THE RELIABILITY OF VASCULAR AND HEMODYNAMIC MEASURES ACROSS THE ORAL CONTRACEPTIVE CYCLE

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ABSTRACT

Jillian Poles: The Reliability of Vascular and Hemodynamic Measures Across the Oral Contraceptive Cycle
(Under the direction of Lee Stoner)

The oral contraceptive (OC) cycle is often controlled for in physiological studies by measuring OC users solely in the inactive phase. While this practice seeks to reduce potential measurement variability due to the impact of hormonal fluctuation on physiological function, it also impairs our understanding of women's physiology, conceals sex differences, and decreases generalizability. Since this variability is not consistently demonstrated across the current literature, there is a need for further investigation into whether controlling for the cycle is necessary, by assessing physiological measurement reliability in OC users. This study therefore investigated the reliability of vascular and hemodynamic measures across the OC cycle in young, healthy women.

Our study found acceptable reliability for all vascular and hemodynamic outcomes across the OC cycle. Therefore, it may not be necessary to control for the cycle, as these measures of interest appear equally representative of cardiovascular function in each cycle phase.

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LIST OF ABBREVIATIONS

AIx	Augmentation index
BP	Blood Pressure
bfPWV	Brachial-femoral pulse wave velocity
cfPWV	Carotid-femoral pulse wave velocity
CIMT	Carotid intima-media thickness
CSA	Cross sectional area
cSBP	Central systolic blood pressure
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
E2	Estradiol
ECG	Electrocardiogram
EE	Ethinyl estradiol
ELISA	Enzyme-linked immunosorbent assay
eNOS	Endothelial nitric oxide synthase
ER	Estrogen receptor
faPWV	Femoral-ankle pulse wave velocity
FMD	Flow mediated dilation
GSR	Galvanic skin response
HRV	Heart rate variability
ICC	Intraclass correlation coefficient
LD	Lumen diameter
MAP	Mean arterial pressure
MDC	Minimal detectable change
OC	Oral contraceptive
PNS	Parasympathetic nervous system

PR	Progesterone receptor
PTT	Pulse transit time
PWA	Pulse wave analysis
PWV	Pulse wave velocity
Q	Cardiac output
RMSSD	Root mean square of successive differences
SBP	Systolic blood pressure
SEM	Standard error of the mean
SMC	Smooth muscle cell
SNS	Sympathetic nervous system
SV	Stroke volume

CHAPTER I: INTRODUCTION

INTRODUCTION

This thesis comprises 5 chapters. **Chapter 1** (this chapter) provides a rationale for this thesis. **Chapter 2** is a literature review, which briefly outlines the significance of the proposed research. **Chapter 3** provides rationale for each aspect of the study design. **Chapter 4** details the study methodology. **Chapter 5** provides the results of the study. **Chapter 6** provides a discussion of the results, including limitations and strengths of the study, as well as future avenues for this research.

KEY TERMINOLOGY

Term	Definition
Arterial Stiffness	The rigidity or compliance of a blood vessel, based on its cellular and structural components.
Cardiac Output	Stroke volume times heart rate; the liters of blood per minute ejected by the left ventricle.
Oral contraceptive cycle	A full cycle of a course of oral contraceptives (inactive, early active, and late active phases), usually lasting 28 days.
Overall reliability	The degree to which repeated measures in an individual provide similar results.
Pulse wave velocity	Distance traveled by pulse wave divided by the time it takes for the pulse wave to travel that distance (meters/second). A metric of arterial stiffness.
Pulse Wave Analysis	An algorithm that can derive central hemodynamic metrics, including cSBP and AIx, from a pulse wave measurement.
Stroke Volume	The milliliters of blood ejected by the left ventricle during systole.

THESIS RATIONALE

Physiological cardiovascular studies often exclude women or control for the menstrual/oral contraceptive (OC) cycle by measuring solely during the follicular/placebo phase. This practice aims to minimize variability in results, as cyclic changes in hormonal concentration are assumed to yield variability in vascular function in women.[1] This assumption is based on the presence of ovarian hormone receptors throughout the cardiovascular system.[2] However, current literature exhibits conflicting findings as to the extent of vascular function differences across the cycle with changing hormonal concentration, with some showing significant variability,[3,4] and some showing little to none.[5–9] Furthermore, some

existing studies are poorly controlled (e.g., neglecting to measure hormonal profile to confirm cycle phase, not matching subjects for confounding characteristics such as age and physical activity level) and have small sample sizes, making it difficult to draw definitive conclusions.[10] Thus, controlling for the OC cycle not only limits our understanding of women's cardiovascular physiology, but it also may not even be necessary. Gaining a better understanding of measurement precision in women will be beneficial as it will allow us to more definitively decide whether we should control for the OC cycle.

The long-term goal of this research was to assess the reliability of measures of vascular and hemodynamic function across the OC cycle in young, healthy women, as compared to men. This randomized, two-group repeated measure observational study aimed to recruit a cohort of 40 (20 men, 20 women) young, healthy individuals from a representative state college population. We obtained noninvasive measures of vascular and hemodynamic function during each of three visits (corresponding to each OC cycle phase) across the span of 4 weeks. The aim of the study was to identify the overall (between-day) reliability of these measures across the cycle in OC users. Comparing measurement reliability between men and women allows us to assess whether it is necessary to control for the OC cycle in physiological cardiovascular studies. If measurements remain reliable in the women across the cycle, it is likely that OC users can be measured in any phase.

The hypothesis was that overall reliability of vascular measures will remain acceptably reliable in OC users. To test this hypothesis, participants were tested during the inactive (placebo), early active, and late active phase of their OC cycle (and male participants at corresponding time points). Male participants were used as a comparison group to ascertain the relationship between vascular function and OC usage, as men were not using OCs. Central and peripheral PWV were used as surrogate measures for arterial stiffness. Data from this lab group and scientific literature supports the usage of brachial-femoral (bf)PWV, carotid-femoral (cf)PWV, and femoral-ankle (fa)PWV as viable arterial stiffness outcomes. Other vascular measures included augmentation index (AIx), carotid β stiffness, and carotid intima media thickness (CIMT). Hemodynamic measures included central and peripheral blood pressure (BP), cardiac output (Q), and stroke volume (SV). Other measures pertaining to hemodynamic control included heart

rate variability (HRV) and galvanic skin response (GSR). Data was collected during each phase (inactive/placebo, early active, late active) of the OC cycle, to address out study aim. Blood estrogen was also measured in both sexes at every visit to be used as a covariate for each measure to determine if there are associations between changes in estrogen concentration and changes in vascular and hemodynamic function.

OVERALL OBJECTIVE AND APPROACH

The long-term goal of this research was to increase our understanding of how OC usage may impact measurement variability across the cycle. To support this goal, this project aimed to assess the overall (between-day) reliability of vascular and hemodynamic measures across the 4-week OC cycle in healthy young women. This aim is important because it will help to clarify whether it is necessary to control for the OC cycle (i.e., measure women only in the inactive phase) during studies of cardiovascular function.

INNOVATION AND SIGNIFICANCE

We hypothesized that the overall reliability of vascular and hemodynamic measures will remain acceptably reliable in healthy young women using OCs. If this hypothesis is proven to be true, it may add clarification to the conflicting results within the literature pertaining to variability in measures of vascular and hemodynamic function across the OC cycle. This will provide support for eliminating the control of the cycle (i.e., measuring women only during the inactive phase) in studies of cardiovascular function, which will increase generalizability and make it simpler to include women in such studies moving forward.

CHAPTER II – LITERATURE REVIEW- SIGNIFICANCE

INTRODUCTION

An estimated 9 million women in the United States are oral contraceptive (OC) users,[11] leading to fluctuations in ovarian hormones (estrogen and progesterone) across the OC cycle. When conducting physiological studies, the cycle is often controlled for by measuring during the inactive (placebo) phase where ovarian hormone concentration is lowest, to decrease measurement variability.[12] Recently, however, some have argued for no control of the cycle, as limiting data collection to certain portions of the cycle decreases external validity and conceals potentially important physiological sex differences. Additionally, results within the literature are not consistent; some studies[4,7,8] have shown that these changes in hormone concentration cause acute changes in physiological measures (including measures of vascular and hemodynamic function) across the cycle, while others[5,6,13] have demonstrated no significant variability. Since the extent of these changes varies between studies, there is no consensus on the physiological impacts of hormonal fluctuation, and thus potentially no strong justification for controlling for the cycle. Furthermore, many of these studies have small sample sizes or fail to address key considerations (e.g., measuring hormonal concentration to confirm cycle phase, matching subjects for age and physical activity), making it difficult to draw definitive conclusions.[10]

Current literature has illustrated the acute impacts of OC usage, as conceptualized in **Figure 1,** which this literature review will later expand upon. However, significant gaps in the knowledge exist regarding the extent to which these phenomena occur, due to methodological flaws and conflicting findings in the existing literature. For these reasons, there is a clear need for further investigation, using internally robust studies, to arrive at a more conclusive consensus. To determine whether it is necessary to

control for the OC cycle in studies of cardiovascular function, we must evaluate the reliability of vascular and hemodynamic measures across cycle phases.

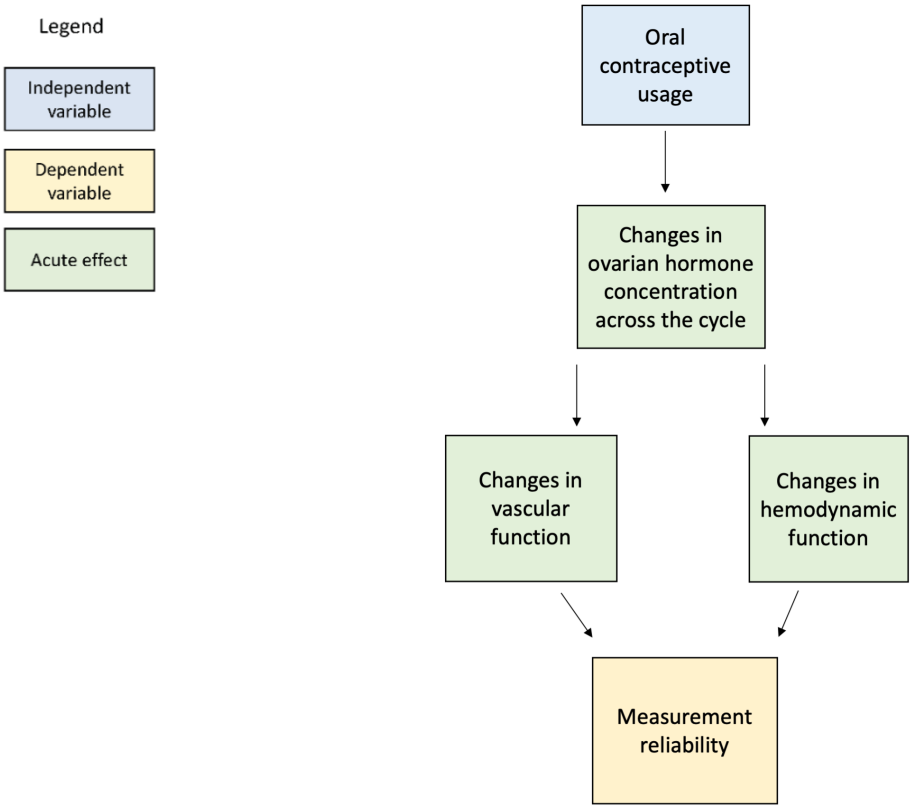

FIGURE 1. A visualization of the acute impacts of OC usage on vascular function, and subsequently, reliability

The following key considerations will be discussed in this chapter.

Consideration	Why Important	Page
Ovarian hormone overview	Define the physiological roles of ovarian hormones	13-15
Oral contraceptive cycle and vascular health	Outline why the cycle is theorized to impact physiological function	15-19
Methodology & rigor	Explain how the study will address the questions at hand	19-29

OVARIAN HORMONE OVERVIEW

MECHANISMS OF OVARIAN HORMONES

To understand the impact of ovarian hormone concentration on vascular function, we must first understand the role they play in the cardiovascular system.

Estrogen exerts its effects via binding to estrogen receptor (ER)a and ERb.[1] Each receptor has been shown to differentially regulate gene expression, and therefore has its own unique function.[14] Functions of ERa include activation of endothelial nitric oxide synthase (eNOS),[15] prevention of vascular smooth muscle cell (SMC) proliferation,[16] and inhibition of medial thickness.[17] Therefore, estrogens appear to have a cardioprotective effect, mediated in part by ERa. The functions of ERb are less clear, but studies in animals have shown that treatment with ERb selective agonist improved left ventricular function in ischemia-reperfusion injured mice.[18] Administration of estradiol (E2), the most biologically prominent estrogen, rescued severe pulmonary hypertension in rats, but only in the presence of ERb selective agonist.[19] Finally, cardiac hypertrophy and fibrosis were prevented with E2 administration in wild type, but not ERb knockout mice.[20] These studies point to a cardioprotective role of ERb, but evidence is limited in humans. See **Figure 2** for a summary of the cardioprotective effects of estrogen.

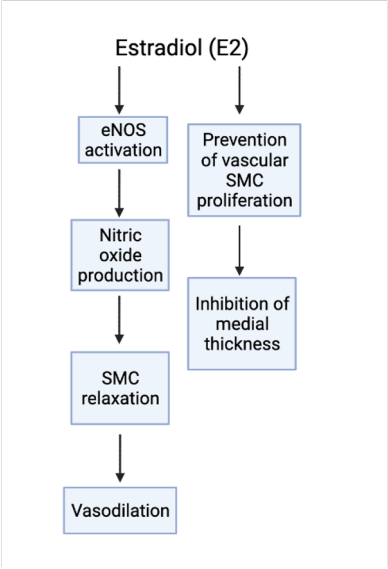

FIGURE 2. Estrogen and cardiovascular function

Progesterone acts via progesterone receptors (PR) which are found in the vascular endothelium.[21] While the role of ERs and estrogen in the cardiovascular system are well recognized, the roles of PRs and progesterone are less widely studied. Similarly to ERs, PRs appear to play a role in the response to vascular injury, as PR knockout mice showed significantly greater vascular SMC proliferation and medial hypertrophy in response to carotid arterial injury, in comparison to wild-type counterparts.[22] Progesterone has also been identified as a vasoactive hormone, inhibiting agonist-induced vasoconstriction by

modulating cellular calcium.[23] Furthermore, in vitro progesterone administration was found to significantly increase eNOS activity via membrane PRs.[24] Therefore, progesterone appears to confer additional cardiovascular benefit.

While each hormone has its own independent role, there is evidence of interplay between the two; estrogens induce synthesis of PRs and are therefore responsible for mediating the effects of progesterone.[25] Progesterone, however, can increase ER turnover in certain tissues, decreasing the effects of estrogens.[26] Accordingly, animal models of atherosclerosis and vascular injury have shown that progestins can reverse the cardioprotective effects of estrogens.[27,28] Therefore, vascular function may also be influenced by the ratio of estrogen to progesterone concentration at a given point in time.

It is evident that estrogen and progesterone play key roles in the cardiovascular system, thus we might expect changes in ovarian hormone concentration to influence vascular function.

ROLE OF OVARIAN HORMONES IN THE CARDIOVASCULAR SYSTEM

ERs and PRs are found throughout the brain, vascular system, and kidneys, giving ovarian hormones a strong influence on vascular homeostasis and endothelial function.[1] Estrogen concentration is directly related to ERa expression, and endothelial ERa expression has been found to be positively associated with the eNOS protein, the activated state of eNOS, and, accordingly, brachial artery flow mediated dilation (FMD).[29] E2 is also involved in the nitric oxide cascade,[30] so estrogen concentration and ER expression play a clear role in influencing vascular tone. Furthermore, both estrogen and progesterone have been found to induce vascular relaxation by inhibiting vascular SMC contraction.[31] Estrogen has also been found to upregulate prostacyclin synthase, improving the response to vascular injury and atherosclerosis.[32]

Ovarian hormones are also involved in blood pressure regulation. Estrogens has been found to directly decrease blood pressure by stimulating the release of endothelium-derived vasodilators and inhibiting the renin-angiotensin system.[33] Administration of progesterone has also been shown to directly lower blood pressure[34] and inhibit the action of angiotensin II.[35,36]

Thus, varying concentrations of ovarian hormones are likely to modify hemodynamics by regulating both vascular tone and blood pressure.

THE ORAL CONTRACEPTIVE CYCLE: IMPLICATIONS FOR VASCULAR HEALTH

OVERVIEW OF THE ORAL CONTRACEPTIVE CYCLE

This section will clarify the structure of the OC cycle and will describe how hormonal concentrations vary across the cycle.

An estimated 9 million women in the United States are using some sort of OC,[11] making OC users the majority of the reproductive-aged female population. However, most of the current literature investigating the physiological impacts of ovarian hormonal fluctuations are in women with a natural menstrual cycle. Thus, we seek to recruit OC users for this study to increase generalizability and fill gaps in the literature. Women with a natural menstrual cycle exhibit larger variation in ovarian hormone concentration between cycle phases, while the amount of exogenous hormone administered during the active phases of a monophasic OC remains constant. Since current literature has not yet assessed reliability (but rather the presence or absence of significant differences between cycle phases), a study in OC users is a good first step towards assessing reliability across the cycle without necessitating as large a sample size as what would be needed with greater variation.

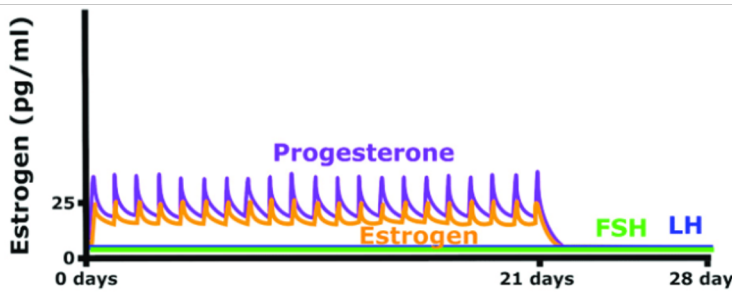

FIGURE 3. Hormonal fluctuation across the OC cycle.[85] Days 0-21: active phase. Days 21-28: inactive (placebo) phase. Abbreviations: FSH = follicle-stimulating hormone; LH= luteinizing hormone

The OC cycle can be divided into three phases: the inactive (placebo) phase (1–5 days after the onset of withdrawal bleeding), early active phase (6–12 days after the onset of withdrawal bleeding), and late active phase (19–27 days after the onset of withdrawal bleeding).

As OC intake suppresses endogenous ovarian production of estrogen and progesterone, circulating levels of these hormones are determined by the concentrations in the pill. Of note, changes in hormone concentration across the cycle vary depending on the type of OC used. OCs also have varying dosages of exogenous estrogen, ranging anywhere from 20 to 90 mcg of estrogen. OCs may also have either a constant (monophasic) or a varying (multiphasic) dose throughout the active phases.[37] This review will focus on monophasic OCs, as these are most commonly used among women in the US.

During the inactive phase, placebo pills (or no pills) are consumed, leading to low estrogen concentration and, as a result, low endothelial ERa expression. In comparison, the early and late active phases are associated with a higher estrogen concentration (with varying degrees, depending on dosage), and a higher endothelial ERa expression.[21] See **Figure 3** for more a depiction of the changes in ovarian hormone concentration that occur across the OC cycle. As we now know, estrogen concentration can modify vascular function, therefore we expect that our measures of interest will differ between the active and inactive phases.

ACUTE CHANGES IN VASCULAR FUNCTION ACROSS THE ORAL CONTRACEPTIVE CYCLE

Some studies have found significant differences in measures of cardiovascular function between cycle phases. Resting heart rate has been found to be significantly higher in the early active, compared to the inactive phase.[8] Both baroreflex sensitivity and mean arterial pressure (MAP) have been found to be higher during the inactive phase, compared to the active phase.[4] These impacts on autonomic blood pressure regulation appear consistent across studies, as higher E2 concentration (during the active phase) is associated with increased with vascular transduction, i.e.; more vasoconstriction per sympathetic burst.[3] In women using OCs containing solely ethinyl E2 (EE), endothelium-dependent vasodilation has been found to be elevated in the active phase compared to the inactive phase. However, this same effect was not seen in a group of women using a combined OC, which also contained levonorgestrel (a progestin).[7] These differential results are unsurprising considering the contrasting effects of estrogen and

progesterone, but they indicate that inadequate control for OC type is a potential methodological weakness in the current literature.

However, not all studies demonstrate this variability across the cycle; studies in OC users have found no significant differences in vascular measures such as HRV,[5] brachial artery FMD,[6] carotid β stiffness, and central and peripheral PWV.[8,13] These inconsistencies illustrate a clear need for more research in OC users. See **Table 1** for a summary of the findings in the current literature.

TABLE 1. Previous findings in oral contraceptive users

Author and year	**Research Question/Aim**	**Study design**	**Findings**
Yu et al. 2014[8,13]	To assess effect of the natural menstrual cycle and OC usage on arterial stiffness, and central and peripheral hemodynamics	Women with a natural menstrual cycle (n=36) compared to women using low-dose OCs (n=24)	Resting heart rate is higher in the early active (65.8±10) phase compared to the inactive (62.1±9.7) phase (p=0.03) No significant change in central and peripheral PWV
Minson et al. 2000[4]	To assess effect of OC usage on resting muscle sympathetic nerve activity (MSNA), and sympathetic and cardiovagal baroreflex sensitivity	Monophasic OCs with 30-35 mcg EE and low-dose progestin (n=9)	MSNA is lower in the active phase compared to the inactive phase (p<0.01) Mean arterial pressure is lower in the active (82±4) phase compared to the inactive (89±3) phase (p=0.01) Diastolic blood pressure is lower in the active (65±3) phase compared to the inactive (72±3) phase (p=0.01) Calf blood flow (mL/100 mL/min) is higher in the active phase (2±0.1) active phase compared to the inactive (1.8±0.2) phase (p=0.02)
Torgrimson et al. 2007[7]	Evaluate endothelial function during the active vs inactive hormonal OC phase (two doses)	OCs containing levonorgestrel and EE Low dose: 150 mcg L/30 mcg EE (n=7) Very low dose: 100 mcg L/20 mcg EE (n=8)	Endothelium-dependent vasodilation (% change in brachial artery diameter from baseline) is lower in the active (5.33±1.77) phase compared to the inactive (7.23±2.6) phase for very low dose OC users (p=0.02) Diastolic blood pressure is higher in the active (71±7) phase compared to the inactive (63±9) phase (p=0.04) for very low dose OC users

Teixeira et al. 2015[5]	To assess the cardiac autonomic modulation (HRV) across phases in OC users	Monophasic OCs with 20-35 mg EE and low-dose progestin (n=17)	No significant change in heart rate variability
Shenouda et al. 2018[6]	To compare changes in endothelial function between cycle phases and between men, women with a natural menstrual cycle, and women using OCs	Men (n=20), women with a natural menstrual cycle (n=18), women using monophasic combined OCs (second, third, or fourth generation) (n=20)	No significant change in brachial artery flow mediated dilation
Priest et al. 2018[13]	To examine the effect of sex, menstrual cycle phase, and OC use on local and central arterial stiffness	Men (n=20), women with a natural menstrual cycle (n=18), women using monophasic combined OCs (second, third, or fourth generation) (n=20)	No significant change in carotid β stiffness No significant change in central and peripheral PWV

LITERATURE REVIEW SUMMARY

WHY IS THIS STUDY NEEDED?

Many physiological studies test women solely in the inactive phase of the OC cycle, but this could conceal potential sex differences and limit external validity. This practice is justified by inconsistent findings regarding variability in vascular measures across the cycle, often resulting from studies with poor methodological quality. Thus, there is a need to conduct an internally robust study to assess the reliability of these measures in women using OCs, as compared to men. To our knowledge, no study has examined reliability across the cycle of our chosen outcomes in women taking OCs.

WHAT IS KNOWN

Ovarian hormones (estrogen and progesterone) play key roles in modulating vascular and hemodynamic function. Since the concentrations of these hormones fluctuate across the cycle in women using OCs, there may be variability in measures of vascular and hemodynamic function across the cycle.

WHAT IS NOT KNOWN

The impact of OC use on the reliability of select vascular and hemodynamic measures has not yet been confirmed, as there are conflicting findings within the literature, and these studies often fail to control for key confounders.

CRITICAL NEED

More information is needed on the extent to which hormonal fluctuations across the OC cycle yield variability in measures of vascular and hemodynamic function. We can use this information to improve measurement accuracy in women, as well as our general understanding of women's cardiovascular physiology, hopefully aiding in the detection and mitigation of CVD risk. To obtain this information, we must study the reliability of vascular and hemodynamic measures across the OC cycle, in a well-controlled study.

CHAPTER III – LITERATURE REVIEW- RATIONALE FOR APPROACH

METHODOLOGICAL AND RIGOR CONSIDERATIONS

This section will outline all considerations made in designing the study, including the study design itself, as well as measures taken to maximize internal and external validity, to provide better understanding into our methodological choices.

STUDY DESIGN CONSIDERATIONS

Several possible study designs were considered in addressing the research question. These designs are summarized in **Table 2** and are discussed below.

TABLE 2: Summary of study design considerations

Consideration	Choices	Selection	Explanation
Study design	Observational study Interventional study	Observational study	Intervention would have been logistically and ethically complicated (e.g., controlled administration of OCs to participants)
Control group	Compare women using OCs to women with a natural menstrual cycle Compare women using OCs to men	Compare women using OCs to men	In order to assess whether between-day variability in hormonal concentration substantially impacts our measures of interest, we must compare to a group with less between-day variability in hormonal concentration

One possibility was to manipulate the use of OCs in our female sample by adding or removing medication; another was to directly manipulate blood estrogen concentrations. However, these experimental designs are too complicated in terms of logistics and ethics. Therefore, we opted for an observational design. This design aims to minimize potential confounders, optimize participant comfort and convenience, and maximize efficiency for investigators. This study was a randomized, two-group repeated measure observational study. This design allows us to compare subjects to themselves at

different time points, as well as compare both experimental groups at each time point. Participants attended three experimental visits, one corresponding to each phase of the OC cycle**.** These visits were spaced apart according to prior literature: the inactive phase was designated as 1-5 days following the onset of withdrawal bleeding, the early active phase was designated as 6-12 days following the onset of withdrawal bleeding, and the late active phase was designated as 19-27 days following the onset of withdrawal bleeding.[9] The control group of men were also assessed at the same time points, to allow direct comparison between groups. Comparisons were made for each subject between time points to determine within-subject reliability, but also between the two groups at for each measure, to determine the specific effects of OC usage.

