). For women, the mean TMT time to completion was 27.52 s (9.12 s) and the reliability across cycle phases was good (ICC = 0.808 [95% CI: 0.593 – 0.922], Table 18). Men completed the TMT in an average of 24.03 s (5.53 s) and across the simulated cycle phases the men displayed a moderate reliability score of 0.700 (95% CI: -0.052 – 0.950).

Table 18. Intraclass Correlation Coefficients of the Trail-Making Test Type B time to completion for the sex-separated groups using absolute agreement assuming an absent interaction effect. Effect of subjects was random and measures effects are fixed. ICC was evaluated based on a mean-rating (k = 3), absolute-agreement, 2-way mixed-effects model.

		95% Confidence Interval		F Test with True Value 0			
	Intraclass Correlation	Lower Bound	Upper Bound	Value	df1	df2	Sig
Female Reliability	0.808	0.593	0.922	5.116	10	20	.001
Male Reliability	0.700	-0.052	0.950	3.220	4	8	.075

FLANKER TASK

The mean response times of correct responses for congruent stimuli in the FT was 667.8 ms (59.4 ms) and the mean response time of correct responses for incongruent stimuli was 707.5 ms (68.4 ms). Using a paired-samples samples t-test, there was a significant difference between the response times of congruent and incongruent stimuli ($t[50] = -6.324$, $p < 0.005$). The mean flanker effect (difference between correct incongruent and correct congruent response times) for each trial of the FT was 39.6 ms (44.7 ms). The average accuracy of all FT responses across trials was 96.20% (3.65%). For both men and women, the reliability was poor with ICC values of 0.000 (95% CI: -1.183 – 0.601) and 0.248 (95% CI: -1.764 – 0.875) respectively (Table 19).

Table 19. Intraclass Correlation Coefficients of the flanker effect using absolute agreement assuming an absent interaction effect. Effect of subjects was random and measures effects are fixed. ICC was evaluated based on a single-rating, absolute-agreement, 2-way mied-effects model.

		95% Confidence Interval		F Test with True Value 0			
	Intraclass Correlation	Lower Bound	Upper Bound	Value	df1	df2	Sig
Female Reliability	0.000	-1.183	0.601	0.656	12	24	.775
Male Reliability	0.248	-1.764	0.875	1.194	4	8	.384

PUPIL DIAMETER

Across both eye recordings and all cognitive tasks, the mean pupil diameter was 32.96 mm (5.02 mm), and the average pupil confidence was 96.09% (4.66%). The mean diameter of both pupils and confidence of the 2-dimentional model in predicting pupil diameter is reported in **Table 20.**

Table 20. The average pupil diameter and pupil confidence for each cognitive test.

	Left Pupil Diameter (mm)	Right Pupil Diameter (mm)	Left Pupil Confidence (%)	Right Pupil Confidence (%)
Trail-Making Test	34.69 (4.87)	32.37 (4.67)	96.31 (5.06)	97.10 (4.04)
Flanker Task	33.22 (5.28)	31.56 (4.82)	95.17 (4.85)	95.80 (4.57)

Abbreviation: mm; millimeter

During the TMT, the left eye measurement in women had good reliability with an ICC of 0.820 (95% CI: 0.604 – 0.931) although the right eye was observed to have moderate reliability with an ICC of 0.505 (95% CI: -0.103 – 0.810, Table 21). In men, the right eye had the stronger reliability with a moderate score of 0.825 (95% CI: 0.391 – 0.971) and the left pupil diameter had very poor reliability (ICC = 0.110 [95% CI: -2.345 – 0.852]).

Table 21. Intraclass Correlation Coefficients of the left and right pupil diameters during the Trail Making Test Type B. Coefficients were calculated using absolute agreement assuming an absent interaction effect. Effect of subjects was random and measures effects are fixed. ICC was evaluated based on a single-rating, absolute-agreement, 2-way mixed-effects model.

		95% Confidence Interval		F Test with True Value 0			
	Intraclass Correlation	Lower Bound	Upper Bound	Value	df1	df2	Sig
Female Reliability (L)	0.820	0.604	0.931	5.655	9	18	.001
Female Reliability (R)	0.505	-0.103	0.810	1.655	9	18	.173
Male Reliability (L)	0.110	-2.345	0.852	1.088	4	8	.424
Male Reliability (R)	0.825	0.391	0.971	5.216	4	8	.023

For women, agreement of pupil diameter during the FT was moderate with a left eye reliability of 0.675 (95% CI: 0.302 – 0.873) and a right eye reliability of 0.646 (95% CI: 0.214 – 0.864, Table 22). For men, left eye pupil diameter had good agreement with an ICC value of 0.889 (95% CI: 0.618 – 0.981) and the right eye had excellent agreement of 0.958 (95% CI: 0.853 – 0.993).

Table 22. Intraclass Correlation Coefficients of pupil diameters during the Flanker Task using absolute agreement assuming an absent interaction effect. Effect of subjects was random and measures effects are fixed. ICC was evaluated based on a single-rating, absolute-agreement, 2-way mixed-effects model.

		95% Confidence Interval		F Test with True Value 0			
	Intraclass Correlation	Lower Bound	Upper Bound	Value	df1	df2	Sig
Female Reliability (L)	0.675	0.302	0.873	3.074	9	18	.020
Female Reliability (R)	0.646	0.214	0.864	2.215	9	18	.072
Male Reliability (L)	0.889	0.618	0.981	11.620	4	8	.002
Male Reliability (R)	0.958	0.853	0.993	23.665	4	8	.000

NEUROVASCULAR COUPLING

The mean NVC response for the TMT was a 1.8% (18.8%) in MCA_V when compared to the supine baseline measurement. On average, a 0.4% (16.1%) increase in MCA_V was observed when comparing the FT to the baseline supine measurement. Women displayed negative changes in MCA_V during the

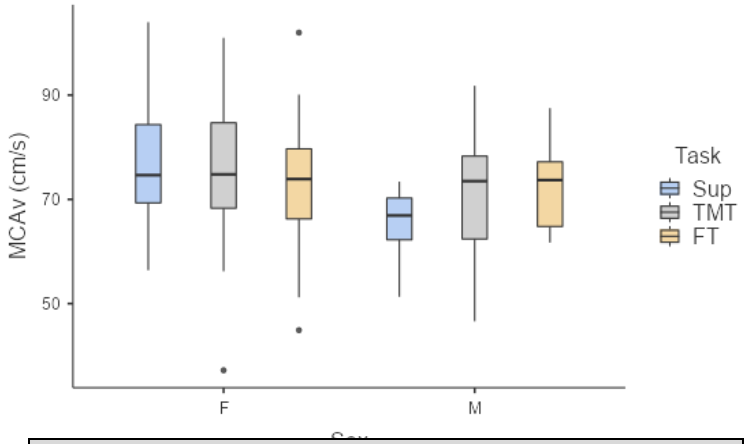

Figure 11. Box plot of the MCAv values in the supine posture, during the TMT, and the FT. Comparison of the supine values to the cognitive test values is the neurovascular coupling response.

cognitive tasks with a -0.2% (15.5%) change in response to the TMT and a -3.1% (13.7%) change in MCA_V. Men responded to the cognitive tasks with increases in MCA_V with a 7.1% (24.4%) increase in response to the TMT and a 9.3% (18.4%) increase in response to the FT. With a one-way ANOVA, there were no significant differences in the MCA_V between the supine posture, the TMT and the FT [$F(2, 51) = 0.0201$, $p = 0.980$]. Reliability of the NVC response during both tests and in men and women was 0.000.

MECHANISTIC OUTCOME: EXOGENOUS OVARIAN HORMONES

Plasma and serum samples were collected at the end of each experimental visit, however the analysis for estrogen was not completed due to the full data set not being collected.

CHAPTER 6: DISCUSSION

The primary aim of this study was to evaluate the reliability of cerebrovascular measurements across the oral contraceptive cycle in women and a simulated cycle in men. The primary aim was to evaluate the reliability of two cerebrovascular measurements, MCA_V and hmPWV. Reliability ratings based on standardized reporting of all outcomes is consolidated in **Table 23**. In both cerebrovascular outcomes, women using OCs had either the same or greater reliability ratings. Women also had better reliability in TMT time to completion than men, and the same very poor reliability of the Flanker Effect. During both cognitive assessments, both men and women had one eye diameter that was better than the other where one was worse. While reliability was not evaluated for the NVC response, there were not significant differences the supine, TMT, and FT MCA_V.

Table 23. The reported reliability of all outcomes accumulated separated by sex groups. ICC values of 0.000 – 0.500, 0.500 – 0.750, 0.750 – 0.900, 0.900 – 1.000 were associated with poor, moderate, good, and excellent ratings respectively.

					Pupil Dilation			
	MCA_V	hmPWV	TMT	Flanker Effect	TMT L	TMT R	FT L	FT R
Women	Moderate	Excellent	Good	Poor	Good	Moderate	Moderate	Moderate
Men	Moderate	Good	Moderate	Poor	Poor	Good	Good	Excellent

Abbreviations: MCA_V, Middle Cerebral Artery Blood Flow Velocity; hmPWV, heart-middle cerebral artery pulse wave velocity; TMT, Trail-Making Test type B

LIMITATIONS AND STRENGTHS

The limitations and strengths of this study are important to understand to be able to contextualize the results. First, this study was only sufficiently powered to evaluate the primary outcome, MCA_V. In addition to this, the total sample size associated with the desired effect size

was not reached for this analysis due to schedule constraints. Another major limitation of this study at time of current analysis is the absence of measurement of exogenous ovarian hormones via biochemical analysis. Previous reviews have stated the importance of confirming estrogen levels in studies that evaluate the impact of ovarian hormones as a part of a mechanism, however this was not conducted due to budget and sample size constraints.[1]

An additional limitation lies within the construct of intra-rater reliability. Within all reliability studies, the context of the specific equipment and technicians has to be considered. As stated previously, MCA_V is a very challenging measurement to obtain and therefore the reliability is somewhat technician dependent. The ICC values reported for MCA_V in this study are reliant of the assumptions that (1) the technician was appropriately trained to conduct these measurements, (2) the reliability study conducted before this trial occurred was sufficient for establishing intra-rater reliability of this participant, (3) the measurement technician remained reliable throughout experimental data collection, and (4) the reliability can be repeated by another technician with appropriate training and intra-rater reliability.

This study does have strengths in addition to limitations. As a novel project, this is the first study to evaluate the reliability of MCA_V across the oral contraceptive cycle, and it is one of only a few studies that has evaluated the emerging outcome hmPWV. Evaluating factors of cerebrovascular health that may impact stroke incidence are helpful additions to the literature that broaden our understanding of how cerebral and cardiovascular diseases may develop.

COMPARISON TO LITERATURE

MIDDLE CEREBRAL BLOOD FLOW VELOCITY

To confirm that quality data was collected and the reliability can be trusted, the means of MCA_V should be compared to those from other studies. Average MCA_V reported in this study

was 69.4 cm/s which was similar to the mean MCAV values in two previous studies which established reference values for MCA_V (71 cm/s, 52.8 cm/s).[70,71] Additionally, in our study women had slightly higher mean MCAV values than men (71.6 cm/s v. 64.2 cm/s) which was reported in each of the two previous reference studies (75 cm/s v 64 cm/s; 53.6 cm/s v. 49.9 cm/s).

