Promoting stretch exercise to reduce cardiovascular health risk in late pregnant women with obesity

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Abbreviations and Definitions of Terms

Abbreviation	Definition
cf-PWV	Carotid femoral pulse wave velocity
eUC	Enhanced Usual Care
GW	Gestation week
HF	High frequency
HRV	Heart rate variability
LF	Low frequency
PA	Physical activity
PEP	Pre-ejection period
RPE	Rate of perceived exertion
SE	Stretch exercise

Study Title	Promoting stretch exercise to reduce cardiovascular health risk in late pregnant women with obesity
Funder	National Institute of Nursing Research (NINR)
Clinical Phase	Phase 2
Study Rationale	This project is important because stretching exercise (SE) may promote health and prevent complications of pregnancy when most pregnant women become sedentary. Stretching exercise may be practical for obese pregnant women, many of whom happen to be minority and underserved. Stretching exercise is known to have high acceptance, better adherence, no known untoward side effects, and promising preliminary evidence of effectiveness.
Study Objective(s)	**Primary** • To evaluate change in blood pressure, sympatho-vagal balance, and arterial stiffness from baseline to 32 and 37 weeks' gestation. **Secondary** • To evaluate 6 maternal outcomes (pre-eclampsia/gestational hypertension, gestational diabetes, preterm delivery, elective or emergency caesarean section) and 4 fetal/neonatal outcomes (intrauterine death, small for gestational age, large for gestational age, admission to NICU).
Test Article(s) *(If Applicable)*	**Stretching Exercise**. The exercise regimen consists of sequential static stretching of large skeletal muscle groups for 20 seconds, 3 times per muscle group for a total of 30 minutes.
Study Design	In this 2-arm randomized control trial, we will determine if a stretch exercise (SE) is superior to a control condition of enhanced usual care (eUC) which includes moderate/vigorous activity, specifically walking, for 30 minutes 5 days/week as recommended by the American College of Obstetricians and Gynecologists. Both the treatment and control arms will receive a supportive behavioral intervention to facilitate adherence. Primary outcomes are BP, sympatho-vagal balance, and arterial stiffness assessed at baseline (27 weeks' gestation), then at 5 and 10 weeks later, and at 32 and 37 weeks' gestation. We will compare SE to eUC (n = 306; 153 per arm) and examine selected cardiovascular functions by which SE may be effective.
Subject Population key criteria for Inclusion and Exclusion:	**Inclusion Criteria** 1. Pregnant at less than 24 weeks gestation 2. Singleton pregnancy 3. BMI ≥ 30 kg/m^2 at their first prenatal care visit 4. English or Spanish speaking **Exclusion Criteria** 1. Women who expect or are scheduled to deliver prior to 37 weeks gestation 2. Women who expect to move from the area during their participation in the study 3. Women who are unable to exercise for 30 or more minutes 3 times per week
Number Of Subjects	Enrollment goal is 352 pregnant women

Study Duration	• Each subject's participation will last 10 weeks (27-37 weeks gestation) • The entire study is expected to last five years
Study Phases Screening Study Treatment Follow-Up	(1) Screening: screening for eligibility and obtaining consent will be conducted at UNC- Women's Health clinics (2) Intervention: the study intervention includes a prescribed exercise regimen for 10 weeks. Treatment condition: Supportive intervention + stretching exercise (SE). Usual Care (UC) condition: UC is enhanced by supportive intervention + walking exercise (WE). (3) Follow up: none
Efficacy Evaluations	Primary evaluation measurements that will be used to assess the efficacy of the intervention are change in blood pressure, sympatho-vagal balance, and arterial stiffness from baseline to 32 and 37 weeks' gestation.
Safety Evaluations	Exercise prescribed in the study will be approved by subject's obstetrical care providers. The care providers will fill out the screening form for physical activities that pregnant women can engage.
Statistical And Analytic Plan	Statistical analyses will be conducted using SAS, and all hypothesis tests will use a two-sided alpha of 0.05. Missing visits and measures of physical activity or physiology will not be imputed, but missing items on psychosocial measures may be imputed according to the scoring rules for the particular measure. **Aim 1**(primary outcomes): We expect that, compared to eUC, participants in the SE group will have lower blood pressure (systolic and diastolic), sympatho-vagal balance (PEP, HF), and arterial stiffness (cf-PWV). We will fit a linear mixed-effects model to each of these outcomes at 32 and 37 gw. We will control for the 27 gw value of the outcome and covariates though to be related to the outcomes (anxiety, age, race/ethnicity, BMI and smoking) and any other covariates that are found to differ between the SE and eUC groups.
DATA AND SAFETY MONITORING PLAN	The PI and the Study Statistician (Dr. Crandell) will oversee and train the project coordinator to establish a system to ensure the verification of source data compliance. The source data will include original records necessary for the reconstruction and evaluation of the clinical trial. It will be clear who documented the data, documentation will be readable, signature identifiable, contemporaneous, original, copy accurate and consistent, long-lasting and durable, available and accessible, complete, credible, and corroborated. All data will be first checked by two separate Research Assistants (RAs) at different times. REDCap will be used as our Research Electronic Data Capture System. The safety of participants in this study will be ensured through a local and impartial Data Safety and Monitoring Committee (DSMC). DSMC will consist of 3 voting faculty members. Study progress and safety will be reviewed monthly (and more frequently if needed). Progress reports, including participant recruitment, retention/attrition, and AEs will be provided to Dr. Schwartz (DSMC) every six months. An annual report will be compiled and will include a list and summary of AEs and SAEs.