OTHER CONSIDERATIONS

PRE-VISIT EXPERIMENTAL VISIT CONTROL

To ensure subjects reported for each visit under standardized conditions, pre-assessment guidelines were established: participants abstained from alcohol, and moderate to vigorous physical activity 24 hours prior to testing, and from food and caffeine 12 hours prior to testing. The time of day at which testing occurred was also standardized as much as possible, both within and between subjects. **Table 3** summarizes our required pre-assessment guidelines.

TABLE 3. Preemptive control considerations.

Consideration	Explanation	Control Procedure
Standardized beginning time for all experimental sessions	Prevent activities of daily living from interfering with data quality	All experimental sessions commenced between 6:00-10:00 AM
Prevent vigorous physical activity before experimental sessions	Prevent prolonged effects of vigorous PA on CV system from influencing data	Individuals were reminded two days prior to an experimental session to refrain from vigorous physical activity for at least 24 hours prior to each visit.
Prevention of alcohol and caffeine consumption before experimental sessions	Prevent effects on CV system and data (e.g., hydration status, heart rate)	Individuals were reminded two days prior to an experimental session to refrain from caffeine and alcohol consumption for at least 12 and 24 hours prior to each visit, respectively.
Prevention of food consumption before experimental sessions	Prevent within- and between-subject variation in CV data due to insulin release and/or different meals composition	Individuals were reminded two days prior to an experimental session to refrain from food consumption for at least 12 hours prior to each visit

INTERNAL VALIDITY

Investigators followed established screening procedures to recruit a homogenous sample group. The aim of this study was to observe changes in cardiovascular measures across the menstrual cycle. A group relatively similar in age, activity, and health simplifies analysis and increases the validity of experimental conclusions. In order maintain internal validity, all measurements occurred between the same hours of the day. Our screening procedures and data collection window are detailed in **Table 4.**

TABLE 4. Maintenance of internal validity.

Consideration	Explanation	Control Procedure
Screening procedures	Ensures homogeneous sample, eliminates potential covariates	During recruitment, participants were required to provide their age and medical history. Women were asked to provide the name and dosage of their oral contraceptive and specify for how long they have been taking that specific medication.
Standardized window for data collection	Prevents within and between subject variation in cardiovascular and hormonal measures due to daily circadian rhythms	All measurements occurred between the hours of 6:00 and 10:00 AM.

POPULATION/SAMPLING

Since we sought to determine reliability of vascular measures in women by comparing them to men, we needed to include subjects of both sexes. Only young, healthy individuals were recruited, to eliminate the confounding effects of cardiovascular disease and advanced age on vascular measures. Smoking can also impact vascular function, so only non-smokers were included. Risk of adverse events with OC use also increases with age, and use is discouraged in women over 35 years of age with cardiovascular risk factors.[38] Thus, male and female subjects were included if they were between 18 and 35 years of age and free of cardiovascular disease. **Table 5** lists our specific inclusion criteria.

TABLE 5. Inclusion criteria

Criteria	Method	Rational
Aged 18-35 years old	Initial email contact screening	This age range includes young, healthy adults, who are not at an increased risk of adverse events due to OC use.
Has been taking OC for at least 6 months	Initial email contact screening; familiarization session screening	Physiological adjustments in early phases of OC use may impact cardiovascular measures. Furthermore, the study aimed to investigate the impacts of chronic OC use, specifically.

Regular cycle	Initial email contact screening; familiarization session screening	A regular cycle allows for predictable progression through phases across the cycle, and for study visits to be properly spaced.

Subjects were excluded if they were pregnant, a smoker, had any cardiovascular disease, or were taking any cardiovascular medications, as these may impact our variables of interest. **Table 6** lists our specific exclusion criteria.

TABLE 6. Exclusion criteria

Criteria	**Method**	**Rational**
Pregnant	Initial email contact screening; familiarization session screening	Women undergo significant physiological changes during pregnancy. Different hormonal profiles could cofound cardiovascular outcomes
Presently smoking	Initial email contact screening; familiarization session screening	Smoking is understood to significant decrease cardiovascular health. This study aimed to characterize physiological responses in a healthy, homogenous population
Taking medication known to alter cardiovascular function	Initial email contact screening; familiarization session screening	**Examples:** beta-blockers, ACE inhibitors. These drugs will significantly alter cardiovascular outcomes in this study. A homogenous population was required to answer research questions in this study.
Diagnosed with cardiometabolic diseases	Initial email contact screening; familiarization session screening	**Examples:** PAD, CAD, T2DM These conditions significantly disrupt healthy cardiovascular function. A homogenous population was required to successfully carry out the aims of this study.

SEX AS A BIOLOGICAL FACTOR

This study sought to recruit a group of twenty females and twenty males. A large sample size of females is beneficial to increase power, especially considering individual variability pertaining to the OC cycle. Males served as a control comparator and we expect less variability in our measures of interest between study visits but sought to recruit an equal number, so not to skew the statistics when comparing sexes.

ETHNICITY/RACE

This study did not exclude potential participants based on race or ethnicity.

EXTERNAL VALIDITY/GENERALIZABILITY

Maintaining internal validity in this study allows for optimal generalizability to the wider population. This was achieved by only allowing primary investigators to collect and analyze data, ensuring quality and consistency. However, due to exclusion criteria, the results of this study may be less generalizable to older individuals and those with cardiovascular disease, although this presents a future avenue for this research.

STATISTICAL CONSIDERATIONS

Various approaches exist for assessing measurement precision/reliability, including the coefficient of variation (CV), intraclass correlation coefficient (ICC), standard error of measurement (SEM), and minimal detectable change (MDC). For this study, between-day reliability was assessed primarily using ICC, with SEM and MDC used as secondary outcomes to confer additional reliability information.

ICC was calculated to assess the strength of the variation in our measures of choice. SEM was calculated to determine the sensitivity of measurements, as it shows what level of variation can be attributed to error within measurement technique or equipment. MDC is an absolute measure of reliability or measurement error, representing the smallest amount of change that can be interpreted as a 'real' change (not due to chance); this is additional information not conferred through the ICC. MDC% as used as a measure of relative reliability, as it is the ratio of the MDC to the mean.

MEASUREMENT CONSIDERATIONS

To fill these knowledge gaps, we will explore changes in a number of vascular and hemodynamic measures such as arterial stiffness, central and peripheral BP, carotid β-stiffness index, CIMT, Q, SV, HRV, and GSR. These measures will help us to get a comprehensive systemic view of how ovarian

hormonal fluctuation due to OC usage might affect the cardiovascular system, including direct changes to the vasculature, changes in fluid balance, and changes to the autonomic nervous system which may mediate hemodynamics. The measures we have chosen are commonly used within the literature and in the clinical setting to investigate vascular and hemodynamic function, as described and referenced in more detail below. See **Table 7** for our rationale behind our outcomes of choice.

TABLE 7. Rationale for selected outcomes

Aim(s)	Construct	Choices	Selection	Explanation
Assess the reliability of vascular measures across the OC cycle	Arterial stiffness	PWV Carotid β-stiffness index	PWV (cfPWV, bfPWV, faPWV) Carotid β-stiffness index	PWV is the gold standard measure of arterial stiffness. Assessing both central and peripheral arterial stiffness through PWV, as well as local arterial stiffness of the carotid, gives us a comprehensive view of how the entire body may be impacted by hormonal fluctuation
	Wave reflection	AIx	AIx	Measurement of AIx confers extra information about the stiffness of smaller arteries, which cannot otherwise be obtained using PWV
	Subclinical atherosclerosis	CIMT	CIMT	Since the carotid is especially prone to plaque formation and subsequent morphological changes, it is important to assess the extent to which these changes occur
Assess the reliability of hemodynamic measures across the OC cycle	Blood Flow	Q SV	Q, SV	Measuring both Q and SV will give us an idea of how hormonal fluctuation may impact whole body blood flow, which is crucial in assessing cardiovascular function
	Hemodynamics	SBP DBP cSBP MAP SVR	SBP, DBP, cSBP	Traditional peripheral BP may be 40 mmHg higher than what occurs in the aorta; it is important to measure both in assessing hemodynamics and vascular tone. SVR is less relevant, as it becomes more clinically significant when a patient is either hypo- or hypertensive; our participants were healthy. SBP and DBP were chosen over MAP, as we wanted to examine the reliability of each component individually, rather than their composite.
Assess the reliability of measures of hemodynamic	Hemodynamic control	RMSSD SDRR	RMSSD	HRV is highly correlated with CVD risk. RMSSD was chosen as a measure of HRV as it has been found to be the most reliable for short ECG

control across the OC cycle				recordings with spontaneous breathing.
	Sympathetic activity	GSR Plasma noradrenaline Baroreflex sensitivity (MSNA)	GSR	GSR is a simple measure to obtain continuously. Obtaining both plasma noradrenaline and MSNA would increase subject burden, as they require injection or blood extraction, and cannot be measured continuously.

Abbreviations: PWV, pulse wave velocity; cfPWV, carotid-femoral pulse wave velocity; bfPWV, brachial-femoral pulse wave velocity; faPWV, femoral-ankle pulse wave velocity; AIx, augmentation index; CIMT, carotid intima-media thickness; Q, cardiac output; SV, stroke volume; SBP, systolic blood pressure; DBP, diastolic blood pressure; cSBP, central systolic blood pressure; MAP, mean arterial pressure; SVR, systemic vascular resistance; RMSSD, root mean square of successive differences; SDRR, standard deviation of the IBIs for all sinus beats; HRV, heart rate variability; GSR, galvanic skin response; MSNA, muscle sympathetic nerve activity.

ARTERIAL STIFFNESS

Arterial stiffness is a novel method of assessing cardiovascular health and has been considered an even better predictor of cardiovascular mortality than blood pressure.[39] PWV is the gold standard measure of arterial stiffness and is an independent predictor of cardiovascular mortality.[40] PWV was chosen as a measure for this study as a means of assessing vascular function as changes in arterial stiffness are an early sign of impaired vascular function and adverse structural changes within the vessel.[41] Therefore, the measurement of PWV allows us to assess acute changes in vascular function across the cycle. Measurement of PWV involves the inflation of cuffs at each measurement site to detect pulse waves. PWV is defined as the distance between measurement sites divided by pulse transit time (PTT). PTT represents the time it takes for a pulse to travel between sites; it is determined by calculating the time between the foot of composite pulse waveforms (detected by the software), collected at two (proximal and distal) measurement sites.[42] PTT is then divided by the distance between the two measurement sites (D) to derive PWV (see **Figure 4**). Central PWV measures such as cfPWV and bfPWV will be used, as well as

peripheral measures such as faPWV, to conceptualize how the entire vascular tree changes throughout the cycle, and to determine if some parts are affected to a greater extent. Arterial stiffness can be expected to change across the cycle, as estrogen has been found to enhance eNOS activity[43] and inhibit calcium influx into vascular SMCs.[44] Therefore, at times of heightened estrogen concentration, we may expect increased arterial compliance.

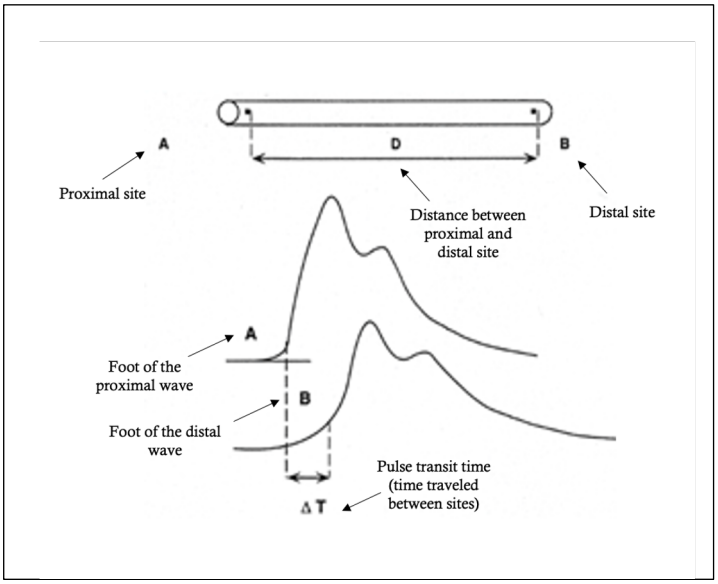

FIGURE 4. Calculation of pulse wave velocity (PWV)

PWV = distance/pulse transit time (PTT)

AUGMENTATION INDEX

AIx is a surrogate measure of arterial stiffness. While similar to PWV, AIx is not the same measure; AIx is determined by large artery PWV, but also by the intensity of the wave reflection, and is therefore also determined by the distensibility of smaller arteries.[45] Therefore, AIx will be used in this study to assess the function of smaller arteries, which PWV may not capture. AIx represents the proportion of the central pulse pressure that can be attributed to the reflected pulse wave and is defined as the augmentation pressure as a percentage of the pulse pressure (see **Figure 5**). Increased wave reflection is independently associated with cardiovascular disease risk and mortality.[46] To obtain AIx, we will use a noninvasive technique known as pulse wave analysis (PWA), which uses the same cuffs and technology

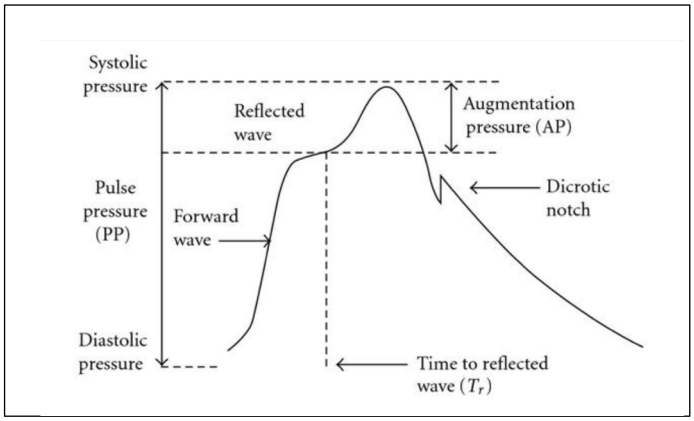

FIGURE 5. Aortic pulse pressure waveform [59]

as PWV, and has been proven to be both simple and reliable.[47] AIx can be expected to change similarly to PWV across the cycle, due to changes in eNOS activity influencing vascular distensibility.

CAROTID INTIMA MEDIA THICKNESS AND β-STIFFNESS INDEX

Use of continuous wave ultrasound is a simple and noninvasive way to assess local vascular function, yielding measures such as β-stiffness index, CIMT. β-stiffness index is a measure of local carotid stiffness, which is especially important in surveying vascular health, considering the heightened risk of atherosclerotic plaque formation at the carotid.[48] Furthermore, due to structural differences in arterial segments, carotid stiffness may not always correspond with aortic stiffness, so both measures must be taken to assess functional changes throughout the arterial tree. Increased carotid stiffness has been found to be associated with incident stroke, independently of aortic stiffness.[13] As with other measures of arterial reactivity, we would expect heightened estrogen concentration to increase carotid artery compliance. CIMT can also be used to assess endothelial function and is a component of intima and medial wall thickness, representing two of the three layers of the arterial wall.[49] This measure was chosen because greater CIMT has been associated with elevated systolic blood pressure (SBP),[50] coronary atherosclerosis,[51] and increased cardiovascular disease risk.[52] CIMT can be expected to fluctuate across the cycle due to changes in endothelin-1 receptor expression modulating vascular SMC proliferation, as studies have supported an inverse relationship between vascular SMC proliferation and CIMT.[53]

HEMODYNAMICS

Blood pressure

The same brachial cuff used to obtain PWV can be used to estimate both peripheral and central blood pressure, which may also fluctuate over the cycle. Traditional brachial systolic pressure may be 40 mmHg higher than what occurs in the aorta,[54] as pressure amplification occurs due to increasing arterial stiffness moving away from the heart. We therefore decided to measure central, in addition to peripheral blood pressure as additional assessments of vascular function and fluid regulation. Central blood pressure is traditionally obtained using cardiac catheterization, but noninvasive techniques are an emerging

alternative. Changes in both central and peripheral blood pressure are expected across the menstrual cycle as estrogen has been found to downregulate the expression of angiotensin 1 receptors, and enhance vascular relaxation,[31] resulting in a lower blood pressure.[55]

Blood flow

Blood flow was assessed across the cycle using Q and SV, in order to assess changes in whole body blood flow. While no studies have examined changes in these measures across the cycle in OC users, literature has shown a greater incidence in lightheadedness with orthostasis during the early follicular (low hormone) phase, compared to the luteal (high hormone) phase, even in healthy women.[56] Orthostatic hypotension (a drop in blood pressure while transferring to the erect posture) is associated with a higher increase in HR and decrease in SV, and is more prevalent in women.[57] Thus, these orthostatic hemodynamic changes with ovarian hormone fluctuation may be mediated by changes in Q and SV (as Q=HR*SV), possibly due to lower sympathetic nerve activity during phases characterized by low hormone concentration,[58] i.e.; the placebo/inactive OC phase. Since chronic OC usage is associated with venous insufficiency and consequent impaired venous return,[59] we might expect these changes to be further exacerbated in OC users.

HEMODYNAMIC CONTROL

Hemodynamic function relies, in part, on the function of the autonomic nervous system. The autonomic nervous system, divided into the sympathetic (SNS) and parasympathetic (PNS) nervous systems, has a strong influence on our stress responses, including heart rate and blood pressure.[60] We might expect fluctuations in ovarian hormones to confer changes in autonomic function, as estrogen has been found to attenuate sympathetic activity.[61,62]

One measure of autonomic function (and therefore hemodynamic control) of interest to this study is HRV. HRV is a measure of beat-to-beat variability in heart rate and is thought to result from adaptation to changes in blood pressure by the SNS and PNS.[63] Low HRV suggests decreased nervous system adaptation and is associated with a 32-45% increased risk of a first cardiovascular event in individuals

without CVD,[64] thus HRV was selected as a measure of hemodynamic control for this study. Chronic OC usage has been found to impair autonomic regulatory capacity, decreasing HRV compared to non-OC users.[65] There are limited studies investigating changes in HRV across the OC cycle, but the existing literature demonstrates little difference between phases.[5]

GSR is another way by which we can quantify autonomic function, as it is an independent index of sympathetic activity.[66] Sweat gland activity is upregulated by the increased sympathetic activity, leading to corresponding increases in GSR.[67] Therefore, as elevated estrogen levels are associated with dampened SNS activity, we might expect an inverse relationship with GSR as well. However, there is currently no data on the relationship between changes in estrogen across the OC cycle and changes in GSR.

HORMONAL PROFILE (BLOOD ESTROGEN)

Blood estrogen levels were also be measured to determine if hormonal fluctuations coincide with changes in our measures of interest, as ethinyl estradiol will change the most between cycle phases, and estradiol is the most biologically active estrogen. It is hypothesized that fluctuations in exogenous estrogen concentration may drive the changes in vascular function seen across the cycle, but this role has not yet been confirmed. Due to suppression of endogenous estrogen with OC use, we can expect significantly higher estrogen during the early and late active phases compared to the inactive phase, but perhaps no significant differences between the early and late active phases.[13,68] However, it is unknown how these changes may be associated with our measures of interest.

WHY IS THIS STUDY NEEDED?

Many studies exclude women or test them solely in the inactive phase of the OC cycle, but this conceals potential sex differences and decreases external validity. Furthermore, current literature exhibits conflicting findings concerning the extent of physiological measurement variability across the cycle, putting into question the necessity of controlling for the cycle. Therefore, we must ascertain the

measurement precision of vascular and hemodynamic measures across the OC cycle. To our knowledge, no study has examined reliability of these measures across the cycle in women using OCs.

To correctly attribute our results to OC usage, we also needed a control group with no OC use for comparison. Thus, we compared this data to that of a group of males at all three phases of the cycle.

POTENTIAL CHALLENGES & ALTERNATIVE STRATEGIES

ATTRITION

Since participants must attend three separate visits over the course of a month, we ran the risk of attrition. To account for this, we opted to include any subjects who attended at least two study visits, as we were still able to calculate reliability statistics using this incomplete data.

UNMET RECRUITMENT TARGETS

Due to the existing pandemic and our inclusion criteria, we expected that we might fail to acquire our desired sample size. This lack of power could inherently reduce the validity of the conclusions drawn from the study. However, the study is ongoing, and will continue until the desired sample size is met.

COVID-19

Due to the COVID-19 pandemic and necessary public health restrictions, certain ultrasound measures such as CIMT and carotid stiffness may require a different approach and extra training, to ensure safety of the participant and data collectors, as the use of probes and gel may increase risk of viral transmission and necessitate the use of specific disinfection procedures.

CARRY- OVER EFFECTS

The necessity for multiple study visits introduces the risk of carry-over effects. While unlikely, there is a small possibility of an impact on vascular measures between visits.

TIMELINE

Table 8 details the timeline of our study, including development and preparation, recruitment and data collection, and the synthesis of the results.

TABLE 8. Project timeline and milestones

Activities	**Start Date**	**End Date**
Pilot Testing	October 15, 2021	January 9, 2022
Equipment SOP's current	May 12, 2022	August 28, 2022
Protocol/IRB	February 12, 2022	October 5, 2022
Staff training	August 2022	November 1, 2022
Study forms/ database	February 12, 2022	Ongoing
Set-up filling system (OneDrive)	October 11, 2022	December 19, 2022
Recruitment	October 8th 2021	February 9, 2022
Data collection	December 5, 2022	March 22, 2022
Aim 1 analysis	March 23, 2022	April 6, 2022
Hand Document to Committee	**APRIL 19, 2022**	
Defend	**MAY 3, 2022**	
Respond to defense changes	May 5. 2022	May 14, 2022
Submit thesis to graduate school	May 17, 2022	May 30, 2022
Authorship order agreement	May 4, 2022	May 18, 2022
Prepare Manuscript	June 1, 2022	July 31, 2022

CHAPTER IV: METHODOLOGY

This study is reported in accordance with CONSORT (Consolidated Standards of Reporting Trials) guidelines. Ethical approval was obtained by the University of North Carolina at Chapel Hill Institutional Review Board (IRB #21-2465). All participants provided written informed consent prior to study participation.

PARTICIPANTS

17 individuals were recruited (12 females, 5 males) between 18-35 years of age from the UNC-CH population. Female participants were included if they had been taking an OC pill for at least 6 months. We excluded individuals with any cardiometabolic disease, pregnant women, current smokers, and those taking any medications known to impact cardiovascular function. Eligibility was assessed using a Qualtrics survey. A follow-up email was sent to gather information regarding OC type and dosage, as well as OC cycle phase dates. No incentives were provided for participation. The UNC-CH Institutional Review Board approved all methods, and all participants provided written informed consent prior to participation.

EXPERIMENTAL DESIGN

This study was a reliability study with 3 between-day and 3 within-day measurements per outcome. Participants were assigned to groups based on sex, as the study compared men (control group) to women taking OCs. 16 of the 17 participants completed 1 familiarization and 3 experimental visits over the course of a month (one participant was only able to complete 2 experimental visits due to time constraints). For men, sessions occurred at the same corresponding time points throughout the month.

Only young and healthy individuals were included in this study to prevent any confounding of our variables of interest due to age or cardiometabolic abnormalities.

Each session lasted approximately 90 minutes, consisting of a rest period, followed by collection of several vascular and hemodynamic measures in the supine, seated and semi-recumbent positions, finishing with venipuncture. (see **Figure 6**).

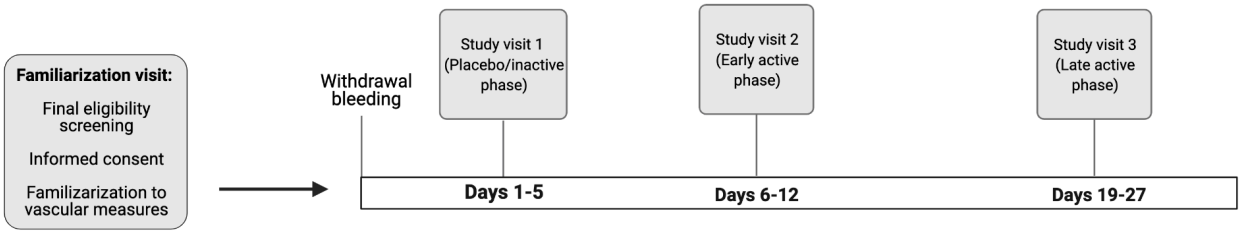

FIGURE 6. Study timeline

All data was collected in a research laboratory setting (UNC-CH Applied Physiology Lab) by trained graduate and undergraduate research students. During the initial familiarization session, written informed consent was obtained, general demographic information such as age, height, weight, and arterial path lengths was recorded, and participants were familiarized to all measures. Before leaving the laboratory, participants were informed of the following pre-assessment guidelines for their study visits, to ensure quality of data: i) no vigorous exercise 24 hours prior to time of experimental session; ii) no alcohol consumption 24 hours prior to time of experimental session; iii) no caffeine or food consumption 12 hours prior to time of experimental session. The familiarization session took place within 1 week prior to the first experimental visit, and within 5 weeks of completing all 3 experimental visits.

RECRUITMENT

The sample was recruited by posting fliers in 2 physical activity centers and 5 classrooms at UNC-CH. Fliers were also shown directly to students enrolled in Lifetime Fitness classes at UNC-CH. The study listing was also posted on Research for Me at UNC automatically after IRB approval. Fliers asked non-smoking men and women, 18-35 years of age, free of cardiovascular disease, if they wanted to participate in a study of their cardiovascular and cerebrovascular function. It was also specified that the

study sought only women taking OCs. Participants expressing interest were instructed to contact the principal investigator, who then provided the Qualtrics survey to ensure they met inclusion criteria.