In our study, moderate reliability of MCA_V was observed overall (ICC = 0.714) with sex specific reliability of 0.718 for women and 0.575 for men. A previous study which evaluated a group of 10 participants found an intra-rater reliability of 0.822 in the seated posture and 0.734 in the supine posture when measuring cerebrovascular reactivity.[3] In just women, reliability of MCA_V in the supine posture was 0.736 which almost exactly matched the previous study while the reliability across postures was just slightly lower (95% CI: 0.431 – 0.894). However, they did not report the sex of their participants so it is hard to determine if this study can be compared well to the sample from the current study. Due to a small sample size, which increase the impact of any variation, the reliability in the supine posture for men was observed as 0.000 (95% CI: -2.838 – 0.834). While the full sample was not collected for either men or women, this suggests that reliability of MCA_V in women using OCs may match reliability that has been observed in previous studies. Another previous study compared resting MCA_V in people with spinal cord injury and people with able-bodies.[104] For the purpose of this comparison, only individuals with able-bodies will be discussed. In their sample of 10 participants, they measured an excellently reliable MCA_V in the seated posture of 0.92 (95% CI: 0.70 – 0.98). Reliability of MCA_V measured in the seated posture was slightly lower in this study with reliability in women measured at 0.675 (95% CI: 0.329 – 0.866) and seated reliability in men being observed at 0.816 (95% CI: 0.359 – 0.969). All of the measurements within this study had large confidence

intervals which suggest greater variability compared to the smaller confidence intervals found in reference studies. Again, a more reasonable reliability statistic may be observed with the full recruitment and data collection in all postures as well as the seated posture.

Within the parallel analysis of this study, reliability of blood pressure, central arterial stiffness, and other cardiovascular measurements were generally found to have excellent reliability in both men and women. A possible mechanistic explanation in this study is that there are greater sources of variability between participants than there are within participants that are observed within a single month. OCs may have slight impact of between-day variance in women taking OCs, however this is less than the total variance observed from numerous other variables including many not observed in this study.

HEART-MIDDLE CEREBRAL ARTERY BLOOD FLOW VELOCITY

Measuring arterial stiffness similarly to hmPWV was completed by Fu et al. who measured stiffness between the carotid artery and the MCA.[75] This study measured intra-observer reliability within the cerebral arterial stiffness measurements. With their measurements having been obtained in a supine posture, the intraobserver reliability they observed in this study was 0.939 ($p < 0.001$) which was excellent. Measurements in all postures of the current study were found to have acceptable reliability with an ICC of 0.897 (95% CI: 0.849 – 0.931) in all participants, and ICC in women and men of 0.928 (95% CI: 0.87 – 0.96) and 0.843 (95% CI: 0.83 – 0.94) respectively. While carotid-cerebral PWV is a slightly different measurement than hmPWV that includes less of the ascending aorta and is measured using two TCD probes as opposed to an ECG, the constructs of these two measurements are comparable. Within the mean values, this study evaluating carotid-cerebral PWV had a lower mean of 499.3 ± 78.6 cm/s compared to the hmPWV mean in this study of 908.9 cm/s (282.7 cm/s). In the current study,

mean velocities were much faster which can likely be explained by the addition of the heart-to-carotid segment of this measurement.

COGNITIVE FUNCTION

Trail-Making Test Type B

The TMT was evaluated in women with Alzheimer's disease taking HRT in the form of conjugated equine estrogens.[105,106] While the women with Alzheimer's disease were much older than the current study population and Alzheimer's disease would definitely impact the results of the TMT, the previous study evaluates similar cognitive mechanisms to those tested in the current study. The women with Alzheimer's were given estrogens (1.25 mg/day) for 16 weeks and the TMT type B was evaluated. After one month of estrogen therapy, women had improved performance on the TMT, with non-significant differences after four and nine-months of treatment. In the current population of women who had been on their OC prescription for at least 6 months, we could expect that changes in the ability to perform on cognitive tasks due to OCs may have already taken place.

Flanker Task

In this study, multiple trials of the FT resulted in a large amount of within subjects variability which caused extremely poor reliability in the Flanker Effect. While learning effects were expected, the impact of changes between visits of the Flanker Effect had unforeseen impact on the reliability construct. While reliability was not observed for the Flanker Effect, there were not main significant differences observed between phases with a one-way ANOVA [$F(2, 48) = .182$, $p = .834$]. Other studies evaluating different versions of the FT report RT which is a more easily comparable outcome.

Previous studies have evaluated a modified FT using letters as opposed to arrows, however many of these studies conducted a much larger number of trials with one evaluated two blocks of 240 for 480 trials, and one conducting 420 trials.[108,109] One study was evaluating children and adults, and the letter stimuli they used included the letters H, and S (HHHHH, SSSSS, HHSHH, SSHSS). With a much longer trial duration, the adults in this study had a total accuracy rate between two sessions of 92.71% which was lower than the accuracy rate observed in this study of 96.20%. In two visits the reliability of accuracy that they observed was moderate with an ICC (ICC = 0.70, $p < 0.001$), whereas the reliability among all participants of the accuracy rate in this study was good with an ICC value of 0.867 (95% CI: 0.745 – 0.937).

The second study used the letters B, D, V, and U with a go, no-go response asked from the participants as opposed to a left or right response.[109] This resulted in either a correct response, or a false positive alarm from the participant. In stimuli where the participant was supposed to withhold from pressing the button, there was a 21% false positive alarm rate. While those specifics are not applicable to this trial based on the details of the test, these investigators did not find significant differences in response times or false positive rates between multiple visits. In the current study there was significant within-subject variation with the flanker effect, however the RT of congruent and incongruent trials were good and moderate with ICC values of 0.761 (95% CI: 0.543 – 0.887) and 0.650 (95% CI: 0.330 – 0.835) respectively. Both of the previous studies are difficult to compare to the current study because of the difference in the lengths of the trials. The accuracy rate especially is different due to a different input required by one test, and especially because in a trial length of 50 the sensitivity of accuracy percentage within this study is a minimum of 2%. However, comparing these studies allows us to view the

results of a modified FT that uses letters as stimuli as opposed to the traditional stimuli of arrows.

IMPLICATIONS

It would be irresponsible to strongly suggest conclusions and discuss the impact of the current results without the full sample size and the total analysis being completed (hormonal assays). However, the implications of this study can be inferred if the current results are observed after final analysis. At minimum, moderate reliability was observed in the hemodynamic cerebral measurements of MCA_V and hmPWV. While the sample size of women was 2.6 times larger than men, reliability of these two measurements was greater in the women than it was in the men. This would challenge the current assumption that has been utilized for deciding to only test women in the placebo phase when women who use OCs are included in physiological studies. Women have just as reliable outcomes or better than men.

At best, MCA_V was found to have moderate reliability in men and women using OCs. However, reliability being higher in the women suggests that for as unreliable as MCA_V may be for all participants, OC usage is not something that significantly causes variation in this measurement. Within the cerebrovascular battery, hmPWV was found to have even higher reliability with excellent reliability being observed in women using OCs, and reliability being higher in women than in men. This suggests that cerebral arterial stiffness may be impacted by a number of factors, but not by OC usage. These implications will be strengthened if the current reliability findings are maintained with the full sample size. In addition, when estrogen can be quantified using assays after the full sample size has been collected, linear regression can be conducted to determine if the changes in MCAV and hmPWV are associated with changes in estrogen.

The results of cognitive assessments in this study suggest also that where changes in cognitive function exists, they are not due to sex differences in men and women using OCs. The results for time to completion in the TMT had good reliability overall and in women, and were moderate in men. While within subjects variability was nearly too strong to measure reliability within the Flanker Effect, the RTs of the congruent and incongruent trials had moderate reliability. Cognitive function is a complex and broad topic to evaluate, and while OC usage may have minimal effects, the current results of this study do not point towards OC usage significantly impacting cognitive function and performance to a point that would make women in any OC phase less reliable than men.

For all measurements, if the reliability in women is found to be similar to men, this would suggest that future studies may not have to control for the OC cycle phase when including women. Instead of the current practice of testing women only during the placebo phase, this would simplify inclusion and scheduling for women which would hopefully increase the ease at which women could be included in physiological studies. A review of the implications from the current study and why the study was conducted can be completed in **Table 24.**

Table 24. Implications of the results from this study and future directions.

What did we know?
• It is know that while homeostasis of cerebral blood flow is important, middle cerebral artery blood flow velocity can change regularly in response to metabolic changes within the brain and other stimuli. • Cognitive function is a broad range of domains with large interindividual variability and a number of covarying factors.
What did we not know?
• We did not know the reliability of middle cerebral blood flow velocity, heart-middle cerebral artery pulse wave velocity, the Trail-Making Test type b, and the Flanker Task in women using oral contraceptives compared to men.
What have we learned?
• In nearly all of the measurements in this study, women using oral contraceptives were measured with greater reliability than men.
Why is this new information useful?
• Women with OCs displaying greater reliability challenges the assumption that women should be tested during the low hormone phase of the menstrual cycle or oral contraceptive cycle in order to best be compared to men.
What do we need to know next?
• The full data sample needs to be completed with a full sample of 20 women and 20 men including using hormonal assays to quantify the blood estrogen of each participant during each visit. • Additional measurements need to be challenged and included. • If future studies include women regardless of OC cycle phase, how do we compare those studies to those that only test women during the low hormone phases.

CONCLUSIONS

This study aimed to assess the reliability of cerebrovascular (MCA_V and hmPWV) and cognitive tasks (TMT and FT) in men and women using OCs, because the hormonal changes within an OC cycle phase have been hypothesize to negatively impact the internal validity of physiological studies. To do this, we observed 13 women and 5 men on three occasions in a multiple-visit cohort crossover study design. Women using combined OCs visited the lab during their placebo phase, and twice during the active phase of their OC (early and late). The group of men visited the lab in a similar time structure. We found a minimum of moderate reliability in MCA_V, hmPWV, TMT time to completion, and FT response times. Generally, the reliability of

the group of women using OCs was better than the reliability of the group of men. These data suggest that women OCs do not negatively impact the reliability of cerebrovascular measurements or cognitive task, and point towards the inclusion of women in research at any time in their OC cycle which would simplify inclusion of women in future studies.

APPENDIX A. SUPPLEMENTAL RELIABILITY STATISTICS

MIDDLE CEREBRAL ARTERY BLOOD FLOW VELOCITY

Table A1. Full reliability statistics for overall agreement of MCA_V in all participants (n = 18), women (n = 13), and men (n =5).

OVERALL

	x	Low 95% CI	High 95% CI
ICC	0.714	0.582	0.810
SEM (cm/s)	8.13	6.63	9.83
MDC (cm/s)	22.53	18.37	27.24
MDC %	32.49	26.48	39.28

Abbreviations: ICC; Intraclass Correlation Coefficient, SEM; Standard Error of the Measurement, MDC; Minimum Detectable Change; MDC %; Minimum Detectable Change Percentage

WOMEN

	x	Low 95% CI	High 95% CI
ICC	0.718	0.553	0.828
SEM (cm/s)	8.23	6.43	10.59
MDC (cm/s)	22.82	17.82	29.36
MDC %	31.84	24.86	40.97

Abbreviations: ICC; Intraclass Correlation Coefficient, SEM; Standard Error of the Measurement, MDC; Minimum Detectable Change; MDC %; Minimum Detectable Change Percentage

MEN

	x	Low 95% CI	High 95% CI
ICC	0.575	0.131	0.816
SEM (cm/s)	6.26	4.12	8.95
MDC (cm/s)	17.35	11.41	24.81
MDC %	27.10	17.83	38.75

Abbreviations: ICC; Intraclass Correlation Coefficient, SEM; Standard Error of the Measurement, MDC; Minimum Detectable Change; MDC %; Minimum Detectable Change Percentage

HEART - MIDDLE CEREBRAL ARTERY PULSE WAVE VELOCITY

Table A2. Full reliability statistics for overall agreement of hmPWV in all participants (n = 18), women (n = 13), and men (n =5).