BACKGROUND AND RATIONALE

1.1 Introduction

More than 50% of pregnant women in the U.S. are obese, increasing their risk for serious complications including preeclampsia, gestational hypertension, and giving birth to preterm and/or low birth weight infants [1-4]. Physical activity (PA) is recommended to manage obesity; PA of moderate or vigorous intensity for at least 150 minutes each week greatly improves health in all stages of life [5]. Although being sedentary can lead to excess risk for maternal and child mortality and morbidity [6], most pregnant women reduce their PA and only 8% meet the recommendations in the 3rd trimester [7]. Behavioral approaches to increase moderate and vigorous PA in women late in pregnancy have been tested with limited success [8]. More recently, researchers have shown interest in light intensity physical activity that may improve adherence,[9] lower blood pressure (BP)[10] and improve sympatho-vagal balance.[11] In our randomized controlled trial with sedentary overweight/obese pregnant women with preeclampsia in a previous pregnancy,[10] we found that compared to walking (a moderate PA), to which pregnant women adhered poorly, 10 or more weeks of stretching exercise (a light PA) reduced the incidence of preeclampsia - a significant risk factor for cardiovascular disease - by lowering BP and enhancing antioxidant up-regulation and production. From our pilot study of healthy pregnant women, of which 84% were overweight/obese, (Preliminary Study 3), we reported promising results of the short-term effects of stretching exercise on sympatho-vagal balance (measured by heart rate variability and cardiac impedance), and established the protocol for arterial stiffness, a long-term effect measure. We are now positioned to test if this effect can be extended to broader populations of pregnant women. Thus, we propose to test the effects of a novel stretching exercise (SE) intervention on cardiovascular health in obese pregnant women in the 3rd trimester. Because women often do not adhere to PA recommendations in late pregnancy, we include evidence-based behavioral strategies to enhance adherence.

We define a session of SE as 30 minutes of sequential static stretching of large skeletal muscle groups for 20 seconds, 3 times per muscle group. In our preliminary studies with small samples, we linked SE to improvements in BP and oxidative stress markers,[10,12,13] findings that are highly consistent with a protective effect against preeclampsia. Both systolic and diastolic BPs were 4 mmHg lower in the stretching than the walking condition. Moreover, two antioxidants biomarkers (superoxide dismutase and transferrin) were significantly higher in the stretching than walking condition.[13] Although little is known about cardiovascular effects of stretching exercise, these findings were unexpected yet consistent across our preliminary studies. If they persist, SE would be an effective, accessible, cost-efficient way for obese pregnant women to promote their health and reduce risk of cardiovascular complications of pregnancy. The study of SE interventions is therefore justified, and is especially important because studies of moderate/vigorous PA interventions for women in late pregnancy have yielded poor results, possibly due to low adherence, which we will address. In this 2-arm RCT we will determine if SE is superior to a control condition of enhanced usual care (eUC) which includes moderate/vigorous activity, specifically walking, for 30 minutes 5 days/week; both treatment and control arms will receive supportive behavioral intervention to facilitate adherence. Primary outcomes are BP, sympatho-vagal balance, and arterial stiffness. We will compare SE to eUC (n = 200; 100 per arm) and examine selected cardiovascular functions by which stretching may be effective.