EXPERIMENTAL MEASURES

The timeline for the experimental visits is depicted in **Figure 7**. During experimental visits, participants had their vascular function assessed using the following measures: carotid diameter, CIMT, carotid beta stiffness, pulse wave velocity (cfPWV, bfPWV, faPWV), central and peripheral blood pressure, Q, SV, and HRV. All measures were taken in the supine, then semi-recumbent, then seated posture. Of note, we defined the supine position as a 25° angle, rather than fully supine, as the fully supine position may cause carotid backflow and make it difficult to obtain cfPWV. The semi-recumbent position was defined as a 45° angle, and the seated position was defined as a 90° angle for both the torso and the legs. Measurements were taken in these postures for several reasons: 1) Although not discussed in this document, we will later determine the repeated-measures reliability of our measures; that is, the extent to which changes in a measure remain similar under repeated conditions. To induce these changes, a hemodynamic perturbation was necessary, and postural change is evidenced to induce this perturbation; 2) Measuring in each posture allows us to determine the reliability of each measure in each individual posture. Since these measures are typically obtained in different postures in both the laboratory and the clinic (e.g., blood pressure in the seated posture), it is important to assess their reliability in each posture across the OC cycle; and 3) Obtaining three data points (one data point per posture) per outcome per visit creates greater statistical power. After all experimental outcomes were obtained, participants had their blood drawn using venipuncture to assess blood estrogen levels. Vascular and hemodynamic measurements took approximately 60 minutes in total and were preceded initially by 10 minutes of quiet, supine rest. Each subsequent postural change was also followed by 5 minutes of quiet rest. Venipuncture lasted approximately 10 minutes; thus, the experimental visit lasted approximately 90 minutes in total. All

participants were fasted (both food and caffeine) and arrived at the laboratory between the hours of 6:00 and 10:00 AM, keeping the time of day as consistent as possible between study visits.

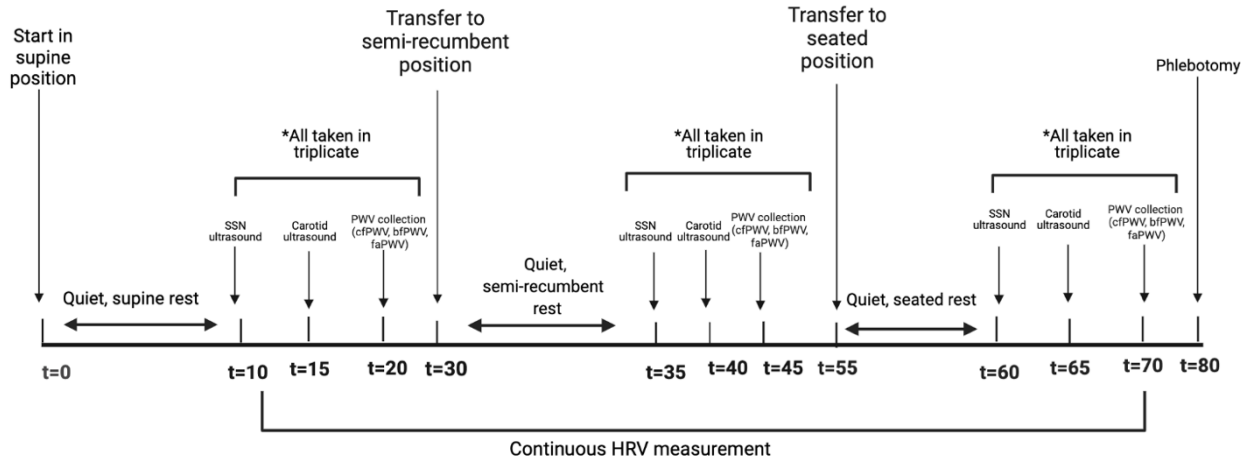

FIGURE 7. Experimental visit timeline

MEASURES OF VASCULAR FUNCTION

A number of measures (PWV, AIx, β-stiffness index, and CIMT) were conducted to assess vascular function, as estrogen and progesterone receptors are abundant throughout the vasculature;[2] therefore. changes in ovarian hormonal concentration across the OC cycle tend to impact vascular characteristics, including arterial stiffness[69] and SMC proliferation.[16]

Pulse wave velocity (PWV)

PWV was chosen for this study as it is the gold standard measure of arterial stiffness and is an independent predictor of cardiovascular mortality.[40] Central and peripheral arterial stiffness were assessed using the VICORDER® device (SMT Medical). The VICORDER® device has been shown to be a valid and reliable measure of PWV.[70] This system acquires these outcomes using an automated, non-invasive blood pressure cuff system. This study measured central arterial stiffness using carotid-femoral (cf)PWV and brachial-femoral (bf)PWV, and peripheral arterial stiffness using femoral-ankle (fa)PWV. These have

all been shown to be reliable measures of arterial stiffness, according to previous research.[71–73] These measures were acquired by wrapping VICORDER® blood pressure cuffs around the participant's arterial sites of interest for each measure (carotid and femoral arteries for cfPWV, brachial and femoral arteries for bfPWV, and femoral artery and ankle for faPWV). The proximal and distal cuffs were placed ipsilaterally on the left side of the body. Pulse transit time is calculated automatically by the VICORDER® system. Straight-line distances between measurement sites were recorded according to manufacturer guidelines to allow the device to calculate PWV. For cfPWV, the straight-line distance was measured from the sternal notch to the superior border of the femoral cuff minus the distance between the carotid artery and the sternal notch. For bfPWV, the straight-line distance was measured between the sternal notch and umbilicus. For faPWV, the straight-line distance was measured between the superior borders of the pressure cuffs placed over the femoral artery and ankle site. All PWV measures were taken after 10 minutes of quiet supine rest, in triplicate, with the closest two values being averaged together and recorded for further analysis. After all supine measures were recorded, participants were passively transferred to the semi-recumbent position (Armedica AM353 Hi-lo Treatment Table; Tiger Medical, TIGER#TM83695) to rest for 5 minutes, and all measures were repeated in the semi-recumbent position. This process was then repeated in the seated position.

Augmentation index (AIx)

AIx was chosen as it is determined by both large artery PWV and the distensibility of smaller arteries,[45] and will thus confer additional information about vascular function. The VICORDER® will conduct PWA in addition to PWV, which can be used to derive AIx: a measure of wave reflection and surrogate measure of arterial stiffness, which has been proven to be an adequate screening parameter of vascular aging and marker of CVD risk.[74] AIx is defined as the percentage of the central pulse pressure that can be attributed to the reflected pulse wave.[75] Determination of central aortic pressure by the VICORDER® has been validated.[54] AIx measures were taken in all three postures in triplicate, with the closest two values being averaged together.

Carotid β-stiffness index and CIMT

Assessing the carotid artery is especially important in surveying vascular health, considering the heightened risk of atherosclerotic plaque formation at the carotid.[48] Furthermore, due to structural differences in arterial segments, carotid stiffness may not always correspond with aortic stiffness, so both measures must be taken to assess functional changes throughout the arterial tree. Ultrasound of the common carotid artery was performed to collect three measures of carotid arterial health: Carotid β-stiffness index, diameter, and CIMT. All three measures were assessed using B-mode ultrasound images of the right common carotid artery using a 12 MHz linear array ultrasound probe attached to a high-resolution ultrasound machine (Vivid Q, GE Medical Systems, Horten, Norway) for 10 consecutive cardiac cycles at 22.5 frames/second. First, ultrasound gel was applied, and the common carotid arterial site was located by placing the probe perpendicular to the common carotid artery and applying a small amount of pressure on the probe to ensure it is an artery (no compression will occur if it is an artery) and not a vein (compression will occur if it is a vein). The probe was then slowly turned to approximately 90°, ensuring the probe notch is oriented towards the heart. Once the artery was located, the arterial segment was identified by locating the carotid bulb, moving it to the right of the screen, and imaging the common carotid artery in a longitudinal section 1 cm proximal to the carotid bulb. Finally, a 10 mm straight arterial segment, free of plaque (preferably on the far wall of the common carotid) was identified, ensuring both the top and bottom vessel walls were clear. This location was marked to ensure consistency across measures. During data collection, the probe was positioned laterally for the best resolution. Three 10-second video recordings will be captured for analysis. Participants were instructed to hold their breath during each video (but not to take a deep breath in beforehand). After 10 seconds, the videographer told the subject to breathe as they stop the video.

Videos were exported to the Carotid-Studio software (Cardiovascular Suite 4.3.0, Quipu, Pisa, Italy) for determination of the β-stiffness index and intima media thickness. Carotid studio has been shown to be reliable in healthy subjects for assessment of these variables, with a coefficient of variation (CV) of 2% for diameter and 6% for CIMT.[76] Carotid Studio provides a chart with the systolic and

diastolic arterial diameters for the duration of the recording (10 cardiac cycles). Carotid arterial diameter was defined as the minimum systolic diameter for each recording. The β-stiffness index was calculated using the following equation: β-stiffness index = ln(SBP/DBP)/[(LDmax−LDmin)/LDmin],[77] where SBP is systolic blood pressure, DBP is diastolic blood pressure (recorded previously using PWA), LDmax is maximum lumen diameter of the carotid artery, and LDmin is minimum lumen diameter of the carotid artery. For CIMT, once the region of interest is identified, the software automatically detects the intima and media layers. When analysis is run, a mean CIMT value is provided in mm. For all ultrasound variables, data was reduced by averaging the closest two measures from the three images.

MEASURES OF HEMODYNAMICS

Central and peripheral blood pressure, Q and SV were chosen to assess hemodynamics, as estrogen and progesterone receptors are found in the vasculature and kidneys;[2] therefore, changes in ovarian hormonal concentration across the OC cycle tend to impact vascular tone[15] and fluid homeostasis.[33,78]

Central and peripheral BP

Traditional brachial systolic pressure may be 40 mmHg higher than what occurs in the aorta,[54] therefore we measured both central and blood pressure as additional assessments of vascular function and fluid regulation. PWA can also be used to estimate central and peripheral blood pressure (BP, cBP). Completion of PWA requires measurements of peripheral oscillatory SBP and DBP at the brachial sites. BP was recorded in all three postures, concurrently with bfPWV. Both central and peripheral measures were taken in triplicate, with the closest two values being averaged together.

Blood flow (Cardiac output and stroke volume)

Hemodynamic measures such as cardiac output and stroke volume have a strong influence on bodily blood flow, and thus tissue perfusion and delivery of oxygen and nutrients. They also confer

unique information about cardiovascular function as they depend on myocardial contractility and fluid balance, which may vary with changes in hormone concentration. A Doppler probe from Ultrasonic Cardiac Output Monitors (USCOM) was applied to locate a pulse signal at the level of the suprasternal notch to capture Q and SV. Q was used as a surrogate measure to approximate changes in aortic blood flow. With USCOM, SV and Q can be reliably determined from the flow profile and the valve cross-sectional area (CSA).[79] Flow velocity can be calculated from the volumetric flow rate equation $v = \frac{Q}{a}$, where Q is cardiac output, a is aortic valve CSA and v is the flow velocity. Aortic wall shear stress can then be estimated from Poiseuille's law: $\gamma = \frac{2\,(2+n)v}{d}$ where γ is wall shear stress, d is internal arterial diameter, v is time-averaged blood flow rate velocity, and n is the velocity profile (in this case, $n = 2$ for a parabolic profile).

MEASURES OF HEMODYNAMIC CONTROL

HRV and GSR were measured to assess hemodynamic control, as estrogen and progesterone receptors are found within the brain;[80] therefore changes in ovarian hormonal concentration across the OC cycle tend to impact autonomic balance, which can indirectly modulate hemodynamics.[62]

Heart rate variability and GSR

Heart rate variability (HRV) was collected as an assessment autonomic nervous system function and cardiovascular health, which may change across the cycle. HRV was measured continuously using the Eq LifeMonitor (Equivital, Cambridge, UK). Participants were fitted for the correct sized vest during the familiarization visit by measuring their bust size with a tape measure. Electrodes were placed on the second and fourth finger over the proximal phalanges to continuously collect GSR data. Galvanic response sensors were connected to the electrodes and connected to the vest's sensory electronics module to allow for data processing in LabChart (ADI Instruments, Sydney, AU). Prior to applying the vest to the subject, electrocardiogram (ECG) sensors lining the band were wet using a damp paper towel to ensure

proper conductance. ECG data was sampled at a frequency of 1000 Hz. R-R interval data was collected from the ECG and exported from LabChart to Kubios HRV Software (Kubios Oy, Kuopio, Finland) as a text file. To determine HRV, we recorded the RMSSD (root mean square of successive differences) from Kubios, as this measurement is appropriate for a 10-minute bout where a subject is breathing normally. The RMSSD reflects the beat-to-beat variance in heart rate, and is the primary measure used to estimate vagally mediated changes in HRV.[81] RMSSD has been shown to be a reliable parameter (ICC=0.76) for assessing HRV from short resting ECG recordings with spontaneous breathing.[82]

HORMONAL PROFILE

Blood estrogen

Blood estrogen was measured in this study as we hypothesized that it is the main driver of vascular and hemodynamic changes in OC users across the cycle. Venipuncture was performed to measure blood estrogen (ethinyl estradiol) concentration and evaluate its relationship with changes in vascular function across the OC cycle. Blood was collected in one 10 mL serum blood collection tube, and one 5 mL plasma blood collection tube. (BD Vacutainer Plus, Red BG Hemogard Closure, Franklin Lakes, NJ). Tubes were prepared with the subject ID, date of the sample, experimental visit (first, second, or third), and time of data collection. Venipuncture was performed on the most optimal vein within the antecubital fossa. Preferably, the middle antecubital or cephalic vein was be used, with the basilic only selected if completely necessary, due to the possibility of heightened subject discomfort. The phlebotomist placed a tourniquet on one arm and had the participant squeeze a ball in their fist to increase pressure within the vein, and thus, visibility. They then surveyed the arm and palpated each vein to determine the optimal vein. This process was repeated on the other arm to ensure the best vein is selected. The area was cleaned with isopropyl alcohol, the tourniquet was applied, and the phlebotomist collected the sample. Once the sample was collected, the tube and needle were removed, the participant was given gauze and told to hold pressure for a few minutes to prevent hematoma.

Due to lack of funding, analysis of blood estrogen has not yet occurred at this point in time. Once possible, ethinyl estradiol levels will be measured using enzyme-linked immunosorbent assay (ELISA) (Estradiol Ultrasensitive ELISA, ALPCO Diagnostics, Salem, NH). The Estradiol Ultrasensitive ELISA has been shown to have very good reliability, with an intra-assay coefficient of variation (CV) of 6.4%, and an inter-assay CV of 7.6%.[83]

RANDOMIZATION

The cycle phase during which all subjects had their first study visit was be randomized due to potential confounding from anxiety/'white coat syndrome'. We used a random number generator (www.randomizer.org), allocating an equal number of participants to '1' (placebo), '2' (early active), and '3' (late active) for their first study visit. Subjects then proceeded in numerical order to the next phase for the next study visit.

QUALITY CONTROL

All measurements and analysis were conducted by a single observer for a given outcome. At the start of the study, the first three data sets were checked by an independent observer. A quality grading score were part of this over-reading so that protocol deviations can be detected. At the conclusion of the study, a random selection of 10% of the data sets (e.g., all data from 3 participants) were re-scored by an independent observer and used to calculate inter-observer reliability.

SAMPLE SIZE

G*Power Statistical Power Analysis Software v3.1 (Heinrich-Heine Universität Düsseldorf, Germany) was used to determine the minimum sample size, assuming an intraclass correlation coefficient (ICC) of at least 0.75 (i.e., acceptable reliability across cycle phases) for cfPWV in women. The

calculation used an α-level of 0.05 and 80% power. The output was 34 participants, but in order to account for unexpected loss to follow-up, we inflated this to 40 participants.

To achieve adequate participant enrollment, we maximized the number of locations in which study fliers are posted. We will also use the service "Research for Me at UNC", which advertises ongoing studies to those who are looking to participate in research within UNC. This has been found to boost enrollment for prior studies.

DATA MANAGEMENT AND STATISTICAL ANALYSIS

HRV analysis was conducted using Kubios HRV Standard Edition v3.3.1. Data management and analysis was completed using several software packages. Storage and aggregation of experimental data will be completed in Microsoft Excel (Excel, IN., Redmond, WA, USA). Data was stored securely within password protected cloud-based data services (OneDrive, Microsoft), as well as on transportable USB drives kept by members of the research team (JP & NA).Data files and subjects were depersonalized with the suffix OC (e.g., Participant 1 = OC01, 2 = OC02, etc.). Outcomes derived from HRV were log-transformed to account for non-standard distribution. The α-level was set a priori for all statistical procedures at $\alpha=0.05$. Summary statistics were reported as mean [SD].

All reliability statistics (ICC, SEM, MDC, and MDC%) were calculated for measures of arterial stiffness, blood pressure, carotid function, and HRV. These statistics allow us to assess the reliability of our outcomes (i.e., the extent to which these outcomes remain the same between study visits), as well as the sensitivity of our measurements (i.e., the smallest amount of change in a measure that can be detected, as well as the variation that can be attributed to user or equipment error). The ICC was used to ascertain between-day reliability (i.e., between the inactive, early active, and late active phases), where ICC values of <0.5, 0.5, 0.75, and >0.9 indicate poor, moderate, good/acceptable, and excellent reliability, respectively.[84] ICC was calculated as $SDb^2 / [SDb^2+SDw^2]$, where SDb^2 and SDw^2 are the between and within-subject variance, respectively. The SEM was then be calculated as $SD*\sqrt{(1-ICC)}$. 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.

CHAPTER V: RESULTS

PARTICIPANTS

Participants were recruited between October 8th, 2021, and February 9th, 2022, through the Research for Me and UNC platform, posters (including a QR code linking to our Qualtrics eligibility survey), emails, and short presentations to classes at UNC Chapel Hill. 58 participants were screened for eligibility through the Qualtrics survey. Out of those 58 participants, 12 were ineligible, and 46 were eligible to participate in the study. Out of those 46 eligible participants, 25 responded to our follow-up email requesting more information (i.e., OC type and dosage, dates of the start of the next 3 inactive phases). Following the receipt of this information, we reached out to all 25 participants to schedule their initial study visit and 20 responded. 2 participants declined to participate due to lack of compensation. Therefore, 18 participants were enrolled in the study, of which 16 completed all three experimental visits. One participant dropped out of the study after one visit due to time constraints, while one subject was unable to attend a study visit during her inactive phase. Due to the nature of the reliability study, participants were only included in the analysis if they completed at least 2 visits, therefore 17 subjects were included in the final analysis. See **Figure 8** for CONSORT flow and reasons for exclusion.

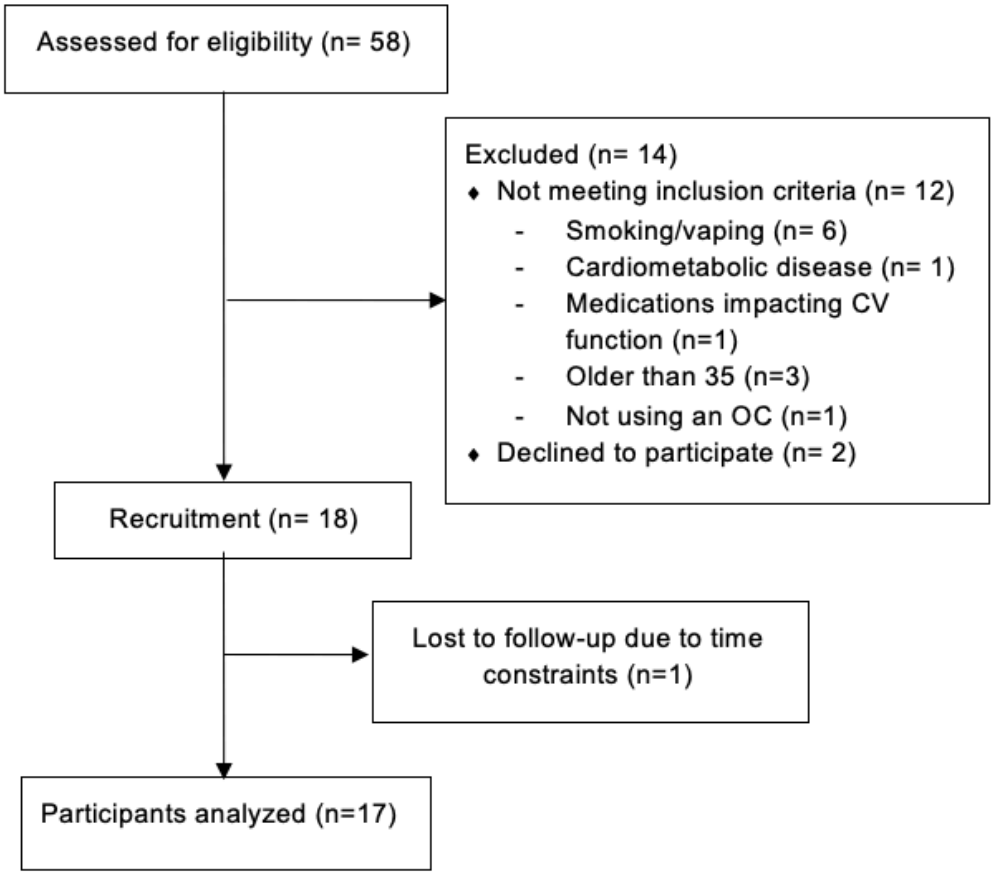

FIGURE 8. CONSORT diagram for recruitment and testing

For OC users, visit 1 occurred 1–5 days after the onset of withdrawal bleeding (placebo pill or no pill), visit 2 occurred 6–12 days after the onset of withdrawal bleeding ("early" active hormonal pills), and visit 3 occurred 19–27 days after the onset of withdrawal bleeding ("late" active hormonal pills).[9] Men attended visits at the same corresponding time points to maintain consistency, although they did not have distinct cycle phases.

BASELINE DATA

Table 9 lists the different OC types and dosages used by the women within our sample, as well as important subject characteristics for both experimental groups.

TABLE 9: Participant characteristics

Experimental Group		n	Age (yrs)	BMI (kg/m^2)
OC Users		12	21.5 [2.4]	22.4 [0.9]
Men		5	21.0 [3.0]	24.4 [2.8]
Overall		17	21.2 [2.8]	23.8 [2.6]
OC type	**OC dosage (mg)**	**n**	**Active ingredients**	
Alyacen	1/35	1	Norethindrone/ethinyl estradiol	
Blisovi	1/20	4	Norethindrone acetate/ethinyl estradiol	
Estarylla	0.25/0.035	1	Norgestimate/ethinyl estradiol	
Larissa	0.1/0.02	1	Levonorgestrel/ethinyl estradiol	
Loestrin	1/10	1	Norethindrone acetate/ethinyl estradiol	
Tri-Lo-Sprintec	0.25/0.035	3	Norgestimate/ethinyl estradiol	
Taytulla	1/20	1	Norethindrone acetate/ethinyl estradiol	

Note: All age and BMI data are recorded as mean [SD]

PRIMARY OUTCOMES

For simplicity, we only report the ICC data for our outcomes below, but our supplementary data within the appendices contains additional SEM and MDC data. In short, **Table 10** is a summary table providing the ICC data for all outcomes. The results for each outcome are described in more detail below.

TABLE 10. Summary of Intraclass Correlation Coefficient data

Outcome	OC users	Men
cfPWV	0.99, 95% CI [0.98, 1.00]*	0.99, 95% CI [0.97, 1.00]
bfPWV	0.98, 95% CI [0.97, 0.99]	0.99, 95% CI [0.98, 1.00]*
faPWV	0.95, 95% CI [0.91, 0.98]	0.98, 95% CI [0.93, 0.99]*
AIx	0.99, 95% CI [0.97, 0.99]*	0.92, 95% CI [0.78, 0.97]
SBP	0.97, 95% CI [0.95, 0.99]*	0.89, 95% CI [0.69, 0.96]
DBP	0.99, 95% CI [0.98, 1.00]*	0.99, 95% CI [0.96, 1.00]
cSBP	0.96, 95% CI [0.92, 0.98]*	0.91, 95% CI [0.74, 0.97]
Q	1.00, 95% CI [1.00, 1.00]*	0.97, 95% CI [0.91, 0.99]
SV	1.00, 95% CI [0.99, 1.00]*	0.99, 95% CI [0.98, 1.00]
β-stiffness	0.98, 95% CI [0.96, 0.99]*	0.94, 95% CI [0.83, 0.98]
CIMT	0.92, 95% CI [0.85, 0.96]	1.00, 95% CI [1.00, 1.00]
HRV	1.00, 95% CI [0.99, 1.00]	1.00, 95% CI [0.99, 1.00]
GSR	0.90, 95% CI [0.76, 0.96]*	0.85, 95% CI [0.27, 0.98]

Note: An asterisk denotes a more reliable measure when comparing experimental groups, with greater reliability quantified by a higher ICC, or by a narrower confidence interval, given an equivalent ICC.

VASCULAR OUTCOMES

All outcomes were acceptably reliable across experimental visits in both sexes.

Arterial stiffness (PWV)

The reliability was excellent (ICC ≥ 0.90) for all measures of PWV (cfPWV, bfPWV, faPWV), in both OC users and men. ICC values for cfPWV were 0.99, 95% CI [0.98, 1.00] 0.99 and 95% CI [0.97, 1.00], respectively. ICC values for bfPWV were 0.98, 95% CI [0.97, 0.99] and 0.99, 95% CI [0.98, 1.00],

respectively. ICC values for faPWV were 0.95, 95% CI [0.91, 0.98] and 0.98, 95% CI [0.93, 0.99], respectively. Mean daily cfPWV remained across cycle phases, while there was a slight decrease in mean daily bfPWV and faPWV as the cycle progressed from the inactive to active phases (see **Figure 10)**.

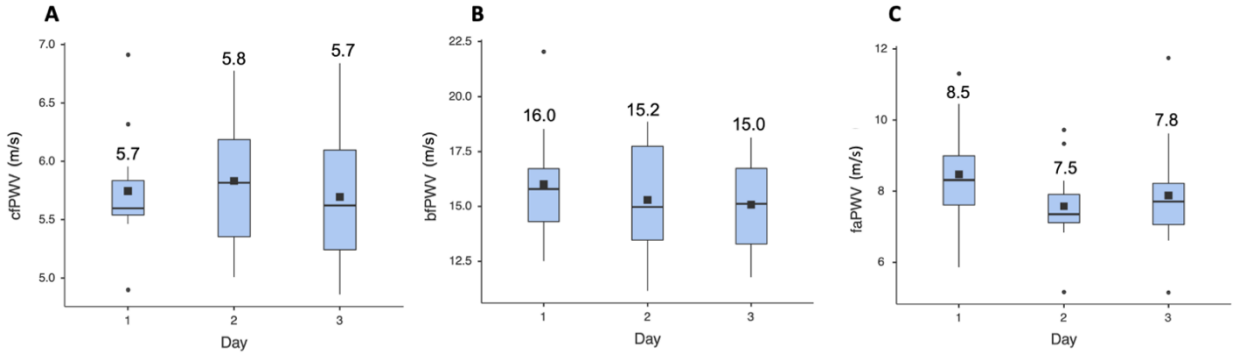

FIGURE 9. Mean cfPWV (A), bfPWV (B), and faPWV (C) values across cycle phases in OC users

Notes: Day 1: inactive phase; Day 2: early active phase; Day 3; late active phase. Error bars represent standard deviation.