OVERALL

	x	Low 95% CI	High 95% CI
ICC	0.897	0.849	0.931
SEM (cm/s)	14.19	11.62	17.19
MDC (cm/s)	39.36	32.22	47.66
MDC %	43.31	35.45	52.44

Abbreviations: ICC; Intraclass Correlation Coefficient, SEM; Standard Error of the Measurement, MDC; Minimum Detectable Change; MDC %; Minimum Detectable Change Percentage

WOMEN

	x	Low 95% CI	High 95% CI
ICC	0.928	0.889	0.956
SEM (cm/s)	11.48	9.04	14.36
MDC (cm/s)	31.83	25.06	39.80
MDC %	36.02	28.35	45.04

Abbreviations: ICC; Intraclass Correlation Coefficient, SEM; Standard Error of the Measurement, MDC; Minimum Detectable Change; MDC %; Minimum Detectable Change Percentage

MEN

	x	Low 95% CI	High 95% CI
ICC	0.843	0.681	0.932
SEM (cm/s)	19.00	12.54	27.17
MDC (cm/s)	52.66	34.77	75.30
MDC %	55.33	36.53	79.13

Abbreviations: ICC; Intraclass Correlation Coefficient, SEM; Standard Error of the Measurement, MDC; Minimum Detectable Change; MDC %; Minimum Detectable Change Percentage

TRAIL-MAKING TEST

Table A3. Full reliability statistics for overall agreement of TMT time to completion in all participants (n = 18), women (n = 13), and men (n =5).

OVERALL

	x	Low 95% CI	High 95% CI
ICC	0.800	0.621	0.905
SEM (cm/s)	5.37	3.70	7.39
MDC (cm/s)	14.88	10.25	20.48
MDC %	56.14	38.69	77.29

Abbreviations: ICC; Intraclass Correlation Coefficient, SEM; Standard Error of the Measurement, MDC; Minimum Detectable Change; MDC %; Minimum Detectable Change Percentage

WOMEN

	x	Low 95% CI	High 95% CI
ICC	0.808	0.593	0.922
SEM (cm/s)	5.696	3.631	8.294
MDC (cm/s)	15.78	10.06	22.98
MDC %	57.37	36.56	83.52

Abbreviations: ICC; Intraclass Correlation Coefficient, SEM; Standard Error of the Measurement, MDC; Minimum Detectable Change; MDC %; Minimum Detectable Change Percentage

MEN

	x	Low 95% CI	High 95% CI
ICC	0.700	-0.052	0.950
SEM (cm/s)	4.38	1.79	8.21
MDC (cm/s)	12.15	4.96	22.75
MDC %	50.54	20.63	94.65

Abbreviations: ICC; Intraclass Correlation Coefficient, SEM; Standard Error of the Measurement, MDC; Minimum Detectable Change; MDC %; Minimum Detectable Change Percentage

FLANKER TASK

Table A4. Full reliability statistics for overall agreement of the Flanker Effect in all participants (n = 18), women (n = 13), and men (n =5).

OVERALL

	x	Low 95% CI	High 95% CI
ICC	0.000	-0.933	0.530
SEM (cm/s)	44.73	30.67	62.19
MDC (cm/s)	123.99	85.00	172.38
MDC %	367.98	252.27	511.61

Abbreviations: ICC; Intraclass Correlation Coefficient, SEM; Standard Error of the Measurement, MDC; Minimum Detectable Change; MDC %; Minimum Detectable Change Percentage

WOMEN

	x	Low 95% CI	High 95% CI
ICC	0.000	-1.183	0.601
SEM (cm/s)	48.02	30.32	70.92
MDC (cm/s)	133.04	84.04	196.58
MDC %	358.49	226.44	529.67

Abbreviations: ICC; Intraclass Correlation Coefficient, SEM; Standard Error of the Measurement, MDC; Minimum Detectable Change; MDC %; Minimum Detectable Change Percentage

MEN

	x	Low 95% CI	High 95% CI
ICC	0.248	-1.764	0.875
SEM (cm/s)	35.55	14.50	68.16
MDC (cm/s)	98.54	40.18	188.94
MDC %	258.28	105.32	495.24

Abbreviations: ICC; Intraclass Correlation Coefficient, SEM; Standard Error of the Measurement, MDC; Minimum Detectable Change; MDC %; Minimum Detectable Change Percentage

APPENDIX B. CONSENT FORM

IRB TEMPLATE Version 2.1 - 1/17/2020 - Do not alter this text box

University of North Carolina at Chapel Hill
Consent to Participate in a Research Study
Adult Participants

Consent Form Version Date: 10/1/21
IRB Study # 21-2465
Title of Study: Reliability of cardiovascular and cerebrovascular measures across the oral contraceptive cycle
Principal Investigator: Jillian Poles
Principal Investigator Department: Exercise and Sport Science
Principal Investigator Phone number: (919) 962-0396
Principal Investigator Email Address: jpoles@email.unc.edu
Faculty Advisor: Lee Stoner
Faculty Advisor Contact Information: (919) 962-0534

CONCISE SUMMARY

We are looking to examine the reliability of measures of vascular, cerebrovascular, and executive function across the oral contraceptive (OC) cycle. It is currently believed that the OC cycle may introduce variability in these measures, making them unreliable across cycle phases, but it is currently known whether this is true, and to what extent it may occur. The devices used in this study are non-invasive and no known adverse events have occurred with use of the stated devices. The findings from this study may result in better understanding of how hormonal fluctuation impacts vascular and cerebrovascular measurement reliability in OC users and help to determine reliable protocol for collecting these measures in this population. The purpose of the study is to measure the changes in the heart, vasculature, brain perfusion and cognitive function across different OC cycle phases.

We seek healthy adults 18-35 years of age, free of cardiometabolic disease, and who do not smoke nor vape. Pregnant women and those who take medications known to alter cardiovascular function are not eligible. A total time commitment of 335 min is required: the study consists of four visits, the first being a familiarization visit (20 min duration) and three experimental visits (100 min duration each).

During all four study visits, participants will be asked to rest in three different postures (supine, semi-recumbent, and seated), while noninvasive, resting cardiovascular and cerebrovascular measurements will be obtained. Participants will then be asked to complete two short cognitive assessments. After these assessments are performed, a small sample of blood will be collected from your arm at each of the three experimental visits.

No significant risks will occur should you take place in this study. Your participation will benefit the scientific body on the assessment of women's health. There is no benefit to you for completing this study, however, we are happy to provide a summary of your results,

including blood pressure, cardiac output, arterial stiffness, carotid blood flow, and cognitive measures, in comparison to group means after the completion of the study.

What are some general things you should know about research studies?

You are being asked to take part in a research study. To join the study is voluntary.

You may choose not to participate, or you may withdraw your consent to be in the study, for any reason, without penalty.

Research studies are designed to obtain new knowledge. This new information may help people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies. Deciding not to be in the study or leaving the study before it is done will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study.

You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?

The purpose of this research study is to explore the (1) overall reliability of measures of cardiovascular, cerebrovascular, and cognitive function across the oral contraceptive cycle and (2) repeated-measures reliability of measures of cardiovascular and cerebrovascular function across the oral contraceptive cycle.

Are there any reasons you should not be in this study?

You should not be in this study if you are a woman not taking an oral contraceptive (birth control pill), if you have known cardiovascular or metabolic diseases (e.g. Congestive heart failure, peripheral artery disease, type I and II diabetes, etc.), you use tobacco or nicotine, take medications known to affect cardiovascular function (e.g. beta-blockers, ACE inhibitors) or you are pregnant.

How many people will take part in this study?

Approximately 40 people at UNC-Chapel Hill will take part in this study.

How long will your part in this study last?

Should you wish to participate in the study, you will be required to attend the Applied Physiology Laboratory at University of North Carolina at Chapel Hill on four occasions across one month. The first visit will last approximately 20 minutes, and three experimental visits last approximately 100 minutes.

What will happen if you take part in the study?

If you would like to take part in the study, you would be required to visit the Applied Physiology Laboratory at UNC, Chapel Hill on four occasions. See below for overall study design:
Visit 1 - The first visit will be a familiarization session during which all experimental procedures will be described to you in full. You will provide informed consent before the study begins, then complete a brief questionnaire on your medical history to ensure you are eligible for this study. If you meet the requirements, we will then show you how each device is prepared for this study, how it functions and where it will be placed on the body for data collection. At the conclusion of the visit, we will take your baseline cognitive assessment for the study. The following devices will be used for study purposes:

- Transcranial Doppler (TCD) – A headset snugly placed on top of the head
- VICORDER® – Non-invasive device using blood pressure cuffs to assess arterial health
- Ultrasound Probe – Small probe lightly placed over several arteries running up the neck to assess blood flow to the brain
- Non-invasive Blood Pressure cuff (NIBP) – Device wrapped around the wrist with small cuffs encircling the middle and index fingers
- Equivital – Chest-worn device and strap that is placed on the skin under a shirt
- USCOM – small, specialized doppler ultrasound that is pressed just above the suprasternal notch (approximately where the neck meets the sternum)
- Pupil Core – eye-tracking device worn like eyeglasses

This visit should take approximately 20 minutes.
Visit 2, 3, and 4 - During the experimental visits (inactive, early active, and late active phases), you will be required to rest quietly for a period of 10 minutes in a supine (lying) position. After, measures of cardiovascular function will be taken by the VICORDER® and Ultrasound devices. Then, you will be passively shifted to a semi-recumbent position where the VICORDER® and Ultrasound measurements will be taken again. Then, you will be passively shifted to a seated position (with legs down) where the VICORDER® and Ultrasound measurements will be taken again. At the end of the cardiovascular measurements in each experimental condition, you will be moved to the supine posture and we will conduct a battery of cognitive tests.
The cognitive tests involved in these three visits are called the Trail-Making Test (TMT) and the Flanker Test. In the TMT, you will complete two short puzzles on an iPad with your finger. You will be presented with numbers and letters placed semi-randomly. You will then connect 25 numbers and numbers, alternating between numbers and letters in numerical or alphabetical order. For example, one would connect "1" to "A", then connect "A" to "2", and "2" to "B" until 25 symbols have been connected. The Flanker Test repeats a singular task to test the domain of

attention. You will be asked to focus on the middle of the screen, and select the direction of a center arrow, from a row of five arrows. Both tests will be conducted electronically, and last less than five minutes.
After cognitive tests, you will have a small sample of blood (30 mL) drawn from a vein in your arm to assess hormonal status.
Prior to attending the Lab for visits 2, 3, and 4, you will have to adhere to the following pre-assessment guidelines:

- Fasted (at least 8 hours), consuming only water.
- No caffeine consumption 12 hours prior to testing
- No vigorous exercise 24 hours prior to testing.
- No alcohol consumption 24 hours prior to testing.