The Aims are:

Aim 1 (primary outcomes): To test the efficacy of SE intervention on improving cardiovascular health compared to eUC with respect to selected indicators of cardiovascular function (blood pressure, sympatho-vagal balance and arterial stiffness), controlling for anxiety, age, race/ethnicity, BMI, and

smoking. Hypothesis 1: SE will result in lower BP, reduced sympatho-vagal balance, and lower arterial stiffness compared to eUC.
Aim 2 (secondary outcomes): To test the efficacy of the SE intervention on maternal, fetal, and neonatal composite outcomes compared to eUC. We will use a composite pregnancy outcome that includes preeclampsia, pregnancy induced hypertension, gestational diabetes, preterm delivery, elective and emergency cesarean section, intrauterine death, small or large for gestational age, and admission to NICU.[14] Hypotheses 2: SE will result lower composite score compared to eUC.
Aim 3: To explore adherence and overall physical activity in this group of obese pregnant women: 3a. Compare the two groups with respect to adherence to the recommended exercise and examine the relationships between adherence and the primary and secondary outcomes; 3b. Examine the overall level of physical activity in the two arms as measured by accelerometer, and make adjustments to the analyses in Aims 1 and 2 accordingly. Participants will be recruited from UNC- Women's Health clinics.

1.2 Name and Description of Investigational Product or Intervention

Stretching Exercise. The exercise regimen consists of sequential static stretching of large skeletal muscle groups for 20 seconds, 3 times per muscle group for a total of 30 minutes. A demonstration video, based on one that we developed for preliminary studies,[15] will have an introduction covering safe exercise environment and a posture of the stretches. The sequence of movements shows how to stretch the major muscle groups, namely the lower extremities (quadriceps, hamstring, adductor, and gastrocnemius muscles), upper extremities (pectoralis major and minor, triceps, latissimus dorsi), and neck and trunk (flexion, extension, and rotation) through their full range of motion.[15,16]

1.3 Non-Clinical and Clinical Study Findings

We propose to investigate whether and how a stretching exercise intervention improves cardiovascular function in obese pregnant women. Although we tested small samples, our preliminary data suggest that stretching modifies mechanisms leading to preeclampsia in a high-risk group of pregnant women, i.e., women with a history of preeclampsia.[10,13,17] Scholars agree that preeclampsia is a cardiovascular condition that increases risk for cardiovascular illness in later life.[18-23] Obese pregnant women, a population of public health concern, also are at risk for cardiovascular illness.[24-26] This suggests that a stretching intervention may reduce risk of cardiovascular illness in pregnant women of obesity.[27] Generally, pathophysiology of cardiovascular conditions are explained using frameworks of oxidative stress, systemic inflammation, and enhanced sympathetic activity.[28]Given that stretching exercise enhanced antioxidants better than walking exercise in our prior study,[10,13,17] we will test a new mechanism, sympatho-vagal balance, a theory of which we developed using existing literature[29,30] and our own preliminary data (published[31,32] and unpublished Study 3). We also will evaluate arterial stiffness, a demonstrated measure of overall cardiovascular health.[15, 33-42] Thus, we posit that a stretching intervention will improve cardiovascular function in obese pregnant women, which in turn will result in fewer pregnancy complications. This study addresses weaknesses in prior research. We have an available sample adequate to assure robust power, have explicated a promising physiological framework to explore underlying mechanisms, and have included a rigorous plan to facilitate adherence. This study will advance knowledge about mechanisms for stretching and wellness promotion in obese pregnant women. Shifting the focus from a narrow, very high-risk population of women with a history of preeclampsia to another group at significant risk for cardiovascular illness serves a larger population and changes the focus from illness to wellness. The potential impact is even broader, as a stretching intervention may help reduce cardiovascular risk in other populations who are unable to adhere to recommendations for moderate to vigorous exercise.

1.4 Relevant Literature and Data

Pregnancy Physical Activity Questionnaire (PPAQ): Participants will be asked to fill out the PPAQ

It has been validated against ActiGraph[43](the intraclass correlation coefficients for reproducibility were 0.78 for total activity, 0.82 for moderate activity, and 0.81 for vigorous activity) and has been used for diverse pregnant populations.[44-47] This questionnaire assesses duration (time spent), frequency, and intensity of daily PA during the previous 4 weeks. The PPAQ has 33 items about activities of daily living in 6 domains: household, caring, sedentary non-occupational, occupational, sports/exercise, and transportation. It estimates daily energy expenditure related to physical activity. Anxiety will be measured because it may affect HRV[48], adherence to exercise, and overall daily physical activity.[49] The State-Trait Anxiety Inventory (STAI) includes self-report scales measuring state and trait anxiety (STAI not included due to copyright restrictions). The state anxiety scale includes statements about the intensity of feeling at a particular moment, while the trait anxiety scale includes statements about the frequency of feelings. The total score for each ranges from 20 to 80, with higher scores indicating more anxiety. The STAI is reliable and valid and has been used with pregnant women.[50,51] Cronbach's alphas are > 0.92 for both state and trait anxiety.