Wave reflection (AIx)

AIx achieved excellent reliability in both OC users and men across experimental visits, with ICC 0.99, 95% CI [0.99, 1.00] and 0.92, 95% CI [0.78, 0.97], respectively. Although changes across the cycle were not sufficient to hinder reliability, there was a slight trend in that mean AIx decreased as the cycle progressed from the inactive to active phases (see **Figure 10)**.

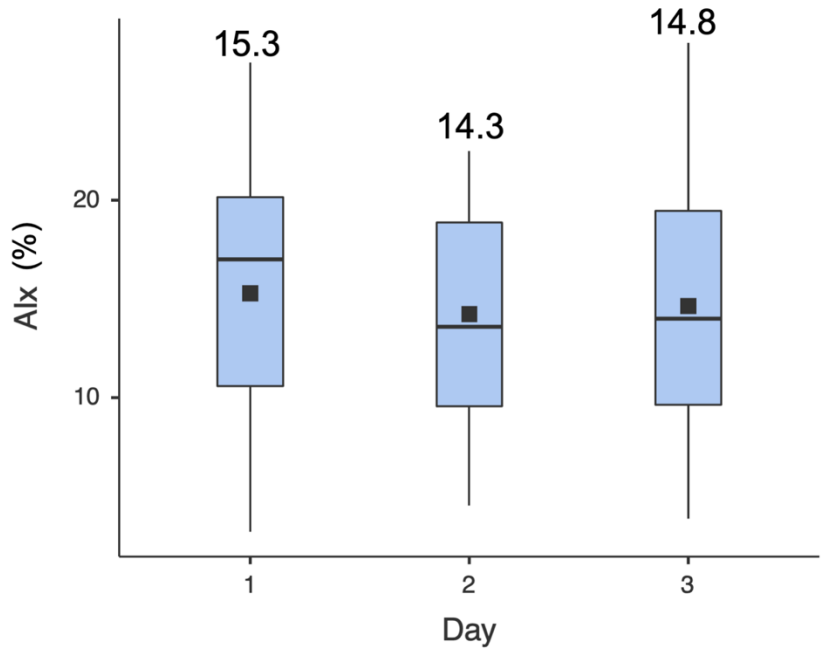

FIGURE 10. Mean AIx values across cycle phases in OC users

Notes: Day 1: inactive phase; Day 2: early active phase; Day 3; late active phase. Error bars represent standard deviation.

Carotid β-stiffness index

Carotid β-stiffness index achieved excellent reliability in both OC users and men across experimental visits, with ICC 0.98, 95% CI [0.96, 0.99] and 0.94, 95% CI [0.83, 0.98], respectively. Additionally, mean daily carotid β-stiffness values across the sample of OC users remained stable across cycle phases (see **Figure 11)**.

CIMT

CIMT achieved excellent reliability in both OC users and men across experimental visits, with ICC 0.92, 95% CI [0.85, 0.96] and 1.00, 95% CI [1.00, 1.00], respectively. Although changes across the cycle were not sufficient to hinder reliability, there was a slight trend in that mean CIMT decreased as the cycle progressed from the inactive to active phases (see **Figure 11)**.

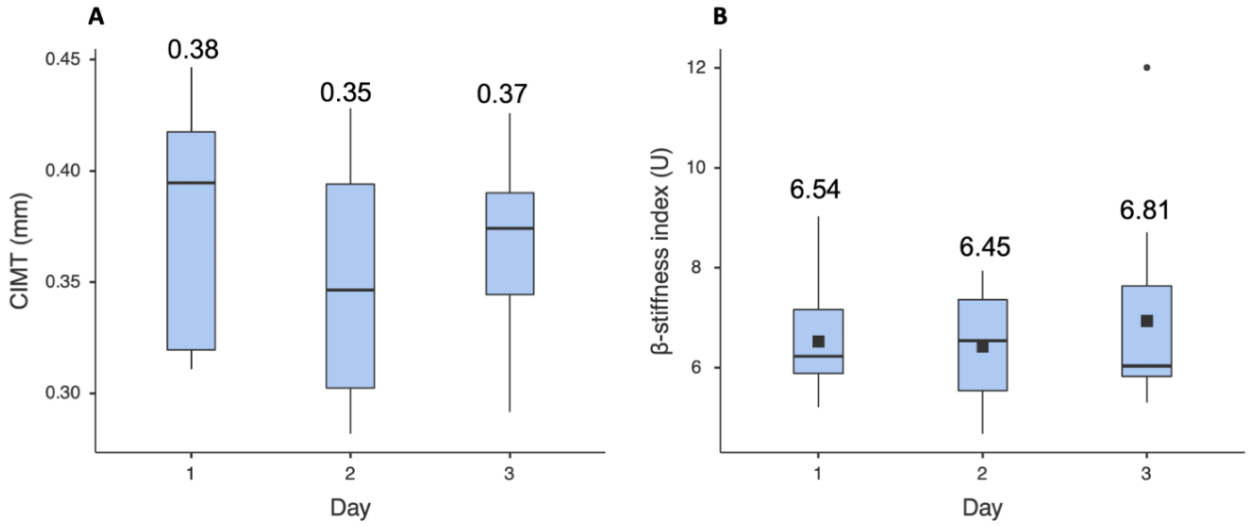

FIGURE 11. Mean carotid ultrasound values across cycle phases in OC users

Notes: Day 1: inactive phase; Day 2: early active phase; Day 3; late active phase. Error bars represent standard deviation.

HEMODYNAMIC OUTCOMES

All outcomes were acceptably reliable across experimental visits in both sexes.

Peripheral blood pressure

SBP achieved excellent reliability in OC users and good reliability in men across experimental visits, with ICC 0.97, 95% CI [0.95, 0.99] and 0.89, 95% CI [0.69, 0.96], respectively. Additionally, mean daily SBP values across the sample of OC users remained stable across cycle phases (see **Figure 12)**.

DBP achieved excellent reliability in both OC users and men across experimental visits, with ICC 0.99, 95% CI [0.98, 1.00] and 0.99, 95% CI [0.96, 1.00], respectively. Additionally, mean daily DBP values across the sample of OC users remained stable across cycle phases (see **Figure 12)**.

Central blood pressure

cSBP achieved excellent reliability in both OC users and men across experimental visits, with ICC 0.96, 95% CI [0.92, 0.98] and 0.91, 95% CI [0.74, 0.97], respectively. Although changes across the cycle were not sufficient to hinder reliability, there was a slight trend in that mean cSBP decreased as the cycle progressed from the inactive to active phases (see **Figure 12)**.

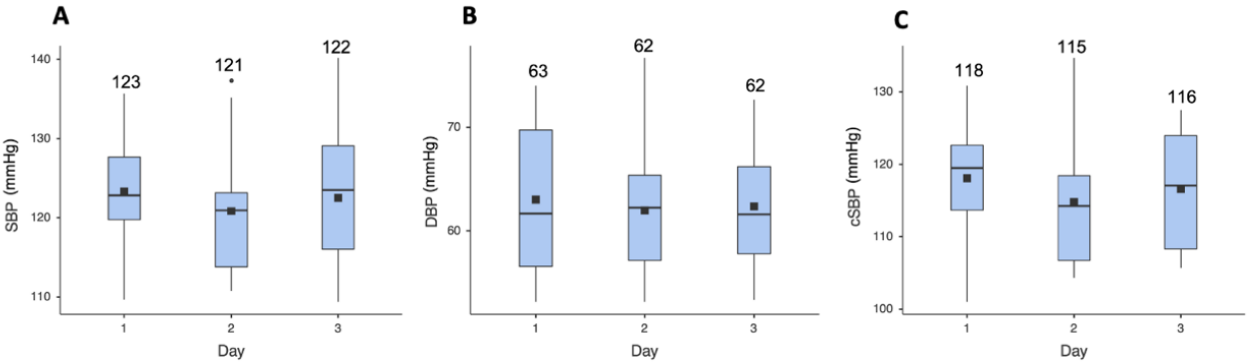

FIGURE 12. Mean SBP (A), DBP (B), and cSBP (C) values across cycle phases in OC users

Notes: Day 1: inactive phase; Day 2: early active phase; Day 3; late active phase. Error bars represent standard deviation.

Q

Q achieved excellent reliability in both OC users and men across experimental visits, with ICC 1.00, 95% CI [1.00, 1.00] and 0.99, 95% CI [0.91, 0.99], respectively. Additionally, mean daily Q values across the sample of OC users remained stable across cycle phases (see **Figure 13).**

SV

SV achieved excellent reliability in both OC users and men across experimental visits, with ICC 1.00, 95% CI [0.99, 1.00] and 0.99, 95% CI [0.98, 1.00], respectively. Additionally, mean daily SV values across the sample of OC users remained stable across cycle phases (see **Figure 13**).

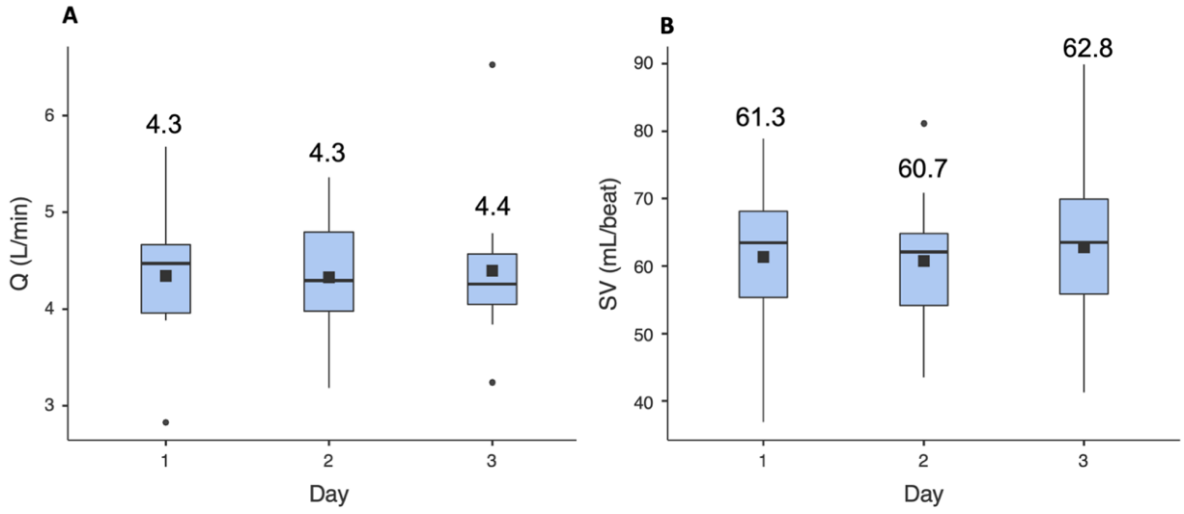

FIGURE 13. Mean blood flow values across cycle phases in OC users

Notes: Day 1: inactive phase; Day 2: early active phase; Day 3; late active phase. Error bars represent standard deviation.

HEMODYNAMIC CONTROL OUTCOMES

Both outcomes were acceptably reliable across experimental visits in both sexes.

HRV (RMSSD)

RMSSD achieved excellent reliability in both OC users and men across experimental visits, with ICC 1.00, 95% CI [0.99, 1.00] and 1.00, 95% CI [0.99, 1.00], respectively. Additionally, mean daily RMSSD values across the sample of OC users remained stable across cycle phases (see **Figure 14)**.

GSR

GSR achieved good reliability (ICC ≥ 0.75) in both OC users and men across experimental visits, with ICC 0.90, 95% CI [0.76, 0.96] and 0.85, 95% CI [0.27, 0.98], respectively. While GSR decreased from the inactive to early active phase, it then increased from the early active to late active phase. Thus, there was no clear trend observed for changes in GSR (see **Figure 14)**.

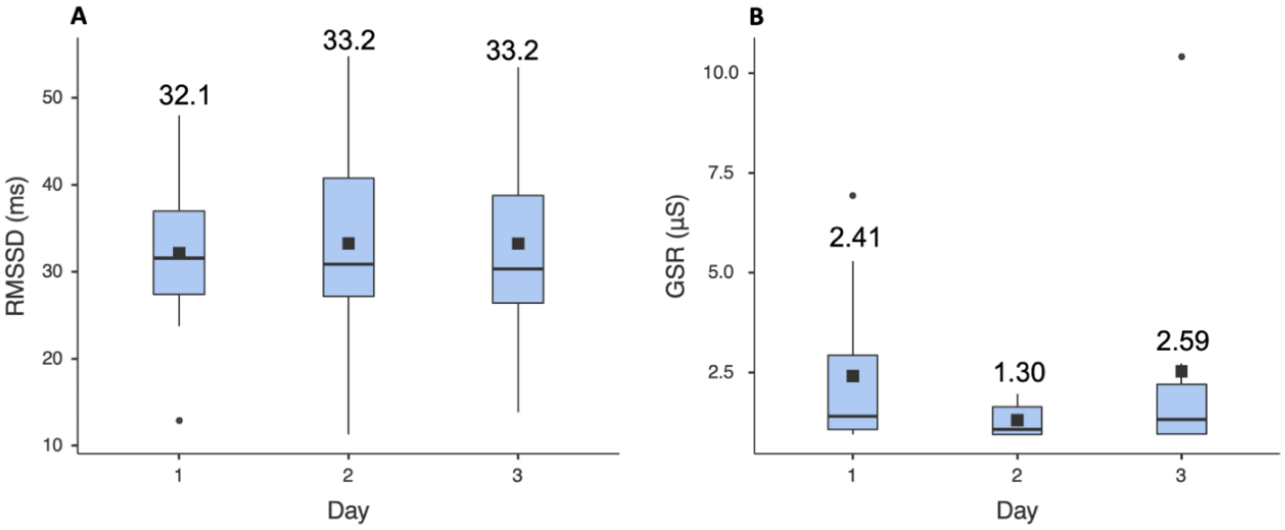

FIGURE 14. Mean HRV (A) and GSR (B) values across cycle phases in OC users

Notes: Day 1: inactive phase; Day 2: early active phase; Day 3; late active phase. Error bars represent standard deviation.

ANCILLARY ANALYSIS

In addition to having acceptable overall between-day reliability, all measures were found to be consistent between study visits, within all postures in OC users (See **Appendix C** for box plots and summary tables displaying individual postural data).

Table 11, Table 12, and Table 13 below show our descriptive statistics (mean, [SD]) for our outcomes of interest, given in each posture (as well as the overall average across postures). Additionally, the table includes the results of a mixed model analysis. We chose to display the interaction effect (i.e.,

condition*day), as this was insignificant for all outcomes of interest, indicating that changes in the given outcome between study visits did not depend on the postural condition. Therefore, due to an insignificant interaction effect, further analysis of the mixed model is unnecessary in assessing the simple effects of postural condition and day.

TABLE 11. Ancillary Vascular Analysis (OC users)

Measure	Day	Supine	Semi-Recumbent	Seated	Overall	P value (condition*day)
cfPWV (m/s)	1	5.1 [0.6]	5.9 [0.6]	6.2 [0.6]	5.7 [0.5]	0.769
	2	5.1 [0.7]	6.2 [0.9]	6.2 [0.5]	5.8 [0.6]	
	3	5.0 [0.6]	5.9 [0.6]	6.2 [0.9]	5.7 [0.7]	
bfPWV (m/s)	1	11.0 [1.4]	17.5 [4.0]	19.5 [3.8]	16.0 [2.6]	0.963
	2	10.8 [1.6]	16.5 [2.7]	18.8 [3.7]	15.3 [2.6]	
	3	10.5 [1.5]	16.7 [2.3]	18.3 [3.3]	15.1 [2.0]	
faPWV (m/s)	1	6.2 [0.9]	8.1 [1.8]	11.1 [2.1]	8.5 [1.5]	0.103
	2	5.7 [1.0]	7.2 [1.3]	9.9 [1.5]	7.6 [1.2]	
	3	6.0 [1.1]	8.1 [2.5]	9.6 [1.7]	7.9 [1.7]	
AIx (%)	1	16.7 [7.9]	14.7 [8.5]	14.5 [8.1]	15.3 [7.7]	0.750
	2	14.5 [6.6]	14.2 [7.4]	13.8 [7.2]	14.2 [6.0]	
	3	14.5 [6.7]	15.7 [9.4]	14.1 [7.2]	14.6 [7.3]	
β-stiffness index (U)	1	6.7 [1.1]	6.2 [1.3]	6.6 [1.5]	6.5 [1.1]	0.935
	2	6.4 [1.2]	6.4 [1.2]	6.5 [1.7]	6.4 [1.1]	
	3	7.1 [2.3]	6.9 [1.5]	6.8 [2.0]	6.9 [1.9]	
CIMT (mm)	1	0.37 [0.1]	0.40 [0.1]	0.36 [0.1]	0.38 [0.1]	0.713
	2	0.34 [0.1]	0.36 [0.1]	0.35 [0.1]	0.35 [0.1]	
	3	0.37 [01]	0.35 [0.1]	0.37 [0.1]	0.37 [0.1]	

Note: All descriptive data are recorded as mean [SD]

TABLE 12. Ancillary Hemodynamic Analysis (OC users)

Measure	Day	Supine	Semi-Recumbent	Seated	Overall	P value (condition*day)
SBP (mmHg)	1	121 [8]	123 [8]	126 [9]	123 [8]	0.301
	2	118 [8]	123 [10]	122 [9]	121 [9]	
	3	123 [14]	123 [10]	122 [10]	124 [9]	
DBP (mmHg)	1	60 [8]	63 [7]	66 [9]	63 [8]	0.754
	2	58 [7]	63 [8]	66 [7]	62 [7]	
	3	57 [5]	63 [8]	66 [8]	62 [7]	
cSBP (mmHg)	1	115 [8]	117 [8]	122 [10]	118 [8]	0.279
	2	112 [9]	117 [12]	116 [8]	115 [9]	
	3	116 [14]	118 [8]	116 [9]	117 [9]	
Q (L/min)	1	5.0 [1.1]	3.9 [0.8]	4.1 [0.8]	4.3 [0.7]	0.929
	2	5.0 [0.7]	4.1 [0.6]	3.9 [1.1]	4.3 [0.6]	
	3	5.1 [0.9]	4.0 [1.0]	4.1 [1.0]	4.0 [0.8]	
SV (mL/beat)	1	72.6 [12.6]	56.7 [13.6]	54.6 [13.1]	61.3 [11.6]	0.817
	2	74.2 [11.5]	57.2 [10.2]	50.8 [15.9]	60.7 [10.1]	
	2	75.8 [14.0]	57.1 [15.1]	55.4 [15.3]	62.8 [12.8]	

Note: All descriptive data are recorded as mean [SD]

TABLE 13. Ancillary Hemodynamic Control Analysis (OC users)

Measure	Day	Supine	Semi-Recumbent	Seated	Overall	P value (condition*day)
RMSSD (ms)	1	34.8 [10.6]	31.2 [11.4]	30.4 [11.4]	32.1 [9.7]	0.997
	2	36.8 [13.9]	31.3 [12.3]	31.7 [13.8]	33.2 [12.0]	
	3	36.6 [16.0]	32.1 [11.9]	31.1 [18.1]	33.2 [11.7]	
GSR (µS)	1	2.16 [2.3]	2.34 [2.2]	2.74 [2.2]	2.41 [2.1]	0.952
	2	1.00 [0.5]	1.30 [0.7]	1.61 [0.9]	1.30 [0.5]	
	3	2.00 [2.7]	2.65 [3.2]	3.18 [3.4]	2.53 [3.0]	

Note: All descriptive data are recorded as mean [SD]

CHAPTER VI: DISCUSSION

The main purpose of this study was to determine the reliability of measures of vascular and hemodynamic function across the OC cycle. Currently, it is believed that best practice for studies of cardiovascular function is to measure women solely during the inactive phase of their OC cycle, as it is believed that fluctuations in ovarian hormones may create variability in certain experimental measures, making them unreliable and unrepresentative of overall health. However, results within the current literature are conflicting, with some studies illustrating significant changes across cycle phases, and others showing no significant change. Thus, there is no current consensus as to whether the practice of controlling for the OC cycle is necessary. Furthermore, measuring women solely in the inactive phase may conceal important sex differences, and hinder external validity, as it only represents one third of a premenopausal OC user's life. Thus, it is important to add to the literature in hopes of ascertaining whether this practice is necessary. We therefore measured both OC users and males on three different occasions (representing the three OC phases) across the course of a month to ascertain the between-day reliability of our variables of interest. The main finding of the study was that all vascular and hemodynamic measures of interest (cfPWV, bfPWV, faPWV, AIx, SBP, DBP, cSBP, carotid β-stiffness index, CIMT, Q, SV, HRV, and GSR) were acceptably reliable (ICC $\geq$ 0.75) across cycle phases in OC users. Since our preliminary findings show acceptable reliability of all measures of interest, it may indicate that it is unnecessary to control for the OC cycle (i.e., measure solely during the inactive phase) during studies of cardiovascular function/health. The same may apply for clinicians measuring these parameters to assess cardiovascular health; measurements during all cycle phases may be equally representative of an OC user's cardiovascular function and disease risk.

LIMITATIONS AND STRENGTHS

To ensure the findings of this study can be interpreted in light of the limitations, they will be addressed here.

One limitation of our study is the small sample size; we aimed to optimize statistical power by recruiting 40 participants (20 of each sex); however, our sample size at this time is 17 total (12 OC users and 5 males). Thus, while our results within OC users were promising, continuation of this project is warranted to see if reliability remains acceptable given a larger sample size of OC users. Our small male sample is especially limiting, as one subject with between-day variation can (and did) appreciably skew the overall reliability. Thus, it is difficult (and likely not useful) to directly compare reliability between sexes at this point in time, as it is possible that men would appear more reliable given a larger sample size. Additionally, it may have been useful to utilize a third experimental group of women with a natural menstrual cycle, as previous similar trials have done.[13] This would have permitted comparisons between OC users and women with a natural menstrual cycle to potentially ascertain the impacts of long-term exposure to exogenous hormones, as well as to examine differences in hormonal fluctuations, as OC users ingest the same amount of hormone between their early and late active phases, while the natural menstrual cycle likely exhibits differences in hormonal concentration between the luteal, follicular, and ovulatory phases. Unfortunately, given time constraints, the recruitment of women with a natural menstrual cycle likely would have been at the expense of our sample size of OC users, who were the main focus of our study.

Another limitation of our study is that it only utilized young and healthy individuals. This was an important facet of our inclusion and exclusion criteria, as this was the first reliability study in this area, and thus it was necessary to prevent the confounding influence of age and disease states on our variables of interest to develop a basic understanding of their reliability. However, these results may not be generalizable to adults over the age of 35, or those with cardiometabolic abnormalities (e.g., cardiovascular disease, hypertension, diabetes, etc).

Finally, although we originally planned to quantify blood estradiol using ELISA, we currently lack the funding to do so. Thus, we were unable to use estradiol as a time-varying covariate in order to determine if changes in estradiol drove any detected changes in vascular and hemodynamic function, or to determine if differences in reliability exist depending on OC dosage. By the end of data collection, we hope to obtain funding for the necessary ELISA kits and include blood estradiol in the analysis.

One major strength of this study is that it is the first to examine reliability statistics (i.e., ICC, SEM. MDC, MDC%), as opposed to techniques such as the analysis of variance or linear regression. Previous similar trials have simply sought to detect significant differences (i.e., p value) in outcomes of interest between cycle phases; our use of reliability statistics allows us to ascertain more confidently whether it is appropriate to measure OC users in phases other than the inactive phase. Additionally, our diverse array of reliability statistics confers additional information that cannot be obtained by simply examining significance: MDC is an absolute measure of reliability, representing the smallest amount of change in a measure that can be detected using its corresponding test, and SEM determines the sensitivity of measurements, quantifying the variation can be attributed to user or equipment error. The use of these statistics in conjunction with the ICC more robustly assesses reliability, especially as it pertains to clinical measurement. Another strength of our study is that it is the first to measure outcomes of interest in multiple postures (i.e., supine, semi-recumbent, and seated). This hemodynamic perturbation allows us to assess more than just static reliability; for physiological studies, more robust information can be derived by additionally ascertaining whether changes in each measure remain reliable in response to a perturbation. Our study is also strong methodologically in that it enacted strict pre-assessment guidelines and inclusion/exclusion criteria to eliminate confounders.

COMPARISON TO LITERATURE

In our study, all measurements were found to be acceptably reliable (ICC at least 0.75) across cycle phases. In agreement with our findings, previous studies have found that PWV[8] and carotid β-stiffness index[13], and HRV[5] did not change significantly across OC cycle phases.

In contrast with our findings, previous studies have shown that mean arterial pressure increased significantly[4,7] and diastolic blood pressure decreased significantly[7] with the cycle progressing from the active to the inactive phase. Differences in blood pressure may be attributed to differences in the samples; the Minson et al. study[4] had an average age of 30 ± 2 years, which was older than our sample. Additionally, all individuals in the study used an OC with 30-35 ug of ethinyl estradiol, whereas many of our subjects were using a lower dosage. Additionally, significant changes in diastolic blood pressure in the Torgrimson et al study[7] were only seen in those taking 'low dose' OCs (30 μg ethinyl estradiol), and not 'very low dose' OCs (20 μg ethinyl estradiol). Since many of our participants were using OCs with a dose of ethinyl estradiol lower than 30 μg (see **Table 8)**, these differences may be attributed to dosage, and this may indicate that reliability may change based on dosage or pill type. Additionally, the Minson et al. study showed that sympathetic activity increased significantly with the cycle progressing from the active to the inactive phase[4]. This contrast may be attributed to the fact that the Minson et al. study measured cardiovagal baroreflex sensitivity (muscle sympathetic nerve activity), while our study used galvanic skin response to quantify sympathetic activity.