The total time commitment that will be required from you is approximately 335 minutes.
Following the analysis of your data, we will happily provide a summary of your results in comparison to the group means.

What are the possible benefits from being in this study?
Research is designed to benefit society by gaining new knowledge. You will not benefit personally from being in this research study.

What are the possible risks or discomforts involved from being in this study?
The devices used in this study are non-invasive and there are no accounts of severe injury due to exposure to the stated devices. Physical harm due to participation in this study is likely very minimal:

VICORDER® - The system requires the placement of pressure cuffs over several arteries for the collection of PWV/A data. Pressure cuffs will only be inflated underneath a level of 65 mmHg. Physical harm or discomfort is unlikely and include, but are not limited to:
Risk 1: Discomfort/unease: Infrequent (1 – 10%) – Application of a slight pressure over the carotid artery may impose a sense of unease for the participant. However, the light pressure used for this experimental protocol will in no way significantly damage cardiovascular structure or place the participant in danger. Investigators will make certain that communication on the procedures during testing session are clearly conveyed to the participant for comfort and safety.

Transcranial Doppler (TCD): Data collection from this system requires the affixation of a headpiece to the participant. Risk of injury due to this device is extremely low. Possible harms may include, but are not limited to:
Risk 1: Mild headache: Infrequent (1 – 10%) – High quality data from this device requires the placement of the probe over the middle cerebral artery (MCA) and posterior cerebral artery (PCA). The slight pressure applied to the area may be slightly discomforting and unusual for the participant.

Blood Collection: Venipuncture blood draws are relatively safe and low risk following standard safety procedures. Cleaning protocols as well as needle safety will be followed including proper disposal of biohazard waste. Adverse effects for subjects are rare, but include:
Risk 1: Pain or bruising at the site of puncture (14%),

Risk 2: Fainting (<3%).
Blood draw will be performed by a trained phlebotomist and care will be taken to avoid these risks.

Protocol: There will be a change in posture, which may pose risk for those with orthostatic intolerance. However, the postural change will be done slowly to mitigate these effects and should be no different to how a participant typically switches from a supine to seated posture.

Possible risks of postural change include, but are not limited to, the following:

- Rare (<1%) and Mild: Episodes of syncope (fainting)
- Rare (<1%) and Mild: Dizziness or headache.
- Rare (<1%) and Mild: Hypotension (low blood pressure) or hypertension (high blood pressure)
- Rare (<1%) and Mild: Nausea.
- Rare (<1%) and Mild: Palpitations and/or change in heart rate.

There may be uncommon or previously unknown risks. You should report any problems to the researcher.

Before each experimental session, female research participants will be required to take a urine pregnancy test before the beginning of data collection session to assure they are not pregnant. Pregnancy tests will be provided by personnel for all females who might be able to get pregnant.

What if we learn about new findings or information during the study?
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.
The imaging we are using in this research study is not the same quality as imaging that you may have as part of your health care. The images will not be reviewed by a doctor who normally reads such images (such as a radiologist). As a result, you may not be informed of any unexpected findings. The results will not be placed in your medical record. Occasionally the technologist or principal investigator may notice something abnormal on the imaging. If this does occur, the images will be reviewed by a qualified doctor to determine if there is anything of clinical importance. If something is found to be important then you, and/or your primary care provider will be notified. Any further follow up and costs associated with the incidental finding will be your responsibility. There may be benefits to learning such results (such as early detection and treatment of a medical condition), but there are risks as well (such as problems with getting insurance or a job, or feeling worried about a finding for which no treatment is required or appropriate).

Do you wish to be informed in case of clinical/relevant unexpected findings? Please initial in the box below if you do not wish to be notified of clinical/relevant unexpected findings. If you do not initial in the box, you will be notified of any findings.

_____ I do not wish to be notified.

Will I receive any other clinical results?
There are no other clinically relevant results of this research that will be communicated with you.

How will information about you be protected?
The data generated from this study will be used for the purpose of scholarly publication and potentially for research presentation. Your personal data will not be identifiable.
However, there is an inherent risk for a breach of confidentiality due to the sharing of personal information with the research team for research purposes.
Breach of confidentiality will be minimized by limiting the number of research team members in the laboratory during any testing session. By needing key card access to the laboratory, we are limiting the number of individuals not on the research team who have access to the lab. Those who do have key card access are exercise physiology professors, PhD candidates, and Master's candidates, and selected undergraduate students who are directly associated with the study and have performed all necessary trainings regarding sample handling, laboratory procedures, and confidentiality. All participants within the study are coded with an individual ID and no names will be identified in any document besides a master key document. This master key document will be kept in a locked drawer in the Cardiometabolic Laboratory within the Applied Physiology Laboratory.
Participants will not be identified in any report or publication about this study. We may use de-identified data from this study in future research without additional consent.
Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies (for example, the FDA) for purposes such as quality control or safety.
Two video recordings from three simultaneous cameras will be taken during each experimental visit using Pupil Core; two individual recordings of the eyes and the immediate area facing away from the eyes. The recordings of the eyes will be taken by cameras attached to glasses that will be positioned ~1 inch away from the eyes, so each camera will only be recording imagery of eye positioning, as well as gaze information. The away facing camera is also attached to the glasses, and will record the relative field of vision of participants. These videos may include parts of your body such as your hands, if held up close to the face. These videos are a requirement of the study. Full recordings of the face will not be taken during any part of the familiarization or experimental visits.
Check the line that best matches your choice:

OK to record me during the study

_____ Not OK to record me during the study

Participants will not be identified in any report or publication about this study. We may use de-identified data and/or specimens from this study in future research without additional consent.

Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies (for example, the FDA) for purposes such as quality control or safety.

What will happen if you are injured by this research?

All research involves a chance that something bad might happen to you. If you are hurt, become sick, or develop a reaction from something that was done as part of this study, the researcher will help you get medical care, but the University of North Carolina at Chapel Hill has not set aside funds to pay you for any such injuries, illnesses or reactions, or for the related medical care. Any costs for medical expenses will be billed to you or your insurance company. You may be responsible for any co-payments and your insurance may not cover the costs of study related injuries.

If you think you have been injured from taking part in this study, call the Principal Investigator at the phone number provided on this consent form. They will let you know what you should do.

By signing this form, you do not give up your right to seek payment or other rights if you are harmed as a result of being in this study.

What if you want to stop before your part in the study is complete?
You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

If you withdraw or are withdrawn from this study all data collected up until the point of withdrawal will be retained, however no additional information will be collected unless you provide additional written permission for further data collection at the time of your withdrawal.

Will you receive anything for being in this study?
You will not receive anything for taking part in this study.

Will it cost you anything to be in this study?
It will not cost you anything to be in this study.

What if you are a UNC student?

You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be offered or receive any special consideration if you take part in this research.

What if you are a UNC employee?

Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

What if you have questions about this study?

You have the right to ask, and have answered, any questions you may have about this research. If you have questions about the study (including payments), complaints, concerns, or if a research-related injury occurs, you should contact the researchers listed on the first page of this form.

What if you have questions about your rights as a research participant?

All research on human volunteers is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject, or if you would like to obtain information or offer input, you may contact the Institutional Review Board at 919-966-3113 or by email to IRB_subjects@unc.edu.

Participant's Agreement:

I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

_____
Signature of Research Participant

_____
Date

_____
Printed Name of Research Participant

_____
Signature of Research Team Member Obtaining Consent

_____
Date

_____
Printed Name of Research Team Member Obtaining Consent

_____
Signature of Witness if applicable; e.g. literacy issues, visually impaired, physically unable to sign, witness/interpreter for non-English speaking participants using the short form)

_____
Date

_____
Printed Name of Witness

APPENDIX C. PRE-ASSESMENT GUIDELINE AND VISIT REMINDERS

FAMILIARIZATION REMINDER

Hello [PARTICIPANT],

This is a reminder email that you have your familiarization visit in the study "Reliability of cardiovascular and cerebrovascular measures across the oral contraceptive cycle" on [DATE] from [TIME]. Please refrain from drinking alcohol 8 hours before your visit.

Please email us if you have any questions,

Nate Adams and Jillian Poles

EXPERIMENTAL VISIT REMINDER

Hello [PARTICIPANT],

This is a reminder email that you have your upcoming visit in the study "Reliability of cardiovascular and cerebrovascular measures across the oral contraceptive cycle" on [DATE] from [TIME]. Please remember to follow the pre-assessment guidelines listed below.

· Fasted (> 8 hours), consuming only water.

· No caffeine consumption 12 hours prior to testing

· No vigorous exercise 24 hours prior to testing.

· No alcohol consumption 12 hours prior to testing.

Please email us if there are any conflicts or you need a parking pass.

Nate Adams and Jillian Poles

APPENDIX D. RECRUITMENT MATERIALS

SCRIPT FOR CLASS RECRUITMENT

Class Recruitment Script:

Hello, my name is (member of research team) and I am a (role in department) in the Exercise and Sport Science department. Thank you for your interest in regards to our study entitled, "Reliability of cardiovascular and cerebrovascular measures across the oral contraceptive cycle." Please see the additional information about the study below:

The Cardiometabolic Lab research team is looking to determine changes in heart and vascular function across the oral contraceptive cycle, both in general and in the arteries that supply the brain. If this system is better understood, the knowledge can be used to determine how to reliably measure women's health, specifically in oral contraceptive users.
To be able to take part in the study, individuals must be **men or women** aged **18 to 35 years**, **non-smokers,** and be **free of cardio-metabolic disorders** (e.g. diabetes). Additionally, women participating in the study must currently be using birth control pills.

If you would like to take part in the study, you would be required to visit the Applied Physiology Laboratory at UNC, Chapel Hill on two occasions:

> Visit 1 - The first visit will be a familiarization session during which all experimental procedures will be described to you in full (approx. 20 mins). We will take some baseline biometric measurements to be used in the experimental sessions, including height and weight.
> Visits 2, 3, and 4 - During visits 2, 3, and 4, you will be required to rest quietly for a period of 10 minutes in a supine (lying) posture, and your cardiovascular and cerebrovascular health will be assessed. Then you will be passively shifted to a semi-recumbent (half upright) position. You will remain in this position for 5 minutes before the prior measurements are repeated. Then you will be passively shifted to a seated (upright, legs down) position. You will remain in this position for 5 minutes before measurements are repeated again. Then, you will undergo a cognitive assessment with simultaneous eye-tracking. Finally, you will have a small blood sample drawn from a vein in your arm. Each visit should last 100 minutes in total. Following the analysis of your data, will happily provide a summary of your results in comparison to the group means.

The total time commitment that will be required from you is approximately 335 minutes. Following the analysis of your data, we will happily provide a summary of your results in comparison to the group means.
If you are interested in participating or have any questions about the study, please contact Jillian Poles or Nathan Adams via Email: jpoles@email.unc.edu or adamsnt@email.unc.edu or Tel: 914-815-7830

Kind Regards,

EMAIL RECRUITMENT

General Information

Email list source: [UNC Email Database]

Target population: Staff and students at UNC, Chapel Hill.

Email frequency: Staff and students at the University will only be emailed details of the study on a single occasion.

Email Subject Information: Seeking Participants for an Exercise and Sport Science Research Study

Researchers in the Department of Exercise and Sport Science (EXSS) at University of North Carolina (UNC) are seeking individuals who would like to participate in a research study. You are receiving this email because you are a staff member or a student at the University of North Carolina, Chapel Hill. Your email address was obtained from the UNC [email database].