1 STUDY OBJECTIVES

1.4 Primary Objective

The primary purpose of this study is to test the efficacy SE intervention on improving cardiovascular health compared to eUC with respect to selected indicators of cardiovascular function (blood pressure, sympatho-vagal balance and arterial stiffness), controlling for anxiety, age, race/ethnicity, BMI, and smoking.

1.5 Secondary Objective

The secondary purpose of this study is to test the efficacy of the SE intervention on maternal, fetal, and neonatal composite outcomes compared to eUC. We will use a composite pregnancy outcome that includes preeclampsia, pregnancy induced hypertension, gestational diabetes, preterm delivery, elective and emergency cesarean section, intrauterine death, small or large for gestational age, and admission to NICU.[14]

2 INVESTIGATIONAL PLAN **(brief overview)**

2.1 Study Design

We propose a two-arm RCT with n=153 per group. The treatment group receives a supportive intervention to promote stretching exercise (SE), with a target of 30 minutes per day, 5 days per week. The control group is an enhanced usual care (eUC) group receiving the same type of supportive intervention to promote adherence to the standard recommendation from the American College of Obstetricians and Gynecologists (ACOG) of moderate/vigorous physical activity (walking) at 30 minutes per day, 5 days per week.

We will use a parallel group design, comparing effects of eUC to stretching on blood pressure, autonomic nervous system activity, arterial stiffness, and pregnancy complications. Participants in both conditions receive an identical behavioral intervention to facilitate adherence; at baseline, participants and interventionists make a behavior plan to adhere to the exercise; participants report their adherence weekly to an intervention facilitator and, in return, receive brief counseling via phone call.

The supportive intervention consists of behavior change strategies for adhering to individual-based home exercise. SE consists of sequential static stretching of a 20 seconds each for 3 repetitions per muscle group of large skeletal muscle groups. Walking consists of a moderate intensity walking; we will instruct participants about their target heart rate and the Rating of Perceived Exertion (RPE), which will be prescribed based on individual fitness. Participants will keep an exercise log and wear a heart rate monitor (Polar H10) when performing their assigned exercise. To monitor their overall physical

activities, all participants will wear ActiGraph for 7 days at 3 time points: baseline (27 weeks' gestation), 32 and 37 weeks' gestation.

2.2 Allocation to Treatment Groups and Blinding (if applicable)

After baseline data collection, we will randomly assign participants to intervention or control groups using a 1:1 ratio with a block size 5-10, which will be randomly sequenced. The statistician will generate the allocation sequence before the study starts, using a computerized randomization program for pre-sequence generation and allocation concealment. The coordinator will reveal group assignment to participants sequentially. Participants are not blinded to arm assignment, but will be blind to the study hypotheses. Group assignments will not be included on data in the computer systems until final analysis.

3

3.1 Study Duration, Enrollment and Number of Subjects

This is a five-year study with expected enrollment of 352 pregnant women.

3.2 Study Population

Inclusion Criteria: 1) Pregnant at less than 24 weeks gestation; 2) Singleton pregnancy**;** 3) BMI ≥ 30 kg/m^2 at their first prenatal care visit**;** 4) English or Spanish speaking

Exclusion Criteria: 1) Women who expect or are scheduled to deliver prior to 37 weeks gestation; 2) Women who expect to move from the area during their participation in the study; 3) Women who are unable to exercise for 30 or more minutes 3 times per week

4 STUDY PROCEDURES

4.1 **Baseline Visit procedures**- Find biobehavioral lab (BBL) procedures at end of document.
4.2 **Intervention/Treatment procedures** – Find intervention procedures at end of document.
4.3 Follow- up procedures (by visits) – Find biobehavioral lab procedure at end of document.

5 STUDY EVALUATIONS AND MEASUREMENTS **(how measurements will be made)**

Self-report physical activity, anxiety, BP, sympatho-vagal balance, arterial stiffness, weight, and daily physical activity will be assessed at 3 time points (27 gestational weeks [gw], 32 gw, and 37 gw). Pregnancy and birth outcomes will be extracted