IMPLICATIONS

Prior to our study, it was known that ovarian hormones (estrogen and progesterone) play key roles in modulating vascular and hemodynamic function. Since the concentrations of these hormones fluctuate across the cycle in women using OCs, there may be variability in measures of vascular and hemodynamic function across the cycle. However, the impact of OC use on the reliability of select vascular and hemodynamic measures was not yet confirmed, as there are conflicting findings within the

literature, and these studies often fail to control for key confounders. In this study, we found that all vascular and hemodynamic measures of interest remained reliable in OC users across cycle phases. These findings are useful, as they tell us that our current practice of measuring women solely in the inactive phase may be unnecessary. Not controlling for the cycle should make it simpler to recruit and include women in studies of cardiovascular function moving forward. It will also increase the generalizability of the findings derived from studies of cardiovascular function and disease risk. Although these findings are promising, important future avenues for this research exist. To make more definitive conclusions regarding the reliability of our measures of interest across the cycle, this study must proceed until recruitment targets are reached. Additionally, this study should be repeated to include OC users over the age of 35 and those with cardiometabolic abnormalities, in order to determine if reliability remains consistent in spite of these factors. Future studies may also include women with a natural menstrual cycle as an additional comparison.

See **Table 14** for a summary of the previous gaps in the knowledge, current discussion topics, and ideas for future study.

TABLE 14. Summary of gaps in knowledge, discussion topics, and future study

What did we know?
• Ovarian hormones influence vascular and hemodynamic function • OC usage leads to ovarian hormone fluctuation • Hormonal fluctuation may lead to changes in vascular and hemodynamic function across the OC cycle
What did we not know?
• The impact of OC use on the reliability of select vascular and hemodynamic measures
What have we learned?
• All vascular and hemodynamic outcomes remained reliable across the OC cycle
Why is this new information useful?
• Current practice of measuring women solely in the inactive phase may be unnecessary • Not controlling for the cycle should make it simpler to recruit and include women in studies of cardiovascular function
What do we need to know next?
• Study must proceed until recruitment targets are reached. • Study should be repeated to include OC users over the age of 35 and those with cardiometabolic abnormalities • Future studies may also include women with a natural menstrual cycle as an additional comparison

CONCLUSIONS

Current best practice for studies of cardiovascular function is to measure OC users solely in the inactive phase, but this may conceal important sex differences and decrease generalizability. It is therefore important to determine whether this practice is necessary by determining the reliability of clinically important vascular and hemodynamic measures. This study has shown that, contrary to some of the current literature, our measures of interest remained acceptably reliable across cycle phases in OC users (as well as in men). Therefore, we demonstrate that it may not be necessary to control for cycle phase in young and healthy OC users. Additional investigation is warranted to see if these results are consistent in older individuals and those with cardiometabolic abnormalities, as well as in women with a natural menstrual cycle.

APPENDIX A. CONSENT

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 B. SUBJECT PRE-ASSESSMENT INSTRUCTIONS

Hello,

This is a reminder email of your appointment at the Cardiometabolic Lab for "Reliability of cardiovascular and cerebrovascular measures across the oral contraceptive cycle on _____ at _____. The following pre-assessment guidelines must be met prior to your familiarization/experimental visit:

For familiarization visit (Visit 1):

- Abstain from alcohol 12 hours prior

The familiarization visit to is expected to last approximately 30 minutes.

For experimental visits (Visit 2, 3, and 4)

- 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.
- Avoid heavy make-up or mineral sunscreen on the forehead or temples
- Have at least one index finger free of nail polish or other varnish on the fingernail
- If possible, wear shorts and a short-sleeve or sleeveless shirt, as cuffs will be placed on your arm and leg.

Each experimental visit to is expected to last approximately 90 minutes.
Please fill out the COVID-19 screening survey prior to each visit, either by scanning the QR code on the lab door upon arrival or filling out this link here:
https://unc.az1.qualtrics.com/jfe/form/SV_9FxzSKmV2ezrU69
The Cardiometabolic Lab is part of the Applied Physiology Lab, located in the basement of Fetzer Hall. From the front entrance, go straight past Gym A and down the stairs on the right. From the bottom of the stairs, move down the hall in the direction of the Student Recreation Center (SRC). Before moving up the ramp to the SRC, turn left and look for the sign for Applied Physiology Lab on the right. Make this right turn and you will find a small lobby to the lab on the left, where you will be greeted by a member of the Cardiometabolic Lab.
If you have trouble finding the location, please call or text Jillian at 914-815-7830.

APPENDIX C. SUPPLEMENTAL DATA

Introduction to supplement

Supplementary data for both experimental groups (OC users and males) are provided in this section for further interpretation. The male group was primarily used as a control group for comparison to OC users, and therefore, box plots were not provided, and data was not discussed independently in chapters IV and V. The data in Tables S1-S13 provide all reliability data (ICC, SEM, MDC, %MDC) for both experimental groups taken in each posture, as well as the overall reliability data (i.e., averaged across postures). Figures S1-S13 show mean values in OC users for each individual outcome, separated by both posture and day. Figures S14-S26 show mean values in men for each outcome overall per day (i.e., averaged across postures), and Figures S27-S39 show mean values men for each individual outcome, separated by both posture and day. For all supplementary box plots displaying data from OC users, day 1 represents the inactive phase, day 2 represents the early active phase, and day 3 represents the late active phase. For all box plots, the error bars represent standard deviation.

RELIABILITY DATA TABLES

Table S1. Reliability of cfPWV in OC users and men

OC	Seat			Semi			Supine			Total		
	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI
ICC	0.99	0.99	1.00	0.94	0.88	0.97	0.99	0.98	0.99	0.99	0.98	1.00
SEM	0.04	0.03	0.06	0.15	0.11	0.21	0.06	0.04	0.09	0.06	0.04	0.08
MDC	0.12	0.09	0.17	0.42	0.30	0.58	0.17	0.12	0.24	0.15	0.11	0.22
%MDC	2.10	1.49	2.94	7.25	5.20	10.05	3.00	2.13	4.20	2.67	1.90	3.75
MALE	Seat			Semi			Supine			Total		
	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI
ICC	0.96	0.88	0.99	0.79	0.47	0.93	0.91	0.75	0.97	0.99	0.97	1.00
SEM	0.22	0.13	0.38	0.51	0.30	0.82	0.33	0.19	0.56	0.11	0.07	0.20
MDC	0.62	0.35	1.06	1.42	0.84	2.26	0.92	0.53	1.55	0.32	0.18	0.56
%MDC	9.68	5.53	16.69	22.26	13.12	35.48	14.40	8.30	24.26	4.99	2.84	8.74

Abbreviations: OC, oral contraceptive; cfPWV, carotid-femoral pulse wave velocity; ICC, intraclass correlation coefficient; SEM, standard error of the mean; MDC, minimal detectable change; LCI, lower confidence interval; UCI, upper confidence interval.
Interpretation: cfPWV achieved excellent reliability (ICC ≥ 0.90) in all postures in OC users, and either excellent (seated and supine) or good (semi-recumbent) reliability (ICC ≥ 0.75) in men.

Table S2. Reliability bfPWV in OC users and men

OC	Seat			Semi			Supine			Total		
	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI
ICC	0.97	0.94	0.98	0.96	0.91	0.98	0.93	0.86	0.96	0.98	0.97	0.99
SEM	0.51	0.36	0.71	0.59	0.42	0.82	0.76	0.54	1.05	0.37	0.26	0.52
MDC	1.41	1.01	1.97	1.64	1.17	2.29	2.10	1.51	2.90	1.03	0.73	1.44
%MDC	8.81	6.29	12.29	10.26	7.34	14.28	13.13	9.42	18.15	6.40	4.56	8.97
MALE	Seat			Semi			Supine			Total		
	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI
ICC	0.98	0.95	0.99	0.93	0.81	0.98	0.96	0.88	0.99	0.99	0.98	1.00
SEM	0.92	0.53	1.61	1.88	1.08	3.21	1.49	0.85	2.57	0.64	0.36	1.12
MDC	2.56	1.46	4.46	5.22	3.00	8.90	4.12	2.36	7.11	1.78	1.01	3.12
%MDC	11.77	6.70	20.56	24.06	13.82	40.98	18.99	10.86	32.74	8.18	4.65	14.35

Abbreviations: OC, oral contraceptive; bfPWV, brachial-femoral pulse wave velocity; ICC, intraclass correlation coefficient; SEM, standard error of the mean; MDC, minimal detectable change; LCI, lower confidence interval; UCI, upper confidence interval.
Interpretation: bfPWV achieved excellent reliability (ICC ≥ 0.90) in all postures in both women and men.

Table S3. Reliability of faPWV in OC users and men

OC	Seat			Semi			Supine			Total		
	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI
ICC	0.80	0.64	0.89	0.92	0.85	0.96	0.90	0.82	0.95	0.95	0.91	0.98
SEM	0.69	0.51	0.93	0.44	0.31	0.60	0.49	0.35	0.67	0.33	0.24	0.46
MDC	1.92	1.40	2.58	1.21	0.87	1.67	1.35	0.97	1.85	0.92	0.66	1.28
%MDC	22.71	16.56	30.50	14.24	10.22	19.66	15.92	11.46	21.88	10.89	7.78	15.14
MALE	Seat			Semi			Supine			Total		
	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI
ICC	0.85	0.60	0.95	0.89	0.69	0.96	0.88	0.68	0.96	0.98	0.93	0.99
SEM	0.31	0.18	0.51	0.27	0.16	0.45	0.28	0.16	0.46	0.12	0.07	0.21
MDC	0.87	0.51	1.42	0.75	0.44	1.26	0.77	0.45	1.28	0.33	0.19	0.58
%MDC	10.76	6.27	17.63	9.33	5.40	15.54	9.55	5.54	15.88	4.14	2.36	7.21

Abbreviations: OC, oral contraceptive; faPWV, femoral-ankle pulse wave velocity; ICC, intraclass correlation coefficient; SEM, standard error of the mean; MDC, minimal detectable change; LCI, lower confidence interval; UCI, upper confidence interval.
Interpretation: faPWV achieved either excellent (semi-recumbent and supine) (ICC ≥ 0.90) or good (seated) reliability (ICC ≥ 0.75) in OC users, and good reliability in all postures in men.

Table S4. Reliability of AIx in OC users and men

OC	Seat			Semi			Supine			Total		
	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI
ICC	1.00	0.99	1.00	0.99	0.98	1.00	0.97	0.95	0.99	0.99	0.99	1.00
SEM	0.35	0.25	0.49	0.62	0.44	0.87	1.00	0.71	1.40	0.45	0.32	0.63
MDC	0.98	0.69	1.37	1.72	1.22	2.41	2.77	1.98	3.87	1.24	0.88	1.74
%MDC	6.39	4.54	8.98	11.25	8.01	15.79	18.13	12.93	25.35	8.11	5.77	11.39
MALE	Seat			Semi			Supine			Total		
	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI
ICC	0.97	0.90	0.99	0.94	0.83	0.98	0.84	0.59	0.95	0.92	0.78	0.97
SEM	1.21	0.69	2.10	1.62	0.93	2.77	2.62	1.53	4.28	1.85	1.07	3.14
MDC	3.36	1.92	5.82	4.49	2.57	7.66	7.26	4.24	11.86	5.14	2.96	8.70
%MDC	35.28	20.15	61.07	47.06	27.00	80.40	76.20	44.46	124.42	53.90	31.02	91.27

Interpretation: AIx achieved excellent reliability (ICC ≥ 0.90) in all postures in both OC users and men.
Abbreviations: OC, oral contraceptive; AIx, augmentation index; ICC, intraclass correlation coefficient; SEM, standard error of the mean; MDC, minimal detectable change; LCI, lower confidence interval; UCI, upper confidence interval.

Table S5. Reliability of SBP in OC users and men

OC	Seat			Semi			Supine			Total		
	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI
ICC	0.89	0.79	0.94	1.00	1.00	1.00	0.94	0.88	0.97	0.97	0.95	0.99
SEM	2.87	2.07	3.93	0.24	0.17	0.34	2.17	1.55	3.00	1.39	0.99	1.94
MDC	7.96	5.74	10.90	0.66	0.47	0.93	6.01	4.31	8.33	3.85	2.75	5.38
%MDC	6.45	4.65	8.84	0.54	0.38	0.76	4.88	3.49	6.75	3.12	2.23	4.37
MALE	Seat			Semi			Supine			Total		
	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI
ICC	0.87	0.65	0.96	0.96	0.88	0.99	0.77	0.42	0.92	0.89	0.69	0.96
SEM	3.19	1.86	5.28	1.79	1.03	3.10	4.32	2.56	6.81	2.99	1.73	4.98
MDC	8.86	5.14	14.64	4.97	2.84	8.58	11.97	7.08	18.89	8.29	4.80	13.81
%MDC	7.06	4.10	11.67	3.97	2.27	6.84	9.54	5.65	15.06	6.61	3.83	11.01

Abbreviations: OC, oral contraceptive; SBP, systolic blood pressure; ICC, intraclass correlation coefficient; SEM, standard error of the mean; MDC, minimal detectable change; LCI, lower confidence interval; UCI, upper confidence interval.
Interpretation: SBP achieved either excellent (semi-recumbent and supine) (ICC ≥ 0.90) or good (seated) reliability (ICC ≥ 0.75) in OC users, and either excellent (semi-recumbent) or good (seated and supine) reliability in men.

Table S6. Reliability of DBP in OC users and men

OC	Seat			Semi			Supine			Total		
	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI
ICC	1.00	1.00	1.00	1.00	0.99	1.00	0.94	0.89	0.97	0.99	0.98	1.00
SEM	0.35	0.25	0.50	0.47	0.34	0.66	1.80	1.29	2.49	0.65	0.46	0.91
MDC	0.98	0.70	1.38	1.31	0.93	1.84	4.98	3.56	6.90	1.80	1.28	2.53
%MDC	1.56	1.11	2.19	2.08	1.48	2.92	7.90	5.66	10.95	2.85	2.03	4.01
MALE	Seat			Semi			Supine			Total		
	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI
ICC	0.84	0.56	0.94	0.98	0.94	0.99	0.97	0.90	0.99	0.99	0.96	1.00
SEM	1.83	1.07	2.98	0.61	0.35	1.06	0.84	0.48	1.46	0.48	0.28	0.85
MDC	5.09	2.97	8.27	1.69	0.96	2.95	2.34	1.34	4.05	1.34	0.76	2.35
%MDC	8.22	4.80	13.36	2.73	1.56	4.76	3.78	2.16	6.54	2.17	1.24	3.80

Abbreviations: OC, oral contraceptive; DBP, diastolic blood pressure; ICC, intraclass correlation coefficient; SEM, standard error of the mean; MDC, minimal detectable change; LCI, lower confidence interval; UCI, upper confidence interval.
Interpretation: DBP achieved excellent reliability (ICC ≥ 0.90) in all postures in OC users, and either excellent (semi-recumbent and supine) or good (seated) reliability (ICC ≥ 0.75) in men.

Table S7. Reliability of cSBP in OC users and men

OC	Seat			Semi			Supine			Total		
	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI
ICC	0.98	0.96	0.99	0.98	0.96	0.99	0.99	0.98	1.00	1.00	1.00	1.00
SEM	0.11	0.08	0.15	0.11	0.08	0.15	0.08	0.05	0.11	0.03	0.02	0.05
MDC	0.30	0.22	0.43	0.30	0.21	0.42	0.21	0.15	0.30	0.10	0.07	0.14
%MDC	6.99	4.98	9.79	6.86	4.89	9.60	4.86	3.46	6.82	2.21	1.57	3.11
MALE	Seat			Semi			Supine			Total		
	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI
ICC	0.91	0.75	0.97	0.99	0.96	1.00	0.92	0.77	0.97	0.97	0.91	0.99
SEM	0.24	0.14	0.41	0.09	0.05	0.16	0.23	0.13	0.39	0.14	0.08	0.24
MDC	0.67	0.39	1.13	0.26	0.15	0.45	0.64	0.37	1.08	0.39	0.22	0.67
%MDC	18.96	10.93	31.95	7.21	4.10	12.61	18.06	10.40	30.55	10.91	6.23	18.93

Abbreviations: OC, oral contraceptive; cSBP, central systolic blood pressure; ICC, intraclass correlation coefficient; SEM, standard error of the mean; MDC, minimal detectable change; LCI, lower confidence interval; UCI, upper confidence interval.
Interpretation: cSBP achieved either excellent (semi-recumbent and supine) reliability (ICC ≥ 0.90) or good (seated) reliability (ICC ≥ 0.75) in OC users, and either excellent (seated) or moderate (semi-recumbent and supine) reliability (ICC ≥ 0.50) in men.

Table S8. Reliability of Q in OC users and men

OC	Seat			Semi			Supine			Total		
	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI
ICC	0.86	0.74	0.93	1.00	0.99	1.00	0.93	0.87	0.97	0.96	0.92	0.98
SEM	3.10	2.24	4.22	0.54	0.39	0.76	2.12	1.52	2.93	1.73	1.23	2.40
MDC	8.59	6.22	11.69	1.50	1.07	2.11	5.87	4.21	8.13	4.78	3.42	6.66
%MDC	7.28	5.27	9.90	1.27	0.91	1.79	4.97	3.57	6.89	4.05	2.90	5.64
MALE	Seat			Semi			Supine			Total		
	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI
ICC	0.92	0.77	0.97	0.68	0.27	0.89	0.69	0.28	0.89	0.91	0.74	0.97
SEM	2.96	1.70	5.00	5.89	3.54	8.99	5.82	3.49	8.90	3.21	1.85	5.39
MDC	8.20	4.72	13.87	16.33	9.81	24.92	16.12	9.67	24.68	8.89	5.13	14.93
%MDC	7.01	4.04	11.86	13.96	8.39	21.31	13.78	8.27	21.11	7.60	4.39	12.77

Abbreviations: OC, oral contraceptive; Q, cardiac output; ICC, intraclass correlation coefficient; SEM, standard error of the mean; MDC, minimal detectable change; LCI, lower confidence interval; UCI, upper confidence interval.
Interpretation: Q achieved excellent reliability (ICC ≥ 0.90) in all postures in both OC users and men.

Table S9. Reliability of SV in OC users and men

OC	Seat			Semi			Supine			Total		
	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI
ICC	0.97	0.94	0.98	1.00	1.00	1.00	0.98	0.96	0.99	1.00	0.99	1.00
SEM	2.40	1.72	3.36	0.30	0.21	0.42	1.88	1.34	2.63	0.94	0.67	1.33
MDC	6.67	4.76	9.30	0.82	0.58	1.15	5.21	3.71	7.29	2.62	1.86	3.68
%MDC	10.88	7.77	15.18	1.34	0.95	1.88	8.50	6.06	11.90	4.27	3.04	6.00
MALE	Seat			Semi			Supine			Total		
	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI
ICC	0.99	0.95	1.00	0.99	0.98	1.00	0.94	0.82	0.98	0.99	0.98	1.00
SEM	1.59	0.91	2.78	1.12	0.64	1.97	3.22	1.85	5.50	1.16	0.66	2.04
MDC	4.41	2.51	7.71	3.11	1.77	5.46	8.92	5.12	15.23	3.23	1.83	5.66
%MDC	6.94	3.95	12.13	4.90	2.79	8.60	14.05	8.06	23.98	5.08	2.89	8.91

Abbreviations: OC, oral contraceptive; SV, stroke volume; ICC, intraclass correlation coefficient; SEM, standard error of the mean; MDC, minimal detectable change; LCI, lower confidence interval; UCI, upper confidence interval.
Interpretation: SV achieved excellent reliability (ICC ≥ 0.90) in all postures in both OC users and men.

Table S10. Reliability of β-stiffness index in OC users and men

OC	Seat			Semi			Supine			Total		
	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI
ICC	1.00	1.00	1.00	0.92	0.84	0.96	0.96	0.91	0.98	0.98	0.96	0.99
SEM	0.06	0.05	0.09	0.42	0.30	0.58	0.31	0.22	0.43	0.22	0.16	0.31
MDC	0.18	0.13	0.25	1.17	0.84	1.62	0.86	0.62	1.20	0.62	0.44	0.87
%MDC	2.73	1.94	3.83	17.97	12.91	24.79	13.18	9.42	18.35	9.48	6.76	13.27
MALE	Seat			Semi			Supine			Total		
	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI
ICC	0.75	0.38	0.91	0.89	0.70	0.96	0.98	0.94	0.99	0.94	0.83	0.98
SEM	0.25	0.15	0.39	0.16	0.09	0.27	0.07	0.04	0.12	0.12	0.07	0.20
MDC	0.69	0.41	1.08	0.45	0.26	0.74	0.19	0.11	0.33	0.32	0.19	0.55
%MDC	11.99	7.12	18.77	7.79	4.51	13.01	3.29	1.88	5.74	5.66	3.25	9.69

Abbreviations: OC, oral contraceptive; ICC, intraclass correlation coefficient; SEM, standard error of the mean; MDC, minimal detectable change; LCI, lower confidence interval; UCI, upper confidence interval.
Interpretation: β-stiffness index achieved excellent reliability (ICC ≥ 0.90) in all postures in OC users, and either excellent (semi-recumbent and supine) or good (seated) reliability (ICC ≥ 0.75) in men.

Table S11. Reliability of CIMT in OC users and men

OC	Seat			Semi			Supine			Total		
	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI
ICC	0.96	0.91	0.98	0.81	0.65	0.90	0.79	0.63	0.89	0.92	0.85	0.96
SEM	0.01	0.01	0.02	0.02	0.02	0.03	0.02	0.02	0.03	0.02	0.01	0.02
MDC	0.03	0.02	0.04	0.07	0.05	0.09	0.07	0.05	0.09	0.04	0.03	0.06
%MDC	8.37	5.98	11.65	17.48	12.73	23.51	18.20	13.28	24.39	11.25	8.08	15.52
MALE	Seat			Semi			Supine			Total		
	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI
ICC	1.00	1.00	1.00	0.94	0.83	0.98	0.96	0.88	0.99	1.00	1.00	1.00
SEM	0.00	0.00	0.00	0.03	0.02	0.05	0.03	0.01	0.04	0.00	0.00	0.00
MDC	0.00	0.00	0.01	0.09	0.05	0.15	0.07	0.04	0.12	0.01	0.00	0.01
%MDC	0.65	0.37	1.15	18.59	10.67	31.78	15.25	8.72	26.31	1.67	0.95	2.93

Abbreviations: OC, oral contraceptive; CIMT, carotid intima-media thickness; ICC, intraclass correlation coefficient; SEM, standard error of the mean; MDC, minimal detectable change; LCI, lower confidence interval; UCI, upper confidence interval.
Interpretation: CIMT achieved either excellent (seated) (ICC ≥ 0.90) or good (semi-recumbent and supine) reliability (ICC ≥ 0.75) in OC users, and excellent reliability in all postures in men.

Table S12. Reliability of HRV (RMSSD) in OC users and men

OC	Seat			Semi			Supine			Total		
	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI
ICC	1.00	1.00	1.00	1.00	1.00	1.00	0.99	0.98	1.00	1.00	0.99	1.00
SEM	0.63	0.45	0.88	0.60	0.43	0.85	1.26	0.90	1.77	0.72	0.52	1.02
MDC	1.74	1.24	2.45	1.67	1.19	2.35	3.49	2.49	4.90	2.01	1.43	2.82
%MDC	5.42	3.85	7.62	5.20	3.70	7.31	10.88	7.74	15.27	6.26	4.45	8.79
MALE	Seat			Semi			Supine			Total		
	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI
ICC	0.96	0.89	0.99	0.99	0.98	1.00	0.98	0.95	0.99	1.00	0.99	1.00
SEM	3.48	1.99	6.01	1.57	0.90	2.76	2.38	1.35	4.15	1.09	0.62	1.92
MDC	9.64	5.51	16.65	4.36	2.48	7.65	6.59	3.76	11.50	3.02	1.72	5.31
%MDC	19.71	11.26	34.04	8.92	5.07	15.65	13.48	7.68	23.52	6.18	3.51	10.86

Abbreviations: OC, oral contraceptive; HRV, heart rate variability; RMSSD, root mean square of successive differences; carotid intima-media thickness; ICC, intraclass correlation coefficient; SEM, standard error of the mean; MDC, minimal detectable change; LCI, lower confidence interval; UCI, upper confidence interval.
Interpretation: CIMT achieved excellent reliability (ICC ≥ 0.90) in all postures in both OC users and men.

Table S13. Reliability of GSR in OC users and men

OC	Seat			Semi			Supine			Total		
	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI
ICC	0.89	0.75	0.95	0.84	0.65	0.93	0.96	0.90	0.98	0.90	0.76	0.96
SEM	0.79	0.51	1.19	0.95	0.62	1.41	0.49	0.32	0.76	0.76	0.49	1.15
MDC	2.19	1.42	3.31	2.63	1.72	3.90	1.36	0.87	2.10	2.11	1.37	3.19
%MDC	109.8	71.2	165.5	131.5	85.97	195.13	68.12	43.7	105	105.4	68.3	159.4
MALE	Seat			Semi			Supine			Total		
	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI	X	LCI	UCI
ICC	0.83	0.22	0.97	0.41	-0.50	0.89	0.81	0.15	0.97	0.85	0.27	0.98
SEM	0.47	0.18	1.03	0.89	0.39	1.42	0.50	0.20	1.07	0.45	0.17	0.99
MDC	1.31	0.51	2.85	2.48	1.08	3.93	1.39	0.54	2.95	1.24	0.48	2.74
%MDC	37.78	14.71	81.95	71.30	31.01	113.29	40.0	15.66	85.1	35.76	13.87	78.96

Abbreviations: OC, oral contraceptive; GSR, galvanic skin response; carotid intima-media thickness; ICC, intraclass correlation coefficient; SEM, standard error of the mean; MDC, minimal detectable change; LCI, lower confidence interval; UCI, upper confidence interval.
Interpretation: CIMT achieved either excellent (supine) (ICC $\geq$ 0.90) or good (seated and semi-recumbent) reliability (ICC $\geq$ 0.75) in OC users, and either good (seated and supine) or poor (semi-recumbent) reliability (ICC < 0.50) in men.