The Cardiometabolic Lab research team is looking to determine changes in heart and vascular function across the oral contraceptive cycle, both in general and in the arteries that supply the brain. If this system is better understood, the knowledge can be used to determine how to reliably measure women's health, specifically in oral contraceptive users.

To be able to take part in the study, individuals must **men or women aged 18-35 years, non vapers/smokers,** and **free of cardio-metabolic disorders** (e.g. diabetes). **Women** participating in the study **must currently be using a birth control pill.** Additionally, individuals must **not be pregnant** nor **take medications known to affect cardiovascular function.**

If you would like to take part in the study, you would be required to visit the Applied Physiology Laboratory at UNC, Chapel Hill on two occasions:

> Visit 1 - The first visit will be a familiarization session during which all experimental procedures will be described to you in full (approx. 20 mins). We will take some baseline biometric measurements to be used in the experimental sessions, including height and weight.

Visits 2, 3, and 4 - During visits 2, 3, and 4, you will be required to rest quietly for a period of 10 minutes in a supine (lying) posture, and your cardiovascular and cerebrovascular health will be assessed. Then you will be passively shifted to a semi-recumbent (half upright) position. You will remain in this position for 5 minutes before the prior measurements are repeated. Then you will be passively shifted to a seated (upright, legs down) position. You will remain in this position for 5 minutes before measurements are repeated again. Then, you will undergo a cognitive assessment with simultaneous eye-tracking. Finally, you will have a small blood sample drawn from a vein in your arm. Each visit should last 100 minutes in total. Following the analysis of your data, will happily provide a summary of your results in comparison to the group means.

Prior to attending the Lab for visits 2, 3, and 4, you will have to perform the following pre-assessment guidelines:

- Fasted (> 8 hours), consuming only water.
- No caffeine consumption 12 hours prior to testing
- No vigorous exercise 24 hours prior to testing.
- No alcohol consumption 12 hours prior to testing.

The total time commitment that will be required from you is approximately 335 minutes. Following the analysis of your data, we will happily provide a summary of your results in comparison to the group means.

If you are interested in participating or have any questions about the study, please contact Jillian Poles or Nathan Adams via Email: jpoles@email.unc.edu or adamsnt@email.unc.edu or Tel: 914-815-7830

Kind Regards,

Jillian Poles

Nathan Adams

RESEARCH FOR ME @ UNC STUDY LISTING

Study Listing Form - Research for Me @UNC

Welcome to your Research for Me @UNC study listing form! Research for Me @ UNC is a resource to improve transparency of research at UNC-Chapel Hill and UNC Health and connect the public with research opportunities. Listings will be publicly accessible on Research for Me @UNC (researchforme.unc.edu).

Basic listings: Required for all UNC research directly involved with human subjects. This listing will display only general information about your study. No contact information will be visible to the public.Recruitment listings: Write your listing to appeal to the target audience for your study. Please be sure to keep information on your listing up-to-date, as potential participants will be able to contact you about taking part in your study. Recruitment Listing PDFs must be attached to your IRB application for review, as with any recruitment material.For studies where UNC is a Data Coordinating Center only or studies where enrollment is closed (and for any other questions), please reach out to our team.

Response was added on 10/01/2021 8:03am.

IRB Reference ID	339015
IRB Number (XX-XXXX)	21-2465

Study Listing Information

Listings will be visible from the time of IRB approval until the time that you indicate to the IRB that the study has officially closed to enrollment.

Study Nickname Only visible to study team on the Researcher Dashboard. Will not display on the public site.	Oral contraceptive reliabillity
Short Study Title This title will display on the public site and should be presented at an 8th grade reading level.	Reliability of heart and brain function across the oral contraceptive cycle (Title should be general (e.g. Diabetes Weight Loss Study), not the protocol or IRB study title.)
Study Purpose Briefly describe the purpose of your study at an 8th grade reading level - what are you doing and why?	People taking birth control pills are sometimes only able to participate in research studies during their inactive phase due to the belief that changes in your hormones may affect physiological measurements. In this study, we want to find out if brain and heart function changes across the oral contraceptive (birth control) cycle. If you are a man or woman currently taking an oral contraceptive (birth control pill), you may be able to participate.
Specific condition related to or the focus of this study If unrelated to a health or behavioral condition, please write "no condition"	No condition (e.g. High Blood Pressure, Pregnancy, Menopause, etc.)

Select all topics that reasonably relate to or describe this study. If you don't feel that any adequately categorize your study, please select the closest one and then enter a suggestion for an alternate category below.	☒ Healthy Volunteer or General Population ☐ COVID-19 ☐ UNC or UNC Health employees ☒ UNC Students (undergrad, grad, professional) ☐ Aging ☐ Allergy ☐ Alternative or Complementary Medicine ☐ Behavior ☐ Blood Conditions ☒ Blood Pressure ☐ Bones, Joints, Muscles ☒ Brain, Head, Nervous System ☐ Cancer ☐ Child and Teen Health ☐ Chronic Conditions ☐ Developmental ☐ Diabetes ☐ Stomach, Digestion and Gut Health ☐ Ear, Nose, and Throat ☐ Eating, Nutrition, and Metabolism ☐ Environment ☐ Eyes and Vision ☐ Genetics and Genetic Disorders ☒ Glands and Hormones ☐ Hands and Feet ☒ Heart and Circulation ☐ HIV/AIDS ☐ Immune System/Infections ☐ Injury/Injury Prevention ☐ Kidneys and Liver ☐ Language and Speech ☐ LGBTQIA+ ☐ Lungs and Breathing ☐ Mental and Emotional Health ☒ Men's Health ☐ Microbiome ☐ Minority Health ☐ Mouth and Teeth ☐ Movement ☐ Obesity ☐ Opinions and Perceptions ☐ Pain ☐ Parents of Children ☐ Physical Disability ☐ Precision Medicine ☐ Pregnancy ☐ Rare Diseases ☒ Sexual and/or Reproductive Health ☐ Skin, Hair, and Nails ☐ Sleep ☐ Social or Workplace Dynamics ☐ Substance Use (tobacco, alcohol, opioids, etc) ☐ Surgery and post-operative healing ☐ Transplant ☐ Urinary and Bladder ☐ Veteran Health / Military ☒ Wellness and Lifestyle ☒ Women's Health (You must select at least 1 topic. You may select as many as are appropriate to your study. This will help people find your study on the website.)
If you didn't see a topic that describes your study well, please suggest an additional category	_____

This study can include participants whose birth-assigned sex is: (select all that apply)	☒ Male ☒ Female
Is your study able to include participants whose gender identity is different from their birth-assigned sex?	○ Yes ⊗ No
Minimum Participant Age	18 (If no limit, enter "0")
	⊗ years ○ months ○ weeks ○ days
Maximum Participant Age	35 (If no limit, enter "99")
	⊗ years ○ months ○ weeks ○ days
Able to consent and complete study activities in these languages: (select all that apply)	☒ English ☐ Spanish ☐ French ☐ German ☐ Chinese ☐ Vietnamese ☐ Arabic ☐ Other language(s)
From where is the UNC study team seeking participants? (select all that apply)	☐ 100% remote (online, phone, text) ☐ North Carolina ☒ United States ☐ International Location (Note: do NOT include other locations if this is a multi-site trial. Only include locations which are run by the UNC study team.)
Are you only able to accept participants from specific NC counties?	○ Yes ⊗ No

Which of the following best describes your study?

Choose one	⊗ Clinical or Medical ○ Behavioral or Social
Choose one	○ Interventional ⊗ Observational ○ Registry

Study Identifiers	
Will this study be listed on ClinicalTrials.gov?	◯ Yes ⊗ No
Existing Study or Departmental Website URL: (please begin with "https://")	_____ (This field should NOT be used for any screening or data collection instruments.)
Do you want potential participants to be able to contact the study team directly to express interest in participating?	⊗ Yes ◯ No (Note: this field can only be changed by selecting a different listing type in your IRB application.)

Extended Recruitment Information

The basic information (above) will display while your study is active, the extended recruitment information (below) will only display during the date range which you specify.

Provide ONLY information that can help a potential participant determine if they might be a good fit for this study. Do not include information which they cannot reasonably evaluate for themselves.

Use language that is understandable at an 8th grade reading level. Avoid jargon, undefined acronyms, or highly technical language.

Recruitment Period Start	2021-09-14 (Enter the date that you would like the additional recruitment information to appear on the listing. If "Today" is selected, recruitment information will appear when the study is IRB approved.)
Recruitment Period End	2022-09-01 (Enter the anticipated date that the visibility of the recruitment information should end.)
Would you like to include a link to an IRB-approved online screening tool, survey link, or registry link?	⊗ Yes ◯ No
Indicate which type:	⊗ Online screening tool ◯ Survey-only study link (with online consent) ◯ Link to Join a Registry (with online consent)
Online screening survey link	_____ (If you have an IRB-approved online study screening tool, you may link to it here. Please begin with "https://" and ONLY include a link in this field)

Introduce the basics of your study

Recruitment Pitch Craft a "pitch" that will show on the search results page. Be direct and focus on what matters to them, hooking their interest enough to click on the link to open up the full listing information.	Are you or a woman currently using an oral contraceptive or a man? You may be able to take part in a study to learn if measurements of your heart and brain function change across the oral contraceptive cycle. (Example: Do you know your blood pressure? Do you sit too much at work? If you have a desk job and higher than normal blood pressure (but are not taking blood pressure medication), you may be able to participate in a research study to find out if sitting less during the workday can lower blood pressure. Compensation provided.)
Total Length of Participation	5 hours over the course of 1 month (Use common blocks of time. e.g. "4 months" instead of "16 weeks")
Will participants need to attend in-person visits (either at a clinic or other physical location)?	⊗ Yes ○ No
Number of study visits done In-Person	4 (If none, enter "0". If a range, enter "x-x")
Number of study visits done completely by Phone or Online	0 (If none, enter "0". if a range, enter "x-x" or "up to xx")
What will you ask of the participant? example: "At each visit, you will meet with a member of the study team and fill out questionnaires. The study also involves 2 blood draws, 1 MRI, and willingness to take the study medication regularly."	At one familiarization visit, you will meet with a member of the study team, fill out questionnaires, have your height and weight taken, and become familiar with the experimental measures. At each of the three experimental visits, you will have your heart and brain function assessed in a series of tests. The study will also involve 3 blood draws. (This should be very broad - hit the highlights of the kinds of things they'll be asked to do so that they are not surprised and can adequately determine if it's something they might be interested in.)
When are visits available?	☒ Standard business hours (M-F, 8-5) ☒ Extended hours (M-F, early morning or evening) ☐ Visits can be combined with regular clinical appointments ☒ Weekend hours, if needed ☐ In-home visits available ☐ Study is 100% remote - no in-person visits
Will participants receive anything for their time in the study?	○ Yes ⊗ No

Who are you recruiting?

You may list up to 5 main inclusion criteria and 5 main exclusion criteria for each cohort. Screening for full eligibility should be completed when interacting with participant or within a screener.