BOX PLOTS (OC USERS)

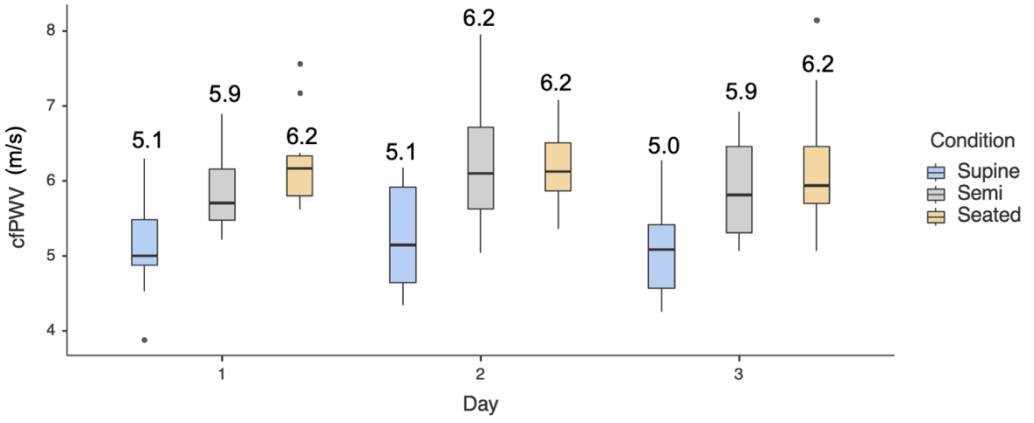

Figure S1. Mean cfPWV values, separated by condition and day

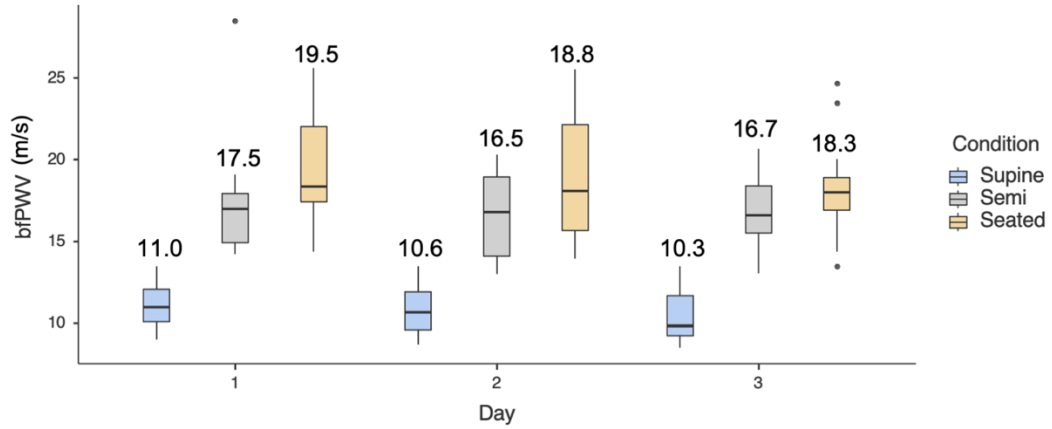

Figure S2. Mean bfPWV values, separated by condition and day

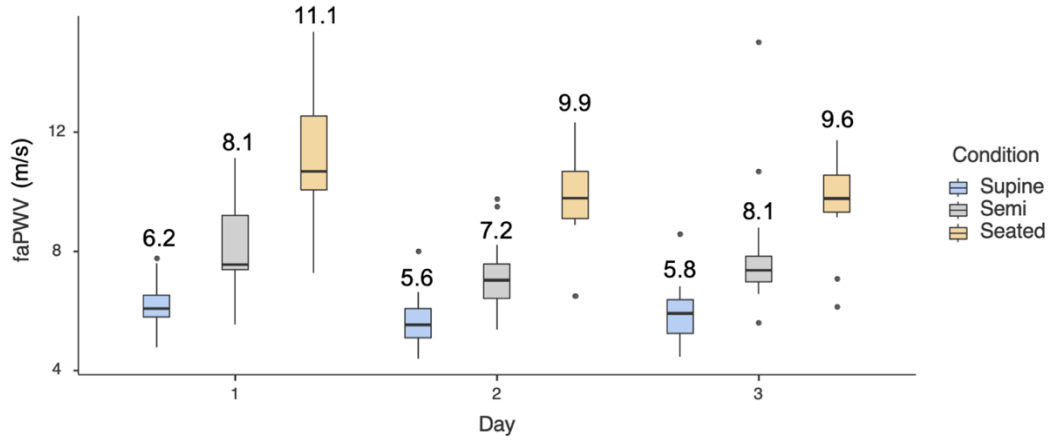

Figure S3. Mean faPWV values, separated by condition and day

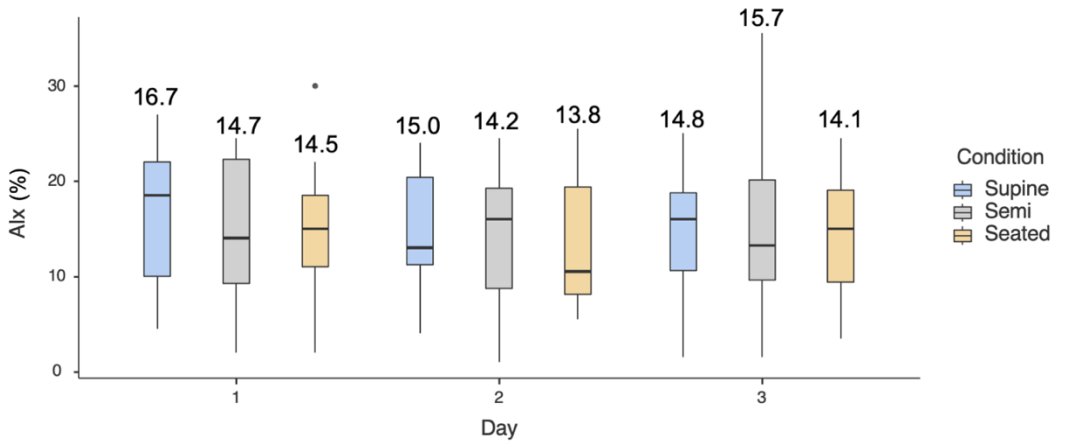

Figure S4. Mean AIx values, separated by condition and day

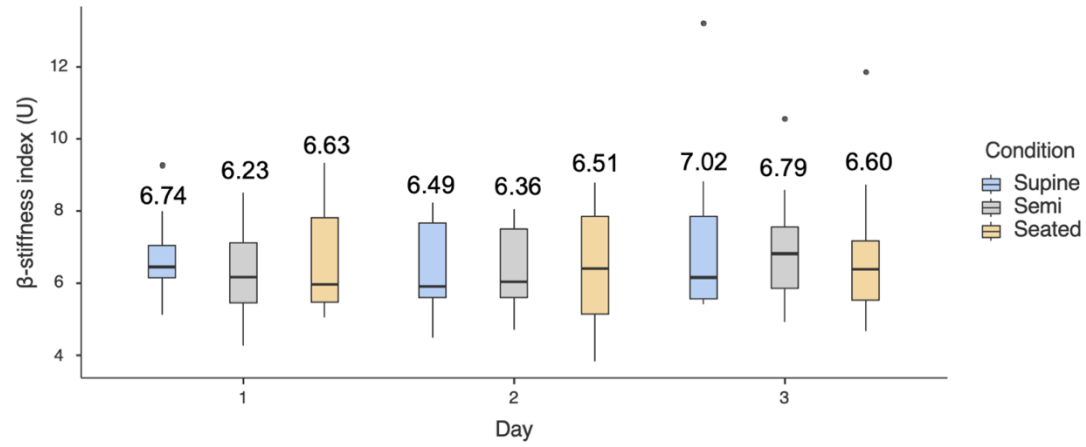

Figure S5. Mean β-stiffness index values, separated by condition and day

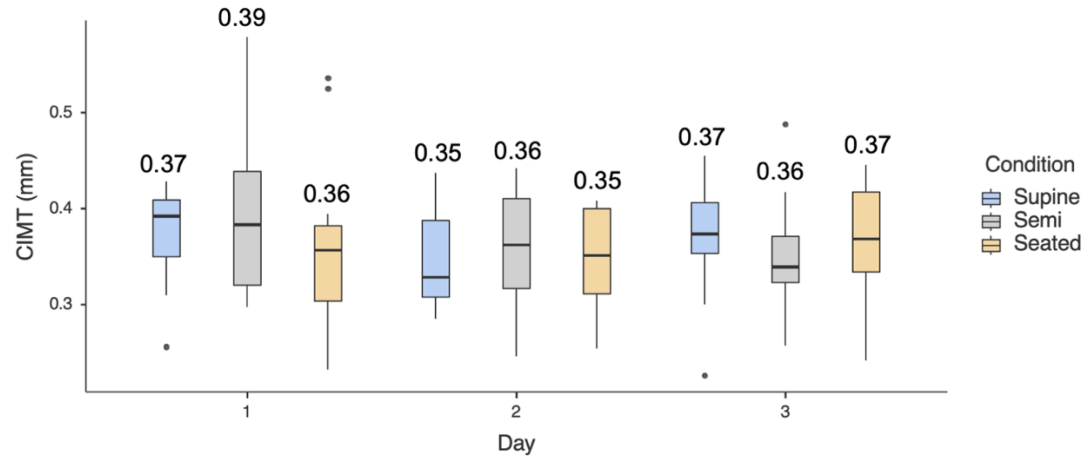

Figure S6. Mean CIMT values, separated by condition and day

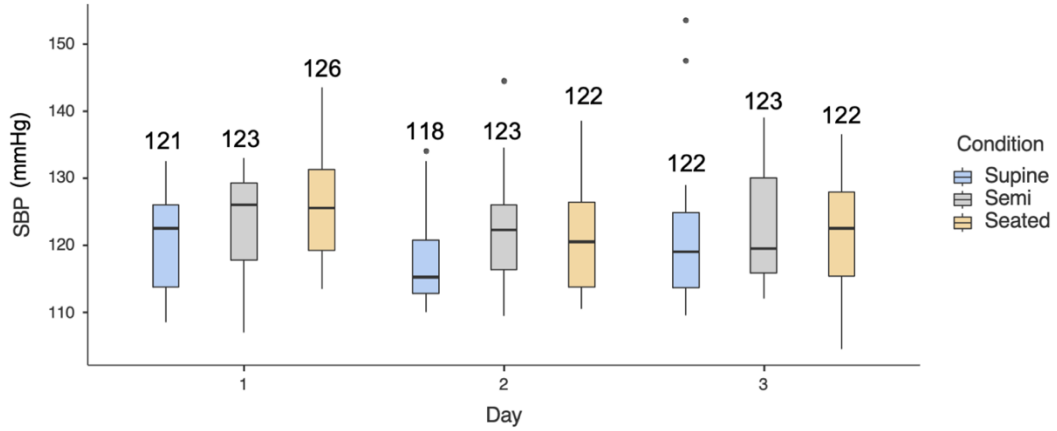

Figure S7. Mean SBP values, separated by condition and day

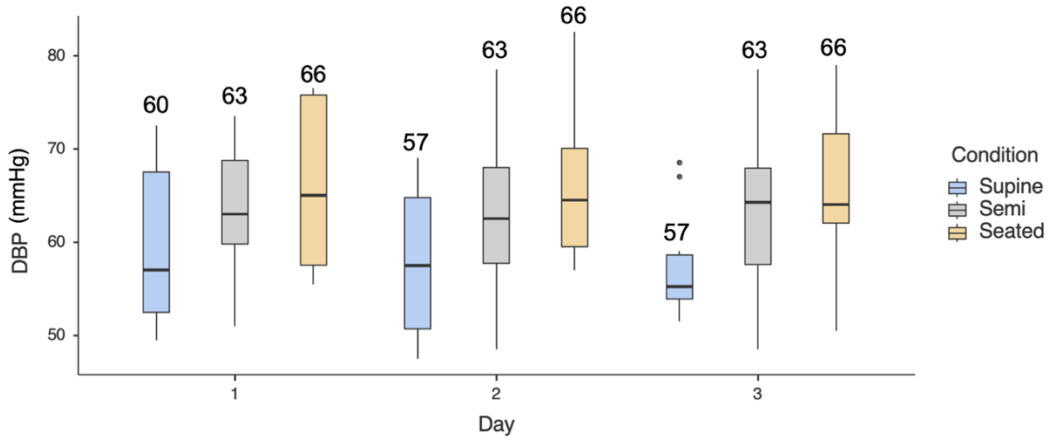

Figure S8. Mean DBP values, separated by condition and day

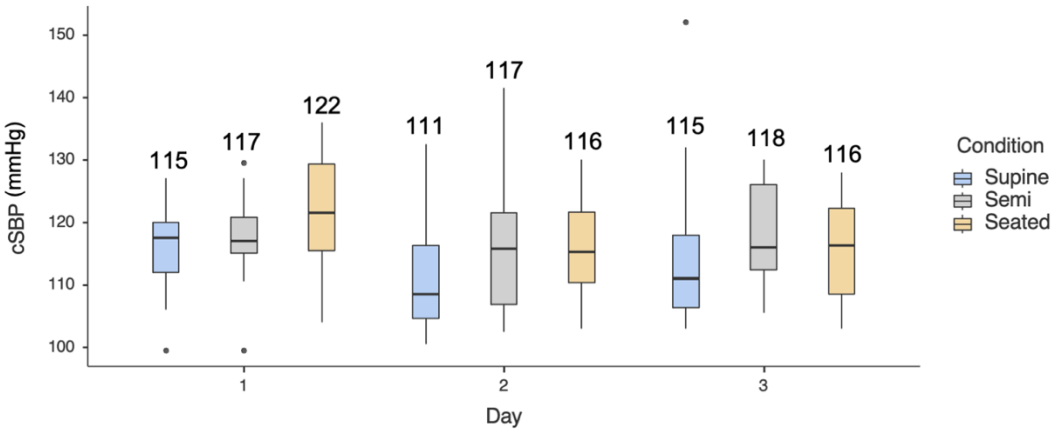

Figure S9. Mean cSBP values, separated by condition and day

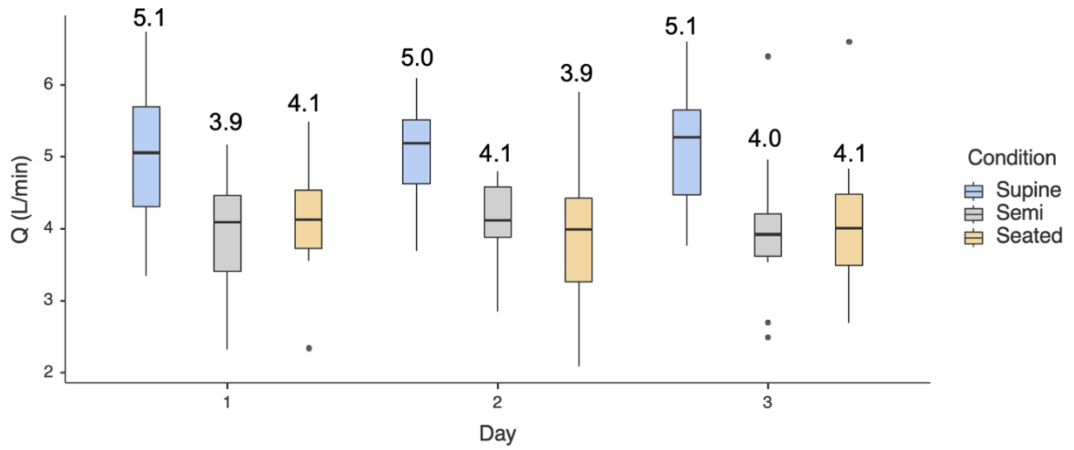

Figure S10. Mean Q values, separated by condition and day

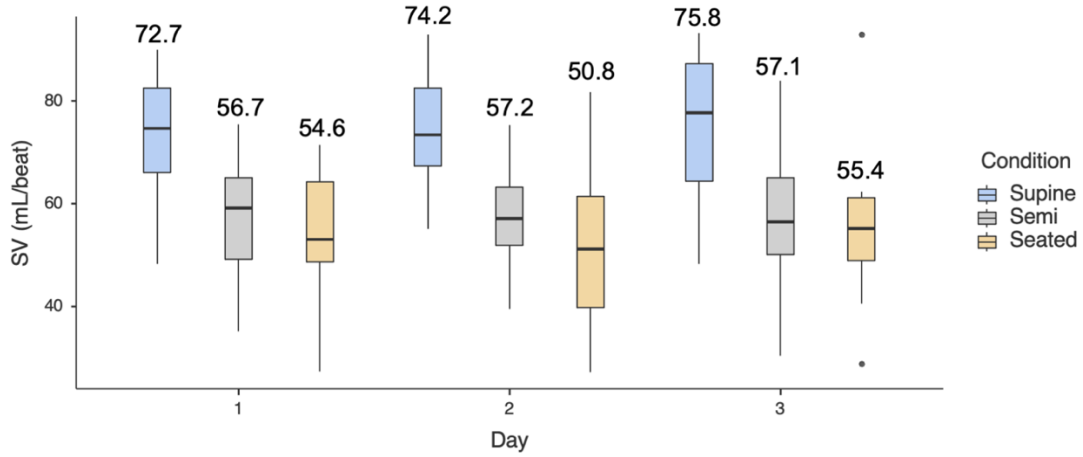

Figure S11. Mean SV values, separated by condition and day

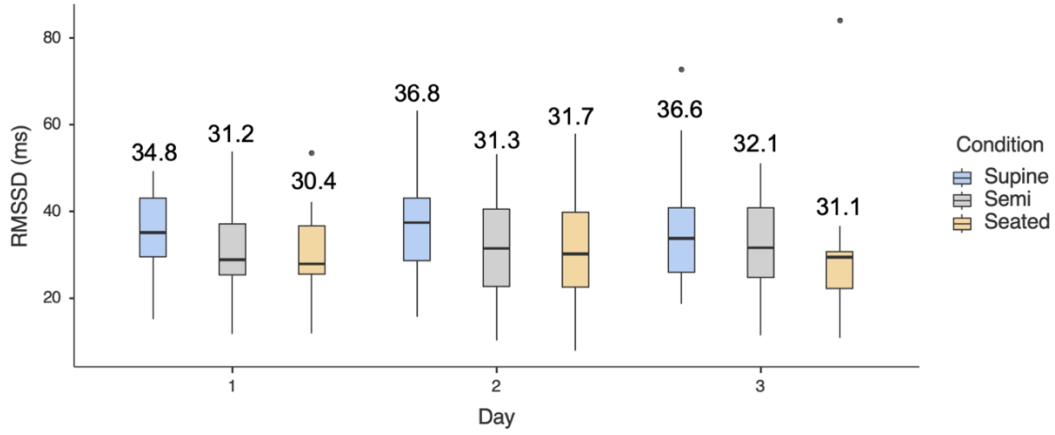

Figure S12. Mean RMSSD values, separated by condition and day

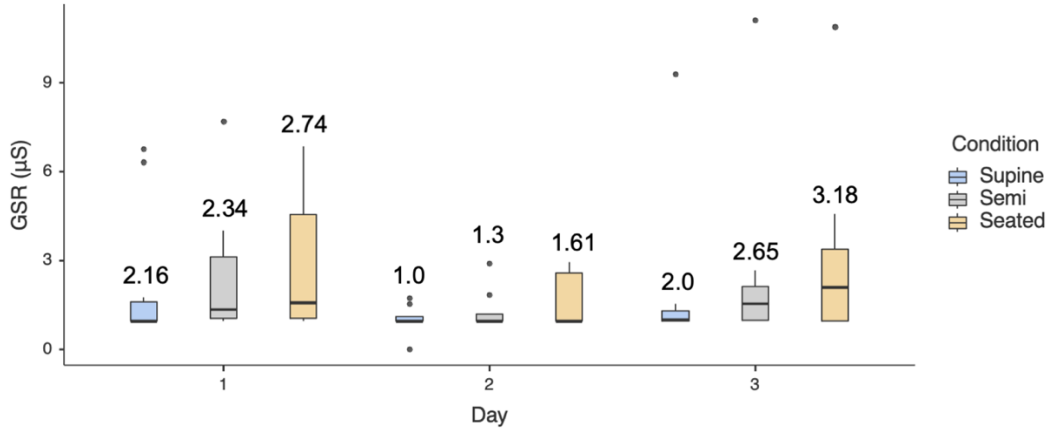

Figure S13. Mean GSR values, separated by condition and day

BOX PLOTS (MALE)

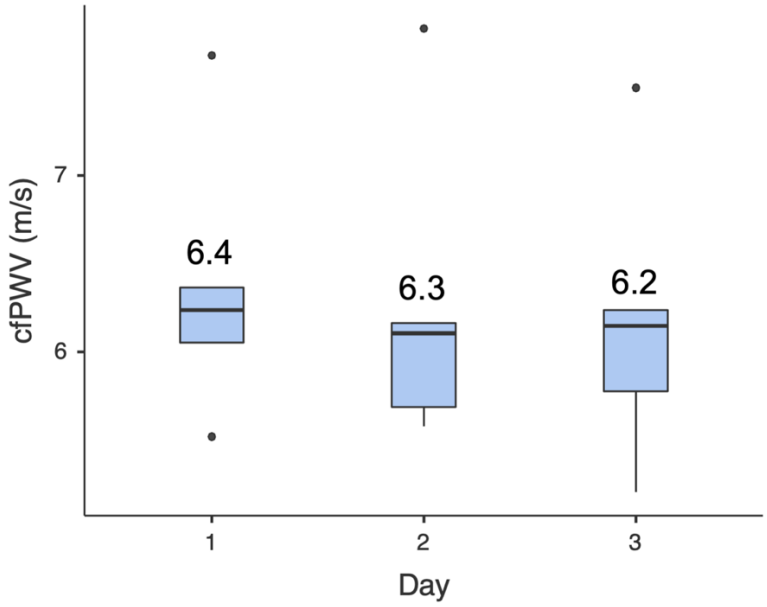

Figure S14. Mean overall daily cfPWV values

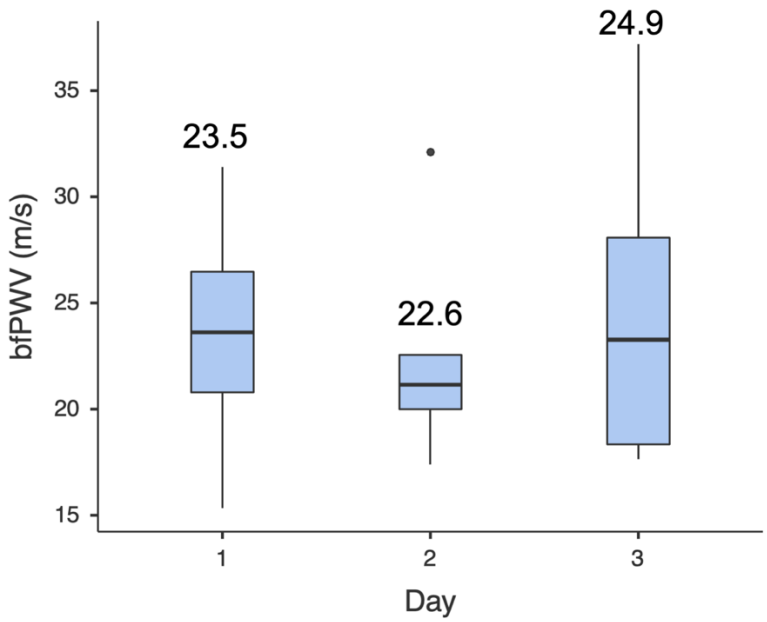

Figure S15. Mean overall daily bfPWV values

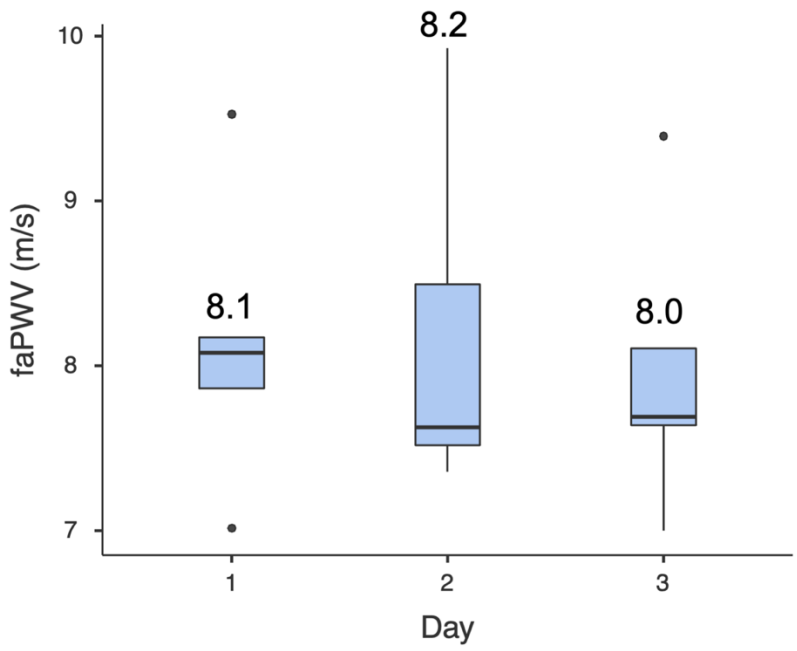

Figure S16. Mean overall daily faPWV values

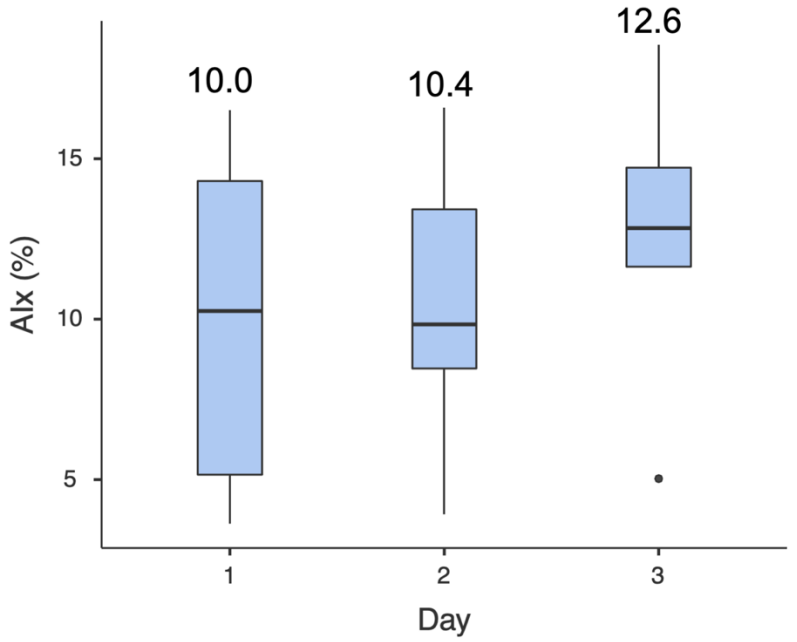

Figure S17. Mean overall daily AIx values

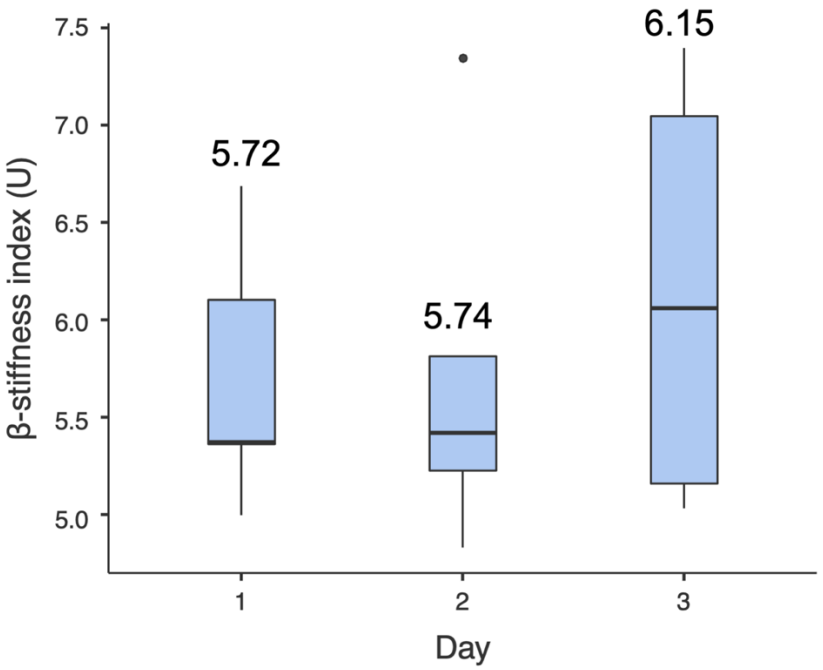

Figure S18. Mean overall daily β-stiffness index values

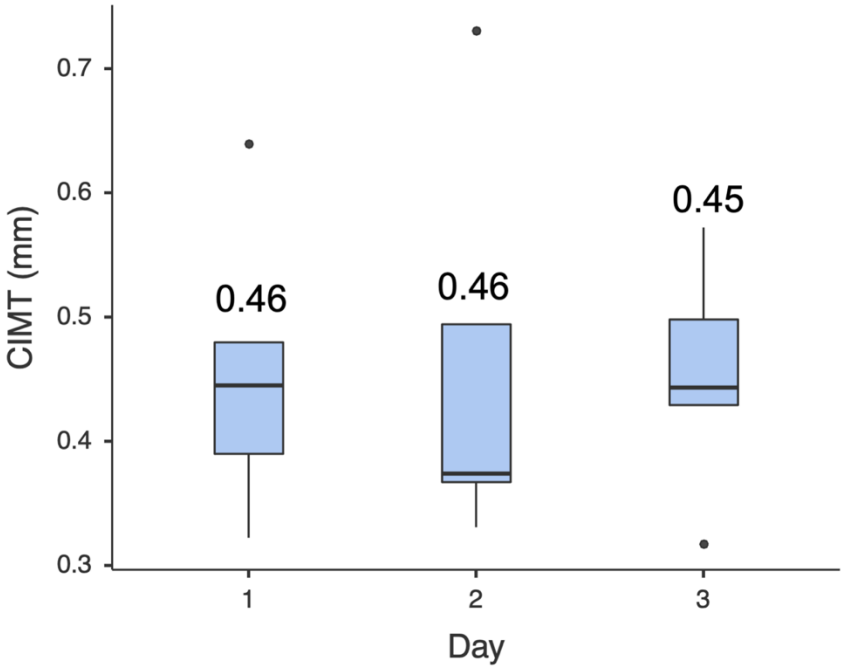

Figure S19. Mean overall daily CIMT values

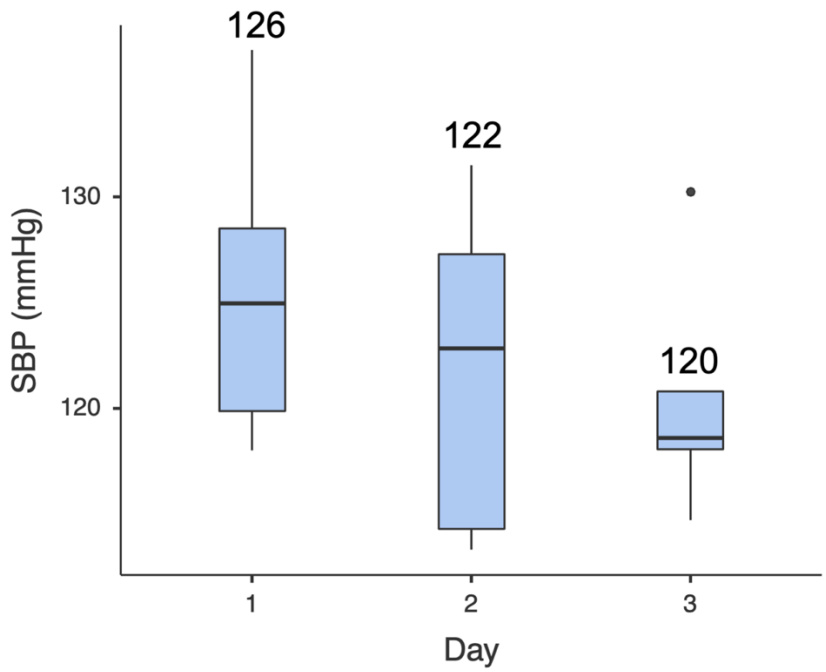

Figure S20. Mean overall daily SBP values

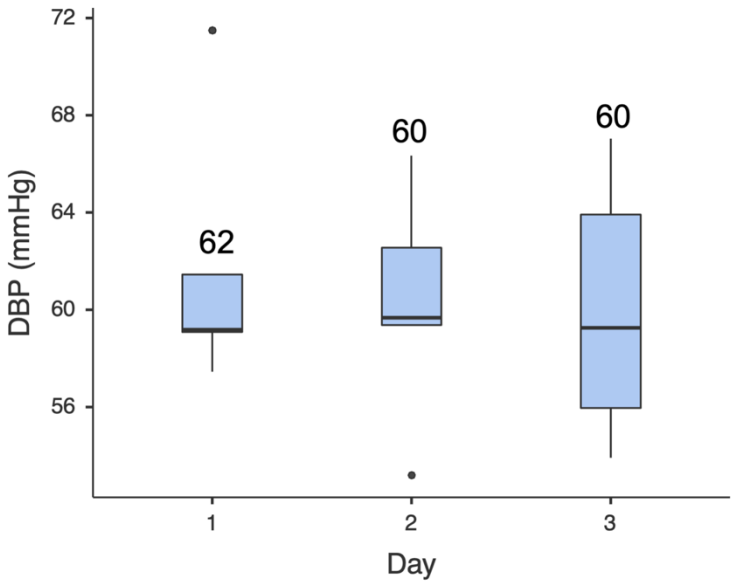

Figure S21. Mean overall daily DBP values

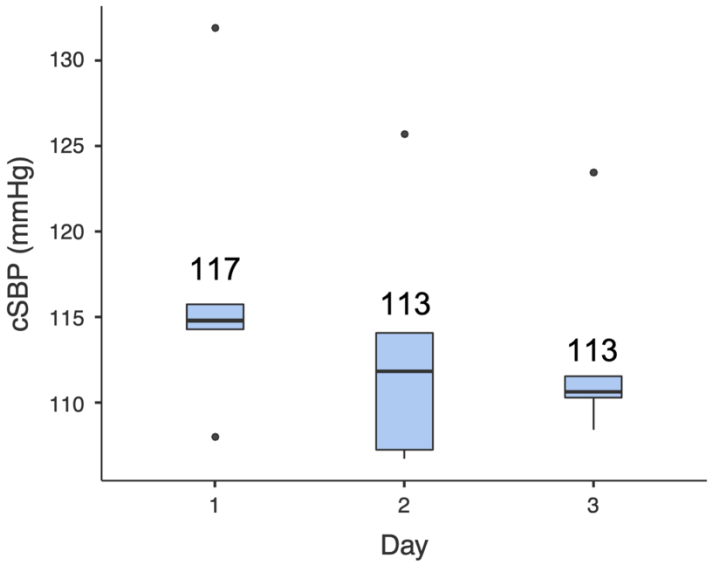

Figure S22. Mean overall daily cSBP values

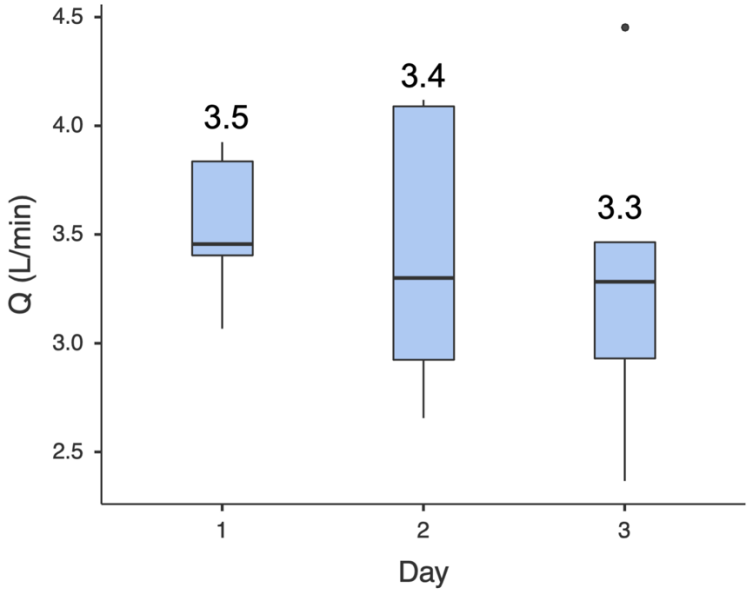

Figure S23. Mean overall daily Q values

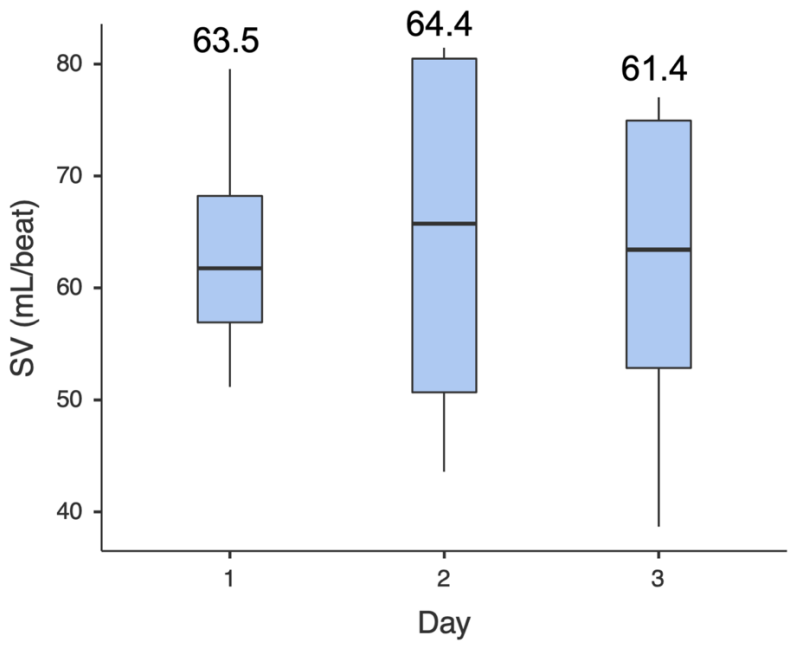

Figure S24. Mean overall daily SV values

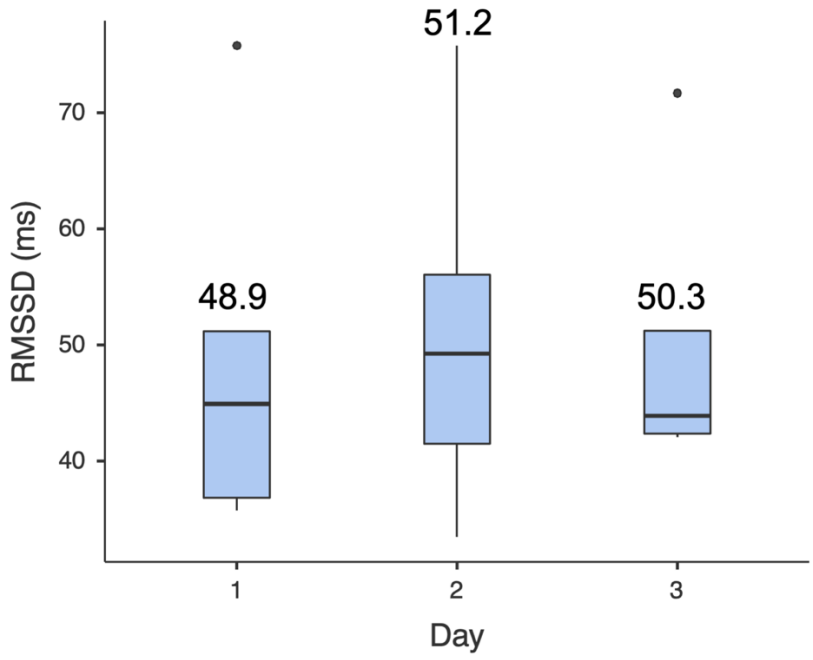

Figure S25. Mean overall RMSSD values

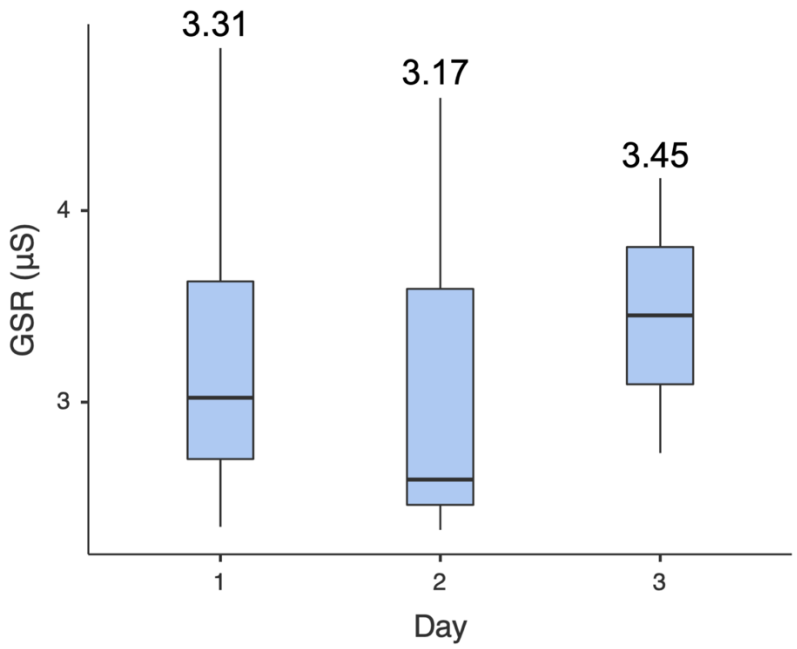

Figure S26. Mean overall GSR values

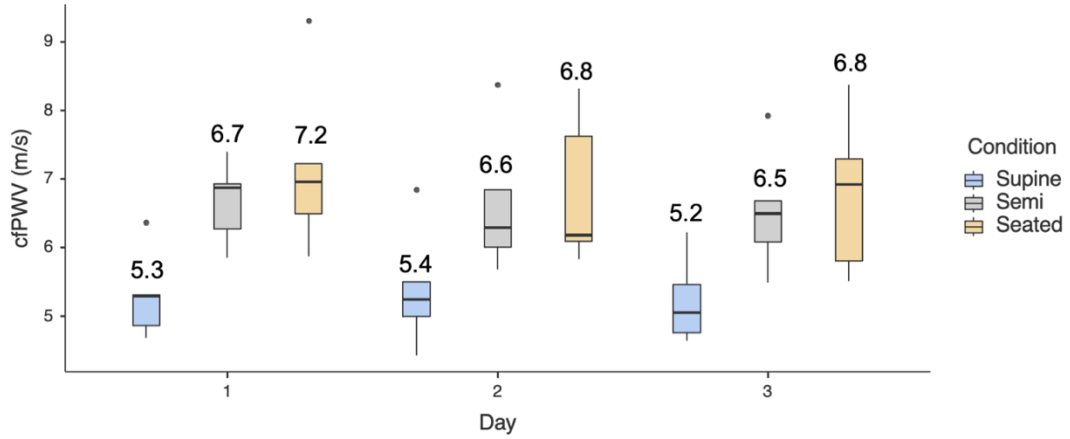

Figure S27. Mean cfPWV values, separated by condition and day

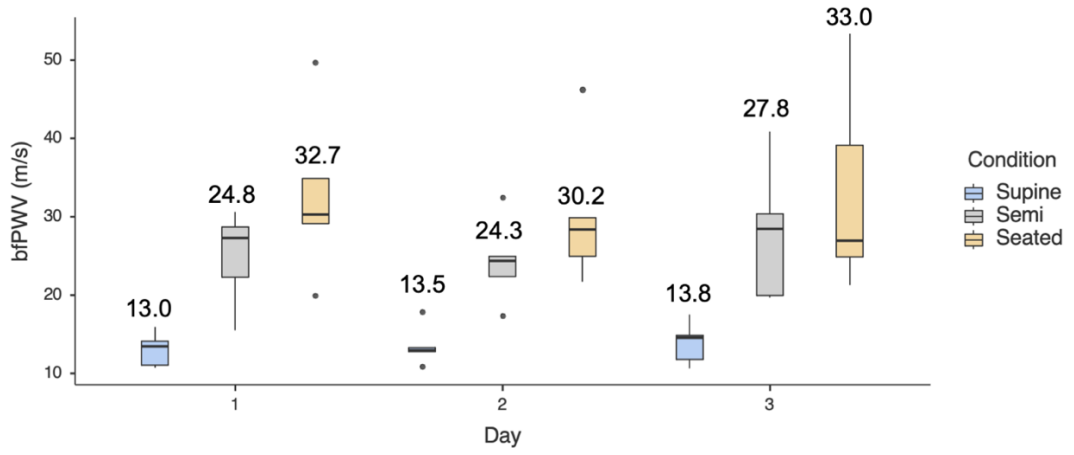

Figure S28. Mean bfPWV values, separated by condition and day

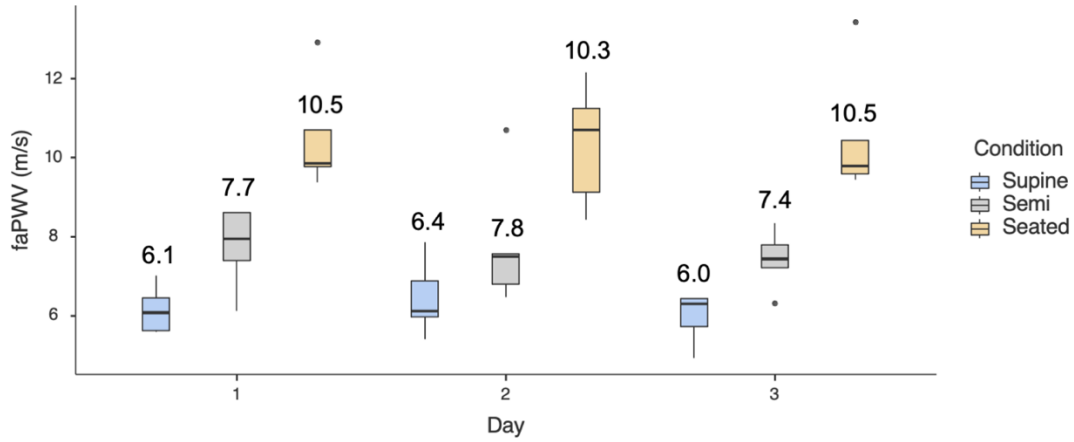

Figure S29. Mean faPWV values, separated by condition and day

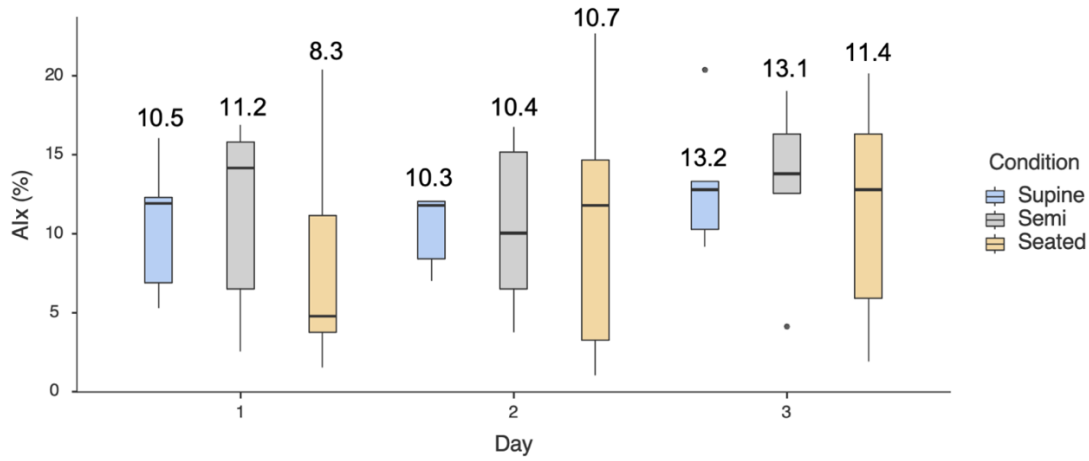

Figure S30. Mean AIx values, separated by condition and day

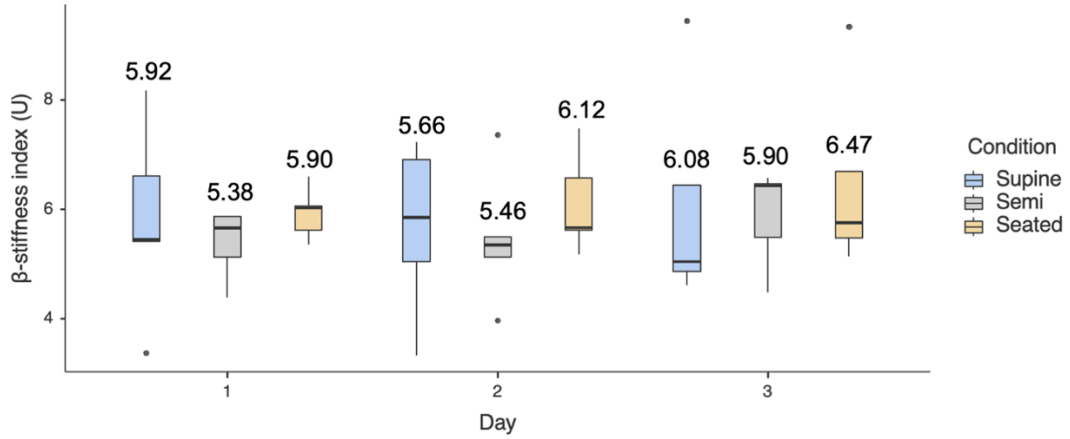

Figure S31. Mean β-stiffness index values, separated by condition and day

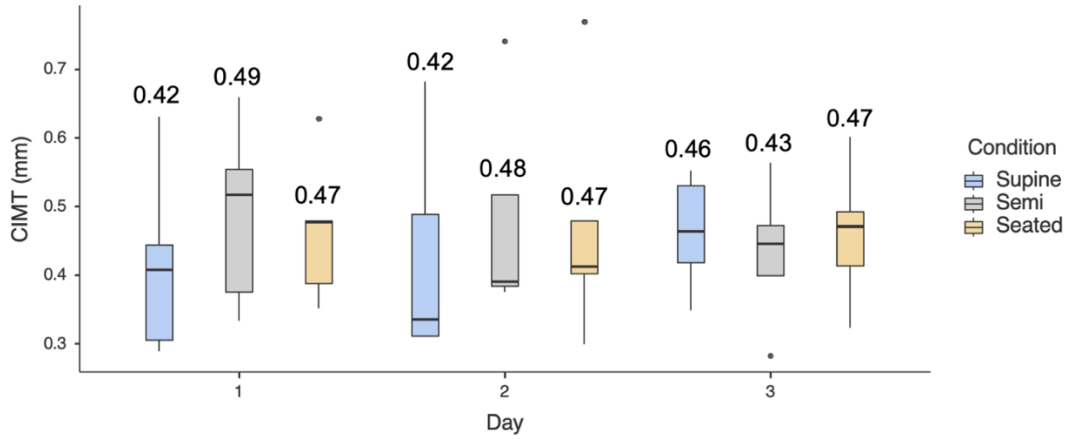

Figure S32. Mean CIMT values, separated by condition and day

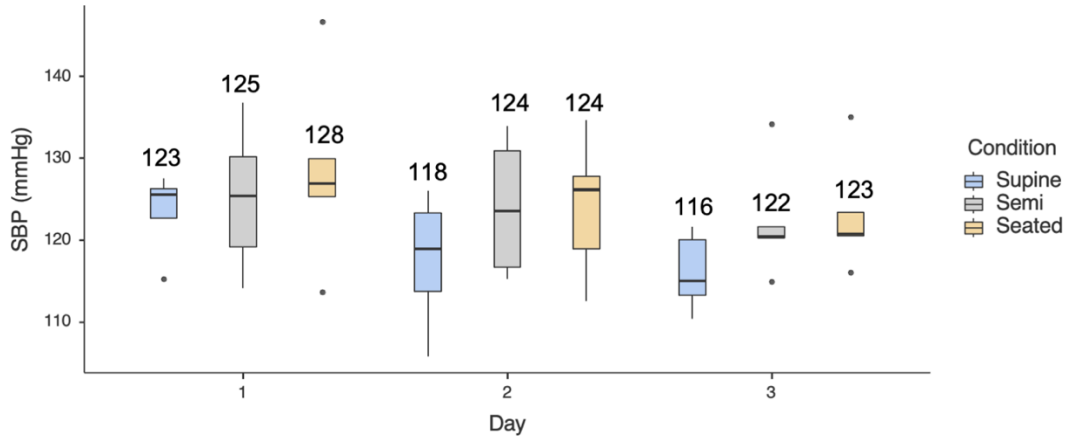

Figure S33. Mean SBP values, separated by condition and day

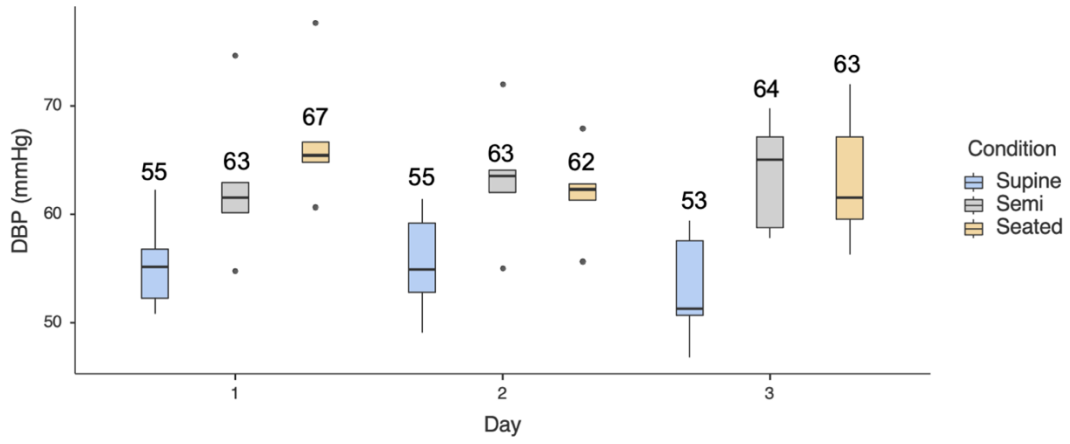

Figure S34. Mean DBP values, separated by condition and day

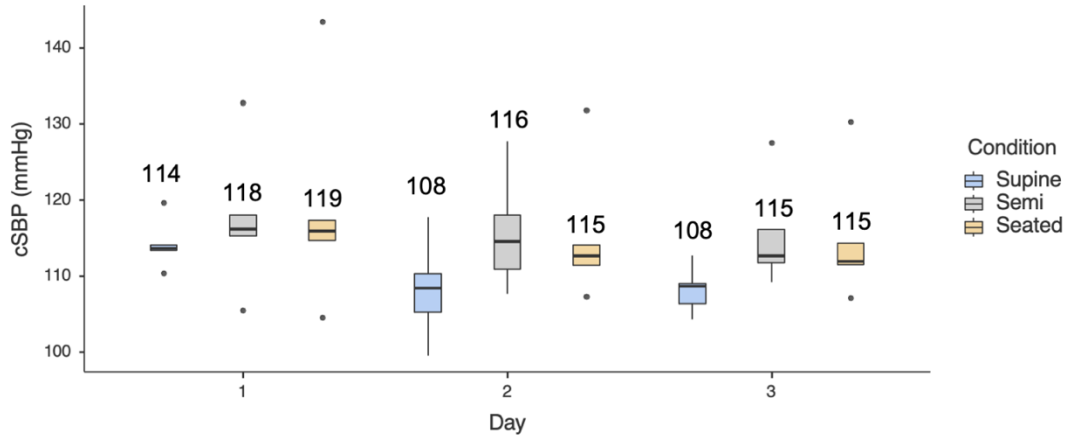

Figure S35. Mean cSBP values, separated by condition and day

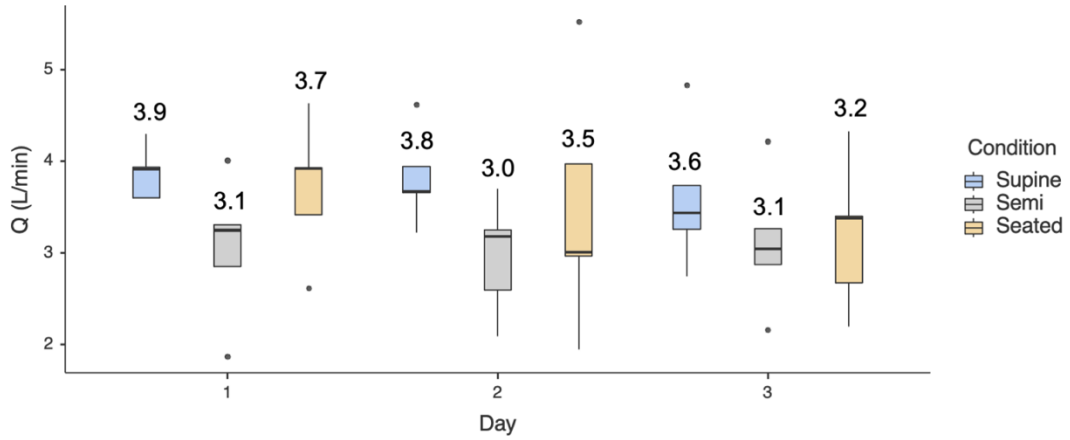

Figure S36. Mean Q values, separated by condition and day

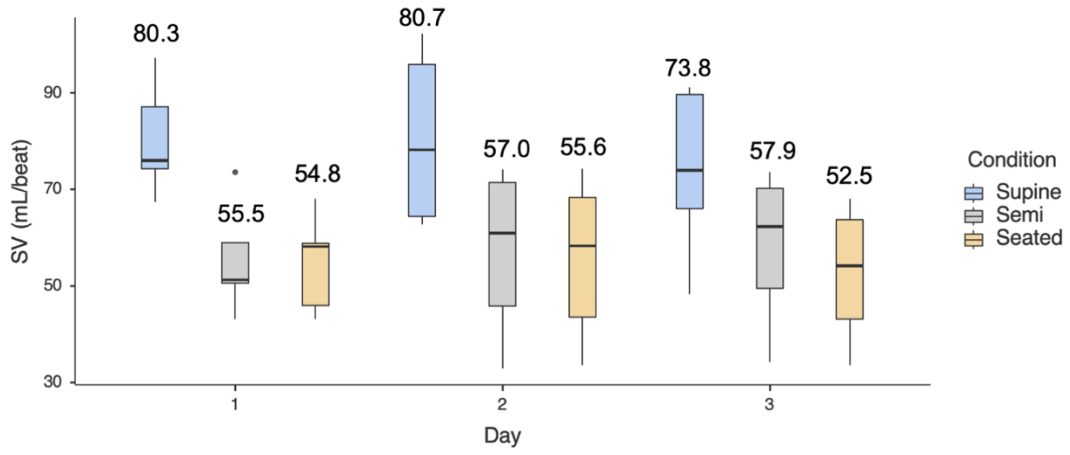

Figure S37. Mean SV values, separated by condition and day

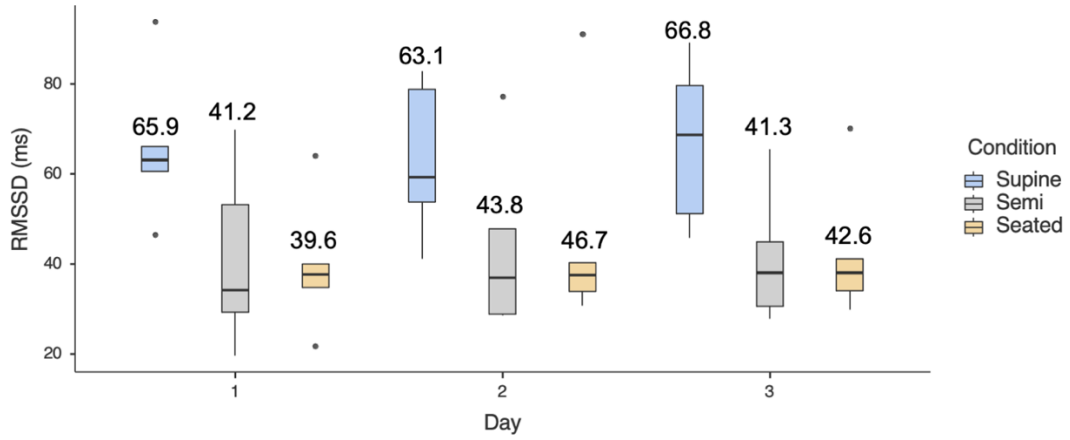

Figure S38. Mean RMSSD values, separated by condition and day

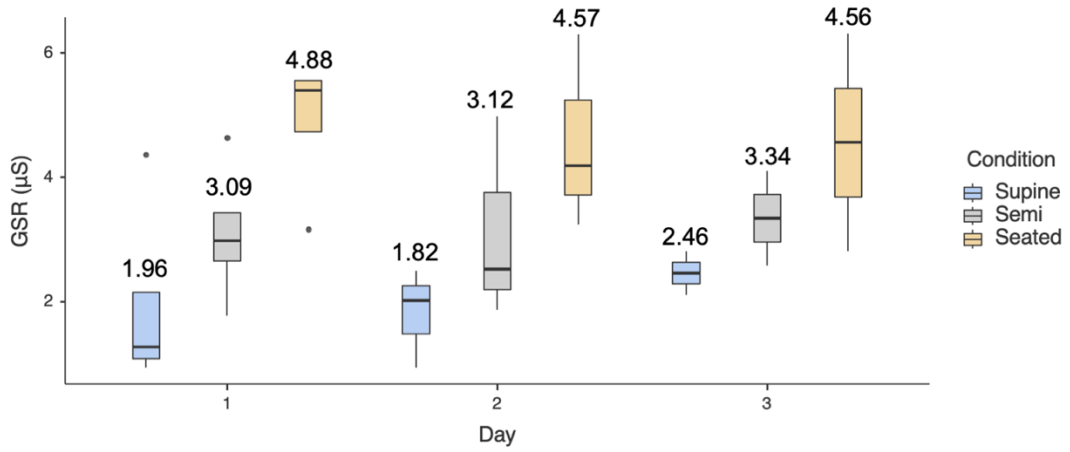

Figure S39. Mean GSR values, separated by condition and day

REFERENCES

1. Gavin, K. M., Seals, D. R., Silver, A. E. & Moreau, K. L. Vascular Endothelial Estrogen Receptor α Is Modulated by Estrogen Status and Related to Endothelial Function and Endothelial Nitric Oxide Synthase in Healthy Women. *The Journal of Clinical Endocrinology & Metabolism* **94**, 3513–3520 (2009).

2. Kelly, D. M. & Jones, T. H. Testosterone: a vascular hormone in health and disease. *Journal of Endocrinology* **217**, R47–R71 (2013).

3. Brunt, V. E. *et al.* Short-term administration of progesterone and estradiol independently alter carotid-vasomotor, but not carotid-cardiac, baroreflex function in young women. *American Journal of Physiology-Heart and Circulatory Physiology* **305**, H1041–H1049 (2013).

4. Minson, C. T., Halliwill, J. R., Young, T. M. & Joyner, M. J. Sympathetic Activity and Baroreflex Sensitivity in Young Women Taking Oral Contraceptives. *Circulation* **102**, 1473–1476 (2000).

5. Teixeira, A. L., Ramos, P. S., Vianna, L. C. & Ricardo, D. R. Heart rate variability across the menstrual cycle in young women taking oral contraceptives. *Psychophysiology* **52**, (2015).

6. Shenouda, N., Priest, S. E., Rizzuto, V. I. & MacDonald, M. J. Brachial artery endothelial function is stable across a menstrual and oral contraceptive pill cycle but lower in premenopausal women than in age-matched men. *American Journal of Physiology-Heart and Circulatory Physiology* **315**, (2018).

7. Torgrimson, B. N., Meendering, J. R., Kaplan, P. F. & Minson, C. T. Endothelial function across an oral contraceptive cycle in women using levonorgestrel and ethinyl estradiol. *American Journal of Physiology-Heart and Circulatory Physiology* **292**, (2007).

8. Yu, A. *et al.* The effect of oral Contraceptive pills and the natural menstrual cYCLe on arterial stiffness and hemodynamICs (CYCLIC). *Journal of Hypertension* **32**, (2014).

9. Priest, S., Shenouda, N. & MacDonald, M. The effect of sex, menstrual cycle phase, and monophasic oral contraceptive 4 pill use on local and central arterial stiffness in young adults. *American Journal of Physiology* (2018).

10. 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. *Sports Medicine* **51**, (2021).

11. United Nations Department of Economic and Social Affairs Population Division. *Trends in Contraceptive Use Worldwide 2020*. (2020).

12. Wenner, M. M. & Stachenfeld, N. S. Point: Investigators should control for menstrual cycle phase when performing studies of vascular control that include women. *Journal of Applied Physiology* japplphysiol.00443.2020 (2020) doi:10.1152/japplphysiol.00443.2020.

13. 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. *American Journal of Physiology-Heart and Circulatory Physiology* **315**, (2018).

14. Menazza, S. & Murphy, E. The Expanding Complexity of Estrogen Receptor Signaling in the Cardiovascular System. *Circulation Research* **118**, (2016).

15. Takahashi, K. *et al.* Both estrogen and raloxifene cause G1 arrest of vascular smooth muscle cells. *Journal of Endocrinology* **178**, 319–329 (2003).

16. Pare, G. *et al.* Estrogen Receptor-α Mediates the Protective Effects of Estrogen Against Vascular Injury. *Circulation Research* **90**, 1087–1092 (2002).

17. Nikolic, I. *et al.* Treatment with an estrogen receptor-beta-selective agonist is cardioprotective. *Journal of Molecular and Cellular Cardiology* **42**, 769–780 (2007).

18. Umar, S. *et al.* Estrogen Rescues Preexisting Severe Pulmonary Hypertension in Rats. *American Journal of Respiratory and Critical Care Medicine* **184**, 715–723 (2011).

19. Pedram, A., Razandi, M., O'Mahony, F., Lubahn, D. & Levin, E. R. Estrogen Receptor-β Prevents Cardiac Fibrosis. *Molecular Endocrinology* **24**, 2152–2165 (2010).

20. Venkov, C. D., Rankin, A. B. & Vaughan, D. E. Identification of Authentic Estrogen Receptor in Cultured Endothelial Cells. *Circulation* **94**, 727–733 (1996).

21. Reed, B. *et al. The Normal Menstrual Cycle and the Control of Ovulation*. (2000).

22. Karas, R. H. *et al.* A complex role for the progesterone receptor in the response to vascular injury. *Journal of Clinical Investigation* **108**, (2001).

23. Barbagallo, M. *et al.* Vascular Effects of Progesterone. *Hypertension* **37**, (2001).

24. Pang, Y., Dong, J. & Thomas, P. Progesterone increases nitric oxide synthesis in human vascular endothelial cells through activation of membrane progesterone receptor-α. *American Journal of Physiology-Endocrinology and Metabolism* **308**, (2015).

25. Knauthe, R., Diel, P., Hegele-Hartung, C., Engelhaupt, A. & Fritzemeier, K. H. Sexual dimorphism of steroid hormone receptor messenger ribonucleic acid expression and hormonal regulation in rat vascular tissue. *Endocrinology* **137**, (1996).

26. Sladek, C. D., Michelini, L. C., Stachenfeld, N. S., Stern, J. E. & Urban, J. H. Endocrine-Autonomic Linkages. in *Comprehensive Physiology* 1281–1323 (2015). doi:10.1002/cphy.c140028.

27. Levine, R. L., Chen, S.-J., Durand, J., Chen, Y.-F. & Oparil, S. Medroxyprogesterone Attenuates Estrogen-Mediated Inhibition of Neointima Formation After Balloon Injury of the Rat Carotid Artery. *Circulation* **94**, (1996).

28. Williams, J. K., Honoré, E. K., Washburn, S. A. & Clarkson, T. B. Effects of hormone replacement therapy on reactivity of atherosclerotic coronary arteries in cynomolgus monkeys. *J Am Coll Cardiol* **24**, (1994).

29. KIM, K., MORIARTY, K. & BENDER, J. Vascular cell signaling by membrane estrogen receptors. *Steroids* **73**, 864–869 (2008).

30. Orshal, J. M. & Khalil, R. A. Gender, sex hormones, and vascular tone. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **286**, R233–R249 (2004).

31. Weiner, C. P. *et al.* Induction of calcium-dependent nitric oxide synthases by sex hormones. *Proceedings of the National Academy of Sciences* **91**, 5212–5216 (1994).

32. 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* **18**, (2014).

33. Stachenfeld, N. S. & Keefe, D. L. Estrogen effects on osmotic regulation of AVP and fluid balance. *American Journal of Physiology-Endocrinology and Metabolism* **283**, E711–E721 (2002).

34. Barbagallo, M., Shan, J., Pang, P. K. T. & Resnick, L. M. Effects of Dehydroepiandrosterone Sulfate on Cellular Calcium Responsiveness and Vascular Contractility. *Hypertension* **26**, (1995).

35. Wang, Z. & Brecher, P. Salicylate Inhibition of Extracellular Signal-Regulated Kinases and Inducible Nitric Oxide Synthase. *Hypertension* **34**, (1999).