Be brief and avoid jargon Please exclude age and gender (displayed elsewhere on the listing) Avoid redundency (e.g. if pregnancy is an inclusion criteria, do not also list "not pregnant" as an exclusion critieria) Do NOT copy and paste the criteria from your protocol - select the main points that can help someone decide if they might be a good fit and write them in understandable language Criteria will be displayed on the site as a bulleted list

Is this study recruiting people with a specific condition(s)?	◯ Yes ⊗ No
Please enter your inclusion exclusion criteria for your study population	
You might be a good fit for this study if: (enter main inclusion criteria)	
1.	You are a cisgender male or female between the ages of 18-35 years of age
2.	You are healthy (no cardiometabolic conditions such as diabetes, not using any medication known to affect cardiovascular function, such as blood pressure medication)
3.	If you are a woman, you have been using an oral contraceptive for at least 6 months
4.	You are not pregnant or planning to become pregnant within the next few months
5.	You are recreationally active (1-10 hours of activity per week)
This study is not right for you if: (enter main exclusion criteria)	
1.	You have any cardiometabolic conditions (such as diabetes) or are using any medication known to affect cardiovascular function (such as blood pressure medication) (If none, enter "None")
2.	You have known cognitive impairment
3	You smoke or vape
4	You are pregnant or planning to become pregnant, or had previous pregnancy complications

5	You are a woman not currently using an oral contraceptive

Where will visits be conducted? Who can they contact to learn more?

Potential participants can express interest in joining your study by calling, emailing, or sending a notification via the website. The individual you name below will be the point of contact for these inquiries.

How many visit locations do you offer?	⊗ 1 ◯ 2 ◯ More than 2 ◯ N/A - no in-person contact (You will only be able to provide details for 2 locations, currently.)
Primary Contact Name	Jillian Poles (Enter the name as entered in the IRB application.)
Primary Contact Phone	(914) 815-7830 (*PLEASE DOUBLE CHECK THIS NUMBER IS CORRECT*)
Primary Contact Email	jpoles@email.unc.edu
Primary Location - Name	UNC Applied Physiology Laboratory (e.g., "Clinical & Translational Research Center," "UNC Urology Clinics," "UNC Health Care", "Local library", "Your home" etc.)
Primary Visit Location - display via Google Map	Fetzer Hall, Chapel Hill, NC, USA (Begin typing your location and select the appropriate option from the drop down. e.g., "Burnett-Womack Building" for CTRC, "UNC Urology", "Chapel Hill Public Library", "Carr Mill Mall", "Davie Hall", etc. If you wish to indicate UNC Hospitals without mentioning a specific clinic, type "UNC Health Care")
Would you be okay with us featuring or posting about your study on Research for Me social media?	⊗ Yes ◯ No

Please review the information below regarding the Research for Me team posting about your study on social media: 1. So we can ensure the language in your listing can also be used on social media, please check the box in the following field for a free listing optimization. 2. The approved language from your listing will be used to write a short description of your study. A link will be provided to your listing. 3. The Research for Me team will select an appropriate photo to be displayed alongside the social media post. 4. A post about your study will go live during the listed recruitment window on this form, but only after IRB approval. We will notify you during the month your listing will be posted on RFM Facebook and Twitter.	☒ I agree
The Recruitment and Retention Program at NC TraCS offers optional, free review and revision of your listing for recruitment optimization. We will turn these around within 2 business days to allow you to confirm updates and attach the revised PDF to your IRB application for approval.	☐ Check here to request listing optimization from Participant Recruitment Specialist (free)
ONYEN or Epic user ID of the person completing this form	jpoles (This allows the listing record to be visible on your Researcher Dashboard before IRB approval, in case revisions are needed.)
Email of person completing this form To receive a confirmation email with the listing PDF. Must be a valid UNC or UNC Health Care address	jpoles@email.unc.edu (If your email is already indicated as either the PI or contact email, you may include the email of an additional team member who should receive an email confirmation of this listing.)
Principal Investigator Email To receive a confirmation email with the listing PDF.	jpoles@email.unc.edu (Enter the PI's Email as listed in the IRB application. It will not be shared with the public.)

Initial IRB application? Need another team member to add information before you submit?

Simply enter the ONYEN or Epic User ID for that team member here to give them access to the form via their Researcher Dashboard. Please only include 1 person's information.

adamsnt
(*For studies that are already IRB approved, all study team members have access to this form via Researcher Dashboard - just let them know they need to go in and edit.)

It is the responsibility of the study team to keep this listing in compliance with changes made to your IRB application. After initial submission, updates to this form should be made via researcherdashboard.unc.edu.

☒ I understand

Please verify your humanity below and then "submit" to save your information to the listing database.

An email confirmation with a PDF of this form will be emailed to the contacts listed above upon submission.

RECRUITMENT FLYER

Volunteers needed for a research study to investigate the effects of oral contraceptives on brain and heart function!

We are looking for healthy men and women who are:

- *Aged 18 - 35 years*
- *Non-smoking*
- *No known cardio-metabolic disorders*
- *Not currently taking medications known to affect cardiovascular function*
- *Not pregnant*
- ***Women using oral contraceptives***

Study participation involves 4 visits to the Applied Physiology Lab, where participants will rest for a 100-minute measurement protocol.

Following the analysis of your data, we will happily provide a summary of your individualized results.

For more information, please contact:

Jillian Poles
Email: jpoles@email.unc.edu
or
Nathan Adams
Email: adamsnt@email.unc.edu

EXSS Exercise and Sport Science

CML UNC CardioMetabolic Lab

IRB: 21-2465

RECRUITMENT FOLLOW UP EMAIL

Follow-up email script:

Hello, my name is (member of research team) and I am a (role in department) in the Exercise and Sport Science department. Thank you for your interest in regards to our study entitled, "Reliability of Cardiovascular and Cerebrovascular Measures Across the Oral Contraceptive Cycle." Please see the additional information about the study below:

The Cardiometabolic Lab research team is looking to determine changes in heart and vascular function across the oral contraceptive cycle, both in general and in the arteries that supply the brain. If this system is better understood, the knowledge can be used to determine how to reliably measure women's health, specifically in oral contraceptive users.
To be able to take part in the study, individuals must be **not use nicotine** (eg. vaping, smoking)**,** aged **18 to 35 years** and be **free of cardio-metabolic disorders** (e.g. diabetes). Additionally, Individuals must **not be pregnant** nor **take medications known to affect cardiovascular function.**

If you would like to take part in the study, you would be required to visit the Applied Physiology Laboratory at UNC, Chapel Hill on two occasions:

Visit 1 - The first visit will be a familiarization session during which all experimental procedures will be described to you in full (approx. 20 mins). We will take some baseline biometric measurements to be used in the experimental sessions, including height and weight.

Visits 2, 3, and 4 - During visits 2, 3, and 4, you will be required to rest quietly for a period of 10 minutes in a supine (lying) posture, and your cardiovascular and cerebrovascular health will be assessed. Then you will be passively shifted to a semi-recumbent (half upright) position. You will remain in this position for 5 minutes before the prior measurements are repeated. Then you will be passively shifted to a seated (upright, legs down) position. You will remain in this position for 5 minutes before measurements are repeated again. Then, you will undergo a cognitive assessment with simultaneous eye-tracking. Finally, you will have a small blood sample drawn from a vein in your arm. Each visit should last 100 minutes in total. Following the analysis of your data, will happily provide a summary of your results in comparison to the group means.

Prior to attending the University for visit 2 and 3, you will have to perform the following:

- Refrain from exercise for a period of 12 hours prior.
- Consume nothing but water for a period of 8 hours prior
- Avoid consuming alcohol and caffeine for 12 hours prior.
- Wear comfortable clothes to the visit, such that your calves can be exposed easily
- Do not wear lotion, sunscreen, or deodorant on the chest
- Avoid heavy make-up or mineral sunscreen on the forehead or temples

- Have at least index finger free of nail polish or other varnish on the fingernail

The total time commitment that will be required from you is approximately 335 minutes. Following the analysis of your data, we will happily provide a summary of your results in comparison to the group means.

If you are interested in participating or have any questions about the study, please contact Jillian Poles or Nathan Adams via Email: jpoles@email.unc.edu or adamsnt@email.unc.edu or Tel: 914-815-7830

Kind Regards,

Jillian Poles

Nathan Adams

SCREENING FORM

OC Screening

Start of Block: Default Question Block

Q1 Please provide us with your name and email:

_____

Q2 What is your current age?

_____

Q3 Do you currently smoke cigarettes or vape?

- ○ Yes (1)
- ○ No (2)

Q4 Have you been diagnosed with a cardiometabolic disease? (e.g.; diabetes, high blood pressure)

- ○ Yes (1)
- ○ No (2)

Q5 Are you currently taking any medications known to impact cardiovascular function? (e.g.; blood pressure lowering medications)

- ○ Yes (1)
- ○ No (2)

Q11 What is your biological sex?

- Male (1)
- Female (3)

Display This Question:

If What is your biological sex? = Female

Q6 Are you currently pregnant or planning to become pregnant within the next year?

- Yes (1)
- No (2)

Display This Question:

If What is your biological sex? = Female

Q8 Are you currently using a birth control pill?

- Yes (1)
- No (2)

Display This Question:

If Are you currently using a birth control pill? = Yes

Q9 Is your birth control pill one of the following:

Yasmin, Ortho-cyclen, Microgestin 1.5/30, Seasonale, Apri, Sprintec, Lo/Ovral, Necon 1/35, Levora, Ortho-Novum 1/35, Desogen, Ortho-Cept, Mononesessa, Seasonique, Zovia 1/50, Loestrin 21 1.5/30, Cryselle, Ovcon 35, Levlen, Nortrel 28, Oegstrel, Low-Ogestel, Portia, Femcon FE, Ortho-Novum 1/50, Zovia 1/35E, Nordette, Norethin 1/35E, Ocella, Nortrel 0.5/0.035, Ovcon 50, Reclipsen, Necon 1/50, Demulen 1/35, Modicon, Kelnor, Jolessa, Demulen 1/50, Junel 30, Junel FE 30, Norinyl 1+35, Ovral, Nortrel, Zenchent, Previfem, Norinyl 1/50, Balziva.

- ○ Yes (4)
- ○ No (5)
- ○ I'm not sure (6)

End of Block: Default Question Block

APPENDIX E. COGNITIVE ASSESMENT INSTRUCTIONS

TRAIL-MAKING TEST INSTRUCTIONS

"This test is called the Trail-Making Test. You are going to see 26 dots on your screen. Your task is to select them by tapping with your finger in an alpha-numeric order starting with 1, going to A, 2, B, and so on until you have connected all of the dots. You will know you have connected the dots when you see a line appear between them. Whenever you are ready, you can hit start test 'type b', skip to test, and the test will count you down."

FLANKER TASK INSTRUCTIONS

"The next task is called the flanker task. This is a test of your reaction time. You are going to see a string of five letters on your screen. They might all be the same, such as C, C, C, C, C, or the one in the middle might be different such as the example you see on your screen, X, X, C, X, X. Your task is to identify the letter in the middle. If you see the letter in the middle is an X or a C, I would like you to hit this button with your left index finger. If you see the letter in the middle is a V or a B, I would like you to hit this button with your right index finger. Whenever you are ready, you can hit one of the start buttons with your right thumb."