36. Wang, D., Yu, X. & Brecher, P. Nitric Oxide and N-Acetylcysteine Inhibit the Activation of Mitogen-activated Protein Kinases by Angiotensin II in Rat Cardiac Fibroblasts. *Journal of Biological Chemistry* **273**, (1998).

37. Miller, V. M. & Duckles, S. P. Vascular Actions of Estrogens: Functional Implications. *Pharmacological Reviews* **60**, 210–241 (2008).

38. Hall, K. S. & Trussell, J. Types of combined oral contraceptives used by US women. *Contraception* **86**, 659–665 (2012).

39. Laurent, S. *et al.* Aortic Stiffness Is an Independent Predictor of All-Cause and Cardiovascular Mortality in Hypertensive Patients. *Hypertension* **37**, 1236–1241 (2001).

40. Bohn, L. *et al.* Sedentary Behavior and Arterial Stiffness in Adults with and without Metabolic Syndrome. *International Journal of Sports Medicine* **38**, 396–401 (2017).

41. Cavalcante, J. L., Lima, J. A. C., Redheuil, A. & Al-Mallah, M. H. Aortic Stiffness. *J Am Coll Cardiol* **57**, (2011).

42. KROEKER, E. J. & WOOD, E. H. Comparison of Simultaneously Recorded Central and Peripheral Arterial Pressure Pulses During Rest, Exercise and Tilted Position in Man. *Circulation Research* **3**, 623–632 (1955).

43. Knot, H. J., Lounsbury, K. M., Brayden, J. E. & Nelson, M. T. Gender differences in coronary artery diameter reflect changes in both endothelial Ca^{2+} and ecNOS activity. *American Journal of Physiology-Heart and Circulatory Physiology* **276**, (1999).

44. Murphy, J. G. & Khalil, R. A. Gender-specific reduction in contractility and $[Ca^{2+}]_i$ in vascular smooth muscle cells of female rat. *American Journal of Physiology-Cell Physiology* **278**, (2000).

45. Stoner, L., Young, J. M. & Fryer, S. Assessments of Arterial Stiffness and Endothelial Function Using Pulse Wave Analysis. *International Journal of Vascular Medicine* **2012**, (2012).

46. Weber, T. *et al.* Arterial Stiffness, Wave Reflections, and the Risk of Coronary Artery Disease. *Circulation* **109**, (2004).

47. Patvardhan, E. *et al.* Augmentation Index Derived from Peripheral Arterial Tonometry Correlates with Cardiovascular Risk Factors. *Cardiology Research and Practice* **2011**, 1–6 (2011).

48. Jensen-Urstad, K., Jensen-Urstad, M. & Johansson, J. Carotid Artery Diameter Correlates With Risk Factors for Cardiovascular Disease in a Population of 55-Year-Old Subjects. *Stroke* **30**, 1572–1576 (1999).

49. Vasankari, T. *et al.* Oxidized LDL and thickness of carotid intima-media are associated with coronary atherosclerosis in middle-aged men: lower levels of oxidized LDL with statin therapy. *Atherosclerosis* **155**, 403–412 (2001).

50. GEROULAKOS, G., O'GORMAN, D. J., KALODIKI, E., SHERIDAN, D. J. & NICOLAIDES, A. N. The carotid intima-media thickness as a marker of the presence of severe symptomatic coronary artery disease. *European Heart Journal* **15**, 781–785 (1994).

51. Wang, L. *et al.* Endogenous sex hormones, blood pressure change, and risk of hypertension in postmenopausal women: The Multi-Ethnic Study of Atherosclerosis. *Atherosclerosis* **224**, 228–234 (2012).

52. Girdler, S. S., Pedersen, C. A., Stern, R. A. & Light, K. C. Menstrual cycle and premenstrual syndrome: Modifiers of cardiovascular reactivity in women. *Health Psychology* **12**, (1993).

53. Sun, H., Wu, S. & Sun, B. MicroRNA-532-5p protects against atherosclerosis through inhibiting vascular smooth muscle cell proliferation and migration. *Cardiovascular Diagnosis and Therapy* **10**, (2020).

54. Pucci, G. *et al.* Evaluation of the Vicorder, a novel cuff-based device for the noninvasive estimation of central blood pressure. *Journal of Hypertension* **31**, (2013).

55. Nickenig, G. *et al.* Estrogen Modulates AT $_1$ Receptor Gene Expression In Vitro and In Vivo. *Circulation* **97**, (1998).

56. Muppa, P. *et al.* Gynecological and menstrual disorders in women with vasovagal syncope. *Clinical Autonomic Research* **23**, (2013).

57. Edgell, H., Robertson, A. D. & Hughson, R. L. Hemodynamics and brain blood flow during posture change in younger women and postmenopausal women compared with age-matched men. *Journal of Applied Physiology* **112**, (2012).

58. Fu, Q. *et al.* Menstrual cycle effects on sympathetic neural responses to upright tilt. *The Journal of Physiology* **587**, (2009).

59. Beebe-Dimmer, J. L., Pfeifer, J. R., Engle, J. S. & Schottenfeld, D. The Epidemiology of Chronic Venous Insufficiency and Varicose Veins. *Annals of Epidemiology* **15**, 175–184 (2005).

60. Wallin, B. G. & Charkoudian, N. Sympathetic neural control of integrated cardiovascular function: Insights from measurement of human sympathetic nerve activity. *Muscle & Nerve* **36**, 595–614 (2007).

61. Liu, C. C., Kuo, T. B. J. & Yang, C. C. H. Effects of estrogen on gender-related autonomic differences in humans. *American Journal of Physiology-Heart and Circulatory Physiology* **285**, H2188–H2193 (2003).

62. Dart, A. Gender, sex hormones and autonomic nervous control of the cardiovascular system. *Cardiovascular Research* **53**, 678–687 (2002).

63. Akselrod, S. *et al.* Hemodynamic regulation: investigation by spectral analysis. *American Journal of Physiology-Heart and Circulatory Physiology* **249**, (1985).

64. Hillebrand, S. *et al.* Heart rate variability and first cardiovascular event in populations without known cardiovascular disease: meta-analysis and dose–response meta-regression. *EP Europace* **15**, (2013).

65. Wilczak, A. *et al.* Relations between combined oral contraceptive therapy and indices of autonomic balance (baroreflex sensitivity and heart rate variability) in young healthy women. *Polish Gynaecology* **84**, (2013).

66. Boucsein, W. *Electrodermal Activity*. (Springer Science+Business Media, LLC, 2012).

67. Sugenoya, J., Iwase, S., Mano, T. & Ogawa, T. Identification of sudomotor activity in cutaneous sympathetic nerves using sweat expulsion as the effector response. *European Journal of Applied Physiology and Occupational Physiology* **61**, 302–308 (1990).

68. Casey, E. *et al.* Influence of Menstrual Cycle and Oral Contraceptive Phase on Spinal Excitability. *PM&R* **8**, (2016).

69. Takada, S. *et al.* Low-intensity exercise can increase muscle mass and strength proportionally to enhanced metabolic stress under ischemic conditions. *Journal of Applied Physiology* **113**, 199–205 (2012).

70. Kis, E. *et al.* Measurement of pulse wave velocity in children and young adults: a comparative study using three different devices. *Hypertension Research* **34**, (2011).

71. Keehn, L., Milne, L., McNeill, K., Chowienczyk, P. & Sinha, M. D. Measurement of pulse wave velocity in children. *Journal of Hypertension* **32**, 1464–1469 (2014).

72. Scandale, G. *et al.* Arterial stiffness and subendocardial viability ratio in patients with peripheral arterial disease. *The Journal of Clinical Hypertension* **20**, (2018).

73. Stone, K. *et al.* Validity and reliability of lower-limb pulse-wave velocity assessments using an oscillometric technique. *Experimental Physiology* **104**, (2019).

74. Nürnberger, J. *et al.* Augmentation index is associated with cardiovascular risk. *Journal of Hypertension* **20**, (2002).

75. O'Rourke, M. F., Pauca, A. & Jiang, X.-J. Pulse wave analysis. *British Journal of Clinical Pharmacology* **51**, (2001).

76. Quipu Srl. Cardiovascular Suite User Manual. (2020).

77. KAWASAKI, T., SASAYAMA, S., YAGI, S.-I., ASAKAWA, T. & HIRAI, T. Non-invasive assessment of the age related changes in stiffness of major branches of the human arteries. *Cardiovascular Research* **21**, (1987).

78. Stachenfeld, N. S. & Taylor, H. S. Progesterone increases plasma volume independent of estradiol. *Journal of Applied Physiology* **98**, 1991–1997 (2005).

79. Tan, H. L., Pinder, M., Parsons, R., Roberts, B. & van Heerden, P. V. Clinical evaluation of USCOM ultrasonic cardiac output monitor in cardiac surgical patients in intensive care unit. *British Journal of Anaesthesia* **94**, (2005).

80. Brinton, R. D. *et al.* Progesterone receptors: Form and function in brain. *Frontiers in Neuroendocrinology* **29**, 313–339 (2008).

81. Electrophysiology, T. F. of the E. S. Heart Rate Variability. *Circulation* **93**, (1996).

82. Pinna, G. D. *et al.* Heart rate variability measures: a fresh look at reliability. *Clinical Science* **113**, (2007).

83. Calbiotech. *Estimation of Estradiol in Mouse Serum Samples: Evaluation of Commercial Estradiol Immunoassays*. (2016).

84. Koo, T. K. & Li, M. Y. A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. *Journal of Chiropractic Medicine* **15**, (2016).

85. Chidi-Ogbolu, N. & Baar, K. Effect of Estrogen on Musculoskeletal Performance and Injury Risk. *Frontiers in Physiology* **9**, (2019).

86. Grazia Modena, M. "Estrogens and the Heart: Do they Help or Hurt?" How Estrogen impacts the Cardiovascular System. *SOJ Gynecology , Obstetrics & Women's Health* **2**, (2016).