APPENDIX F. SAMPLE SIZE CALCULATION

SAMPLE SIZE CALCULATION

[16] *-- Thursday, March 18, 2021 -- 22:27:03*

z tests - Correlations: Two independent Pearson r's

Analysis:	A priori: Compute required sample size		
Input:	Tail(s)	=	One
	Effect size q	=	0.9229133
	α err prob	=	0.05
	Power (1-β err prob)	=	0.80
	Allocation ratio N2/N1	=	1
Output:	Critical z	=	1.6448536
	Sample size group 1	=	18
	Sample size group 2	=	18
	Total sample size	=	36
	Actual power	=	0.8112869

REFERENCES

1. Elliott-Sale, K. J. *et al.* Methodological Considerations for Studies in Sport and Exercise Science with Women as Participants: A Working Guide for Standards of Practice for Research on Women. *Sport. Med.* **51**, 843–861 (2021).

2. Aaslid, R. & Markwalder, T.-M. *Noninvasive transcranial Doppler ultrasound recording of flow velocity in basal cerebral arteries*. *J Neurosurg* vol. 57 (1982).

3. Mcdonnell, M. N. *et al.* Transcranial Doppler ultrasound to assess cerebrovascular reactivity: reliability, reproducibility and effect of posture. *PeerJ* **1**, e65 (2013).

4. Pelligrino, D. A. & Galea, E. Estrogen and cerebrovascular physiology and pathophysiology. *Jpn. J. Pharmacol.* **86**, 137–158 (2001).

5. Abidi, S. *et al.* Influence of sex, menstrual cycle, and oral contraceptives on cerebrovascular resistance and cardiorespiratory function during Valsalva or standing. *J. Appl. Physiol.* **123**, 375–386 (2017).

6. Nili, M., Abidi, S., Serna, S., Kim, S. & Edgell, H. Influence of sex, menstrual cycle, and oral contraceptives on the cerebrovascular response to paced deep breathing. *Clin. Auton. Res.* **27**, 411–415 (2017).

7. Koo, T. K. & Li, M. Y. A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. *J. Chiropr. Med.* **15**, 155–163 (2016).

8. Vanzetta, I. & Grinvald, A. Increased cortical oxidative metabolism due to sensory stimulation: Implications for functional brain imaging. *Science (80-.).* **286**, 1555–1558 (1999).

9. Thompson, J. K., Peterson, M. R. & Freeman, R. D. Single-neuron activity and tissue oxygenation in the cerebral cortex. *Science (80-.).* **299**, 1070–1073 (2003).

10. Offenhauser, N., Thomsen, K., Caesar, K. & Lauritzen, M. Activity-induced tissue oxygenation changes in rat cerebellar cortex: Interplay of postsynaptic activation and blood flow. *J. Physiol.* **565**, 279–294 (2005).

11. Ruitenberg, A. *et al.* Cerebral hypoperfusion and clinical onset of dementia: The Rotterdam study. *Ann. Neurol.* **57**, 789–794 (2005).

12. Sabayan, B. *et al.* Cerebrovascular hemodynamics in Alzheimer's disease and vascular dementia: A meta-analysis of transcranial Doppler studies. *Ageing Res. Rev.* **11**, 271–277 (2012).

13. Lee, C. H., Jeon, S. H., Wang, S. J., Shin, B. S. & Kang, H. G. Factors associated with temporal window failure in transcranial Doppler sonography. *Neurol. Sci.* **41**, 3293–3299 (2020).

14. Chadwick, K. D., Burkman, R. T., Tornesi, B. M. & Mahadevan, B. Fifty Years of "the Pill": Risk Reduction and Discovery of Benefits Beyond Contraception, Reflections, and Forecast. *Toxicol. Sci.* **125**, 2–9 (2012).

15. Fan, J.-L. *et al.* Integrative physiological assessment of cerebral hemodynamics and metabolism in acute ischemic stroke. *J. Cereb. Blood Flow Metab.* 0271678X2110337 (2021) doi:10.1177/0271678x211033732.

16. CDC. FastStats - Contraceptive Use. https://www.cdc.gov/nchs/fastats/contraceptive.htm (2016).

17. Benagiano, G., Bastianelli, C. & Farris, M. Hormonal contraception: Present and future. *Drugs of Today* vol. 44 905–923 (2008).

18. Sims, S. T., Heather, A. K. & Stacy Sims, C. T. Myths and Methodologies: Reducing scientific design ambiguity in studies comparing sexes and/or menstrual cycle phases. *Exp. Physiol.* **103**, 1309–1317 (2018).

19. De Leo, V., Musacchio, M. C., Cappelli, V., Piomboni, P. & Morgante, G. Hormonal contraceptives: pharmacology tailored to women's health. *Hum. Reprod. Update* **22**, 634–646 (2016).

20. Kaunitz, A. M. Menstruation: Choosing whether ... and when. *Contraception* **62**, 277–284 (2000).

21. Hee, L., Ozer Kettner, L., Vejtorp, M. & Lene Hee, C. Continuous use of oral contraceptives: an overview of effects and side-effects. doi:10.1111/aogs.12036.

22. Weisberg, E. *Prescribing Oral Contraceptives*. *Current Therapeutics* vol. OCT (1997).

23. Priest, S. E., Shenouda, N. & MacDonald, M. J. Effect of sex, menstrual cycle phase, and monophasic oral contraceptive pill use on local and central arterial stiffness in young adults. *Am. J. Physiol. - Hear. Circ. Physiol.* **315**, H357–H365 (2018).

24. Peltonen, G. L. *et al.* Cerebral blood flow regulation in women across menstrual phase: differential contribution of cyclooxygenase to basal, hypoxic, and hypercapnic vascular tone. *Am. J. Physiol. Integr. Comp. Physiol.* **311**, R222–R231 (2016).

25. Krejza, J., Rudzinski, W., Arkuszewski, M., Onuoha, O. & Melhem, E. R. Cerebrovascular reactivity across the menstrual cycle in young healthy women. *Neuroradiol. J.* **26**, 413–419 (2013).

26. Krejza, J., Mariak, Z., Huba, M., Wolczynski, S. & Lewko, J. Effect of endogenous estrogen on blood flow through carotid arteries. *Stroke* **32**, 30–36 (2001).

27. Krejza, J. *et al.* Oscillations of cerebrovascular resistance throughout the menstrual cycle in healthy women. *Ultrasound Obstet. Gynecol.* **22**, 627–632 (2003).

28. Ashraf, M. S. & Vongpatanasin, W. Estrogen and Hypertension. (2006).

29. Dehaini, H. *et al.* Estrogen in vascular smooth muscle cells: A friend or a foe? *Vascul. Pharmacol.* **111**, 15–21 (2018).

30. Somani, Y. B., Pawelczyk, J. A., Souza, M. J. De, Kris-Etherton, P. M. & Proctor, D. N. Aging women and their endothelium: probing the relative role of estrogen on vasodilator function. *https://doi.org/10.1152/ajpheart.00430.2018* **317**, H395–H404 (2019).

31. Dubey, R. K., Oparil, S., Imthurn, B. & Jackson, E. K. *Sex hormones and hypertension*. *Cardiovascular Research* vol. 53 www.elsevier.com/locate/cardiores (2002).

32. Novella, S., Dantas, A. P., Segarra, G., Medina, P. & Hermenegildo, C. Vascular Aging in Women: is Estrogen the Fountain of Youth? *Front. Physiol.* **0**, 165 (2012).

33. Kannel, W. B., Hjortland, M. C., McNamara, P. & Gordon, T. Menopause and risk of cardiovascular disease. The Framingham study. *Ann. Intern. Med.* **85**, 447–452 (1976).

34. Reckelhoff, J. F. & Fortepiani, L. A. Novel Mechanisms Responsible for Postmenopausal Hypertension. *Hypertension* **43**, 918–923 (2004).

35. Dos Santos, R. L., Da Silva, F. B., Ribeiro, R. F. & Stefanon, I. Sex hormones in the cardiovascular system. *Hormone Molecular Biology and Clinical Investigation* vol. 18 89–103 (2014).

36. Hulley, S. *et al.* Randomized Trial of Estrogen Plus Progestin for Secondary Prevention of Coronary Heart Disease in Postmenopausal Women. *JAMA* **280**, 605–613 (1998).

37. Writing Group for the Women's Health Initiative Investigators, W. G. for the W. H. I. I. Risks and Benefits of Estrogen Plus Progestin in Healthy Postmenopausal Women: Principal Results From the Women's Health Initiative Randomized Controlled Trial. *JAMA* **288**, 321–333 (2002).

38. Minson, C. T., Halliwill, J. R., Young, T. M. & Joyner, M. J. *Sympathetic Activity and Baroreflex Sensitivity in Young Women Taking Oral Contraceptives*. http://www.circulationaha.org (2000).

39. Yu, A. *et al.* The effect of oral Contraceptive pills and the natural menstrual cYCLe on arterial stiffness and hemodynamICs (CYCLIC). *J. Hypertens.* **32**, 100–107 (2014).

40. Nevo, O., Soustiel, J. F. & Thaler, I. Cerebral blood flow is increased during controlled ovarian stimulation. *Am. J. Physiol. Circ. Physiol.* **293**, H3265–H3269 (2007).

41. Marečková, K. *et al.* Hormonal contraceptives, menstrual cycle and brain response to faces. *Soc. Cogn. Affect. Neurosci.* **9**, 191–200 (2014).

42. Phillips, A. A. *et al.* Neurovascular coupling in humans: Physiology, methodological advances and clinical implications. doi:10.1177/0271678X15617954.

43. Larson, E. B., Kukull, W. A. & Katzman, R. L. Cognitive impairment: Dementia and Alzheimer's disease. *Annu. Rev. Public Health* **13**, 431–449 (1992).

44. Wolters, F. J. *et al.* Twenty-seven-year time trends in dementia incidence in Europe and the United States: The Alzheimer Cohorts Consortium. *Neurology* **95**, e519–e531 (2020).

45. Andersen, K. *et al.* Gender differences in the incidence of AD and vascular dementia: The EURODEM Studies. *Neurology* **53**, 1992–1997 (1999).

46. Seshadri, S. *et al.* Lifetime risk of dementia and Alzheimer's disease: The impact of

mortality on risk estimates in the Framingham Study. *Neurology* **49**, 1498–1504 (1997).

47. Rocca, W. A., Mielke, M. M., Vemuri, P. & Miller, V. M. Sex and gender differences in the causes of dementia: A narrative review. *Maturitas* **79**, 196–201 (2014).

48. Carter, C. L., Resnick, E. M., Mallampalli, M. & Kalbarczyk, A. Sex and gender differences in Alzheimer's disease: Recommendations for future research. *J. Women's Heal.* **21**, 1018–1023 (2012).

49. Levine, D. A. *et al.* Sex Differences in Cognitive Decline among US Adults. *JAMA Netw. Open* **4**, e210169–e210169 (2021).

50. Mordecai, K. L., Rubin, L. H. & Maki, P. M. Effects of menstrual cycle phase and oral contraceptive use on verbal memory. *Horm. Behav.* **54**, 286–293 (2008).

51. Wang, J. & Chen, A. High progesterone levels facilitate women's social information processing by optimizing attention allocation. *Psychoneuroendocrinology* **122**, (2020).

52. Upadhayay, N. & Guragain, S. Comparison of Cognitive Functions Between Male and Female Medical Students: A Pilot Study. *J. Clin. Diagn. Res.* **8**, BC12 (2014).

53. Lokken, K. L. & Ferraro, F. R. The relationship between menopausal status, phase of menstrual cycle, and replacement estrogen on cognition in healthy women without dementia. *J. Psychol. Interdiscip. Appl.* **140**, 533–547 (2006).

54. Salthouse, T. A. The Processing-Speed Theory of Adult Age Differences in Cognition. *Psychol. Rev.* **103**, 403–428 (1996).

55. Jia, L. & Zhang, H. Attention in subjective cognitive decline. *Lancet Neurol.* **19**, 565–566 (2020).

56. Pantsiou, K. *et al.* Inhibitory control, task/rule switching, and cognitive planning in vascular dementia: Are there any differences from vascular aging? *Front. Aging Neurosci.* **10**, (2018).

57. Demolis, P., Chalon, S. & Giudicelli, J.-F. Repeatability of Transcranial Doppler Measurements of Arterial Blood Flow Velocities in Healthy Subjects. *Clin. Sci.* **84**, 599–604 (1993).

58. Hall, K. S. & Trussell, J. Types of combined oral contraceptives used by U.S. women. *Contraception* **86**, 659 (2012).

59. Centers for Disease Control and Prevention. Office on Smoking and Health (OSH). https://www.cdc.gov/tobacco/about/osh/index.htm (2021).

60. Ghuran, A., Wieken, L. R. van der & Nolan, J. Cardiovascular complications of recreational drugs : Are an important cause of morbidity and mortality. *BMJ Br. Med. J.* **323**, 464 (2001).

61. Piano, M. R. Alcohol's Effects on the Cardiovascular System. *Alcohol Res.* **38**, 219 (2017).

62. Pase, M. P., Grima, N. A., Stough, C. K., Scholey, A. & Pipingas, A. Cardiovascular disease risk and cerebral blood flow velocity. *Stroke* **43**, 2803–2805 (2012).

63. Perdomo, S. J. *et al.* Cardiovascular disease risk is associated with middle cerebral artery blood flow velocity in older adults. *Cardiopulm. Phys. Ther. J.* **31**, 38–46 (2020).

64. Pase, M. P., Grima, N. A., Stough, C., Scholey, A. & Pipingas, A. Association of pulsatile and mean cerebral blood flow velocity with age and neuropsychological performance. *Physiol. Behav.* **130**, 23–27 (2014).

65. Mergeche, J. L., Bruce, S. S., Sander Connolly, E. & Heyer, E. J. Reduced middle cerebral artery velocity during cross-clamp predicts cognitive dysfunction after carotid endarterectomy. *J. Clin. Neurosci.* **21**, 406–411 (2014).

66. Marshall, R. S., Pavol, M. A., Cheung, Y. K., Asllani, I. & Lazar, R. M. Cognitive Impairment Correlates Linearly with Mean Flow Velocity by Transcranial Doppler below a Definable Threshold. *Cerebrovasc. Dis. Extra* **10**, 21–27 (2020).

67. Hurn, P. D., Littleton-Kearney, M. T., Kirsch, J. R., Dharmarajan, A. M. & Traystman, R. J. Postischemic cerebral blood flow recovery in the female: Effect of 17β-estradiol. *J. Cereb. Blood Flow Metab.* **15**, 666–672 (1995).

68. Zarrinkoob, L. *et al.* Blood flow distribution in cerebral arteries. *J. Cereb. Blood Flow Metab.* **35**, 648–654 (2015).

69. Serrador, J. M., Picot, P. A., Rutt, B. K., Shoemaker, ; J Kevin & Bondar, R. L. *MRI Measures of Middle Cerebral Artery Diameter in Conscious Humans During Simulated Orthostasis*. http://ahajournals.org (2000).

70. Krejza, J. *et al.* Age and sex variability and normal reference values for the V MCA/VICA index. *Am. J. Neuroradiol.* **26**, 730–735 (2005).

71. Patel, N. *et al.* The Leicester cerebral haemodynamics database: Normative values and the influence of age and sex. *Physiol. Meas.* **37**, 1485–1498 (2016).

72. Aaslid, R., Huber, P. & Nornes, H. *A transcranial Doppler method in the evaluation of cerebrovascular spasm*. *Neuroradiology* vol. 28 (1986).

73. Lindegaard, K. F., Nornes, H., Bakke, S. J., Sorteberg, W. & Nakstad, P. *Cerebral vasospasm diagnosis by means of angiography and blood velocity measurements*. *Acta Neurochirurgica* vol. 100 http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L19247138 (1989).

74. Blackwell, J. The Effects of Prolonged Sitting and Mental Stress: a Synapse to Cerebrovascular Function. (University of North Carolina at Chapel Hill Graduate School, 2020).

75. Fu, X., Huang, C., Wong, K. S., Chen, X. & Gao, Q. A New Method for Cerebral Arterial Stiffness by Measuring Pulse Wave Velocity Using Transcranial Doppler. *J. Atheroscler. Thromb.* **23**, 1004–1010 (2016).

76. Tarumi, T. *et al.* Central artery stiffness, neuropsychological function, and cerebral perfusion in sedentary and endurance-trained middle-aged adults. *J. Hypertens.* **31**, 2400–2409 (2013).

77. Sloten, T. T. van *et al.* Association between arterial stiffness, cerebral small vessel disease and cognitive impairment: a systematic review and meta-analysis. *Neurosci. Biobehav. Rev.* **53**, 121 (2015).

78. Singer, J., Trollor, J. N., Baune, B. T., Sachdev, P. S. & Smith, E. Arterial stiffness, the brain and cognition: A systematic review. *Ageing Res. Rev.* **15**, 16–27 (2014).

79. Mitchell, G. F. Effects of central arterial aging on the structure and function of the

peripheral vasculature: Implications for end-organ damage. *J. Appl. Physiol.* **105**, 1652–1660 (2008).

80. Vlachopoulos, C., Aznaouridis, K. & Stefanadis, C. Prediction of Cardiovascular Events and All-Cause Mortality With Arterial Stiffness. A Systematic Review and Meta-Analysis. *J. Am. Coll. Cardiol.* **55**, 1318–1327 (2010).

81. Weintraub, S. *et al.* Cognition assessment using the NIH Toolbox. *Neurology* **80**, S54–S64 (2013).

82. Miller, D. I. & Halpern, D. F. The new science of cognitive sex differences. *Trends Cogn. Sci.* **18**, 37–45 (2014).

83. Hess, E. H. & Polt, J. M. Pupil size in relation to mental activity during simple problem-solving. *Science (80-.).* **143**, 1190–1192 (1964).

84. Ogoh, S. Relationship between cognitive function and regulation of cerebral blood flow. *J. Physiol. Sci.* **67**, 345–351 (2017).

85. Kottner, J. *et al.* Guidelines for Reporting Reliability and Agreement Studies (GRRAS) were proposed. *J. Clin. Epidemiol.* **64**, 96–106 (2011).

86. Harrer, J. U. *et al.* Transcranial perfusion sonography using a low mechanical index and pulse inversion harmonic imaging: Reliability, inter-/intraobserver variability. *Ultraschall der Medizin* **32**, 95–101 (2011).

87. Brodie, F. G., Atkins, E. R., Robinson, T. G. & Panerai, R. B. Reliability of dynamic cerebral auto regulation measurement using spontaneous fluctuations in blood pressure. *Clin. Sci.* **116**, 513–520 (2009).

88. Wang, Q. *et al.* Environmental ambient temperature and blood pressure in adults: A systematic review and meta-analysis. *Sci. Total Environ.* **575**, 276–286 (2017).

89. Brook, R. D. The Environment and Blood Pressure. *Cardiol. Clin.* **35**, 213–221 (2017).

90. Billinger, S. A. *et al.* Dynamics of middle cerebral artery blood flow velocity during moderate-intensity exercise. *J. Appl. Physiol.* **122**, 1125–1133 (2017).

91. Jorgensen, L. G., Perko, G., Payne, G. & Secher, N. H. Effect of limb anesthesia on middle cerebral response to handgrip. *Am. J. Physiol. - Hear. Circ. Physiol.* **264**, (1993).

92. Ainslie, P. N. *et al.* Elevation in cerebral blood flow velocity with aerobic fitness throughout healthy human ageing. *J. Physiol.* **586**, 4005–4010 (2008).

93. Sato, K., Ogoh, S., Hirasawa, A., Oue, A. & Sadamoto, T. The distribution of blood flow in the carotid and vertebral arteries during dynamic exercise in humans. *J. Physiol.* **589**, 2847–2856 (2011).

94. Szirmai, I., Amrein, I., Pálvölgyi, L., Debreczeni, R. & Kamondi, A. Correlation between blood flow velocity in the middle cerebral artery and EEG during cognitive effort. *Cogn. Brain Res.* **24**, 33–40 (2005).

95. Boban, M., Črnac, P., Junakovic, A. & Malojčic, B. Hemodynamic monitoring of middle cerebral arteries during cognitive tasks performance. *Psychiatry Clin. Neurosci.* **68**, 795–803 (2014).

96. Wallace Deckel, A., Cohen, D. & Duckrow, R. Cerebral blood flow velocity decreases during cognitive stimulation in Huntington's disease. *Neurology* **51**, 1576–1583 (1998).

97. Cho, S. J., Sohn, Y. H., Kim, G. W. & Kim, J. S. Blood flow velocity changes in the middle cerebral artery as an index of the chronicity of hypertension. *J. Neurol. Sci.* **150**, 77–80 (1997).

98. Farhoudi, M. *et al.* Doppler study of cerebral arteries in hypercholesterolemia. *Vasc. Health Risk Manag.* **7**, 203–207 (2011).

99. Karakurt, F. *et al.* Relationship between cerebral arterial pulsatility and carotid intima media thickness in diabetic and non-diabetic patients with non-alcoholic fatty liver disease. *J. Endocrinol. Invest.* **32**, 63–68 (2009).

100. Kim, E. J. *et al.* Relationship between blood pressure parameters and pulse wave velocity in normotensive and hypertensive subjects: Invasive study. *J. Hum. Hypertens.* **21**, 141–148 (2007).

101. Jamovi. The jamovi project. (2021).

102. Corporation, I. IBM SPSS Statistics.

103. Shrout, P. E. & Fleiss, J. L. Intraclass correlations: Uses in assessing rater reliability. *Psychol. Bull.* **86**, 420–428 (1979).

104. Wecht, J. M., Weir, J. P. & Bauman, W. A. Inter-day reliability of blood pressure and cerebral blood flow velocities in persons with spinal cord injury and intact controls. *J. Spinal Cord Med.* **40**, 159–169 (2017).

105. Hogervorst, E., Yaffe, K., Richards, M. & Huppert, F. A. H. Hormone replacement therapy to maintain cognitive function in women with dementia. *Cochrane Database Syst. Rev.* **2009**, (2009).

106. Henderson, V. W. *et al.* Estrogen for Alzheimer's disease in women: randomized, double-blind, placebo-controlled trial. *Neurology* **54**, 295–301 (2000).

107. Pomeroy, A. The Effect of Blood Pooling in the Lower Limbs During Prolonged Sitting on Cerebral Arterial Stiffening: Two Randomized Cross-Over Trials. (The University of North Carolina at Chapel Hill, 2021).

108. Lin, M. H., Davies, P. L., Stephens, J. & Gavin, W. J. Test-Retest Reliability of Electroencephalographic Measures of Performance Monitoring in Children and Adults. *Dev. Neuropsychol.* **45**, 341 (2020).

109. Ip, C. T. *et al.* Pre-intervention test-retest reliability of EEG and ERP over four recording intervals. *Int. J. Psychophysiol.* **134**, 30–43 (2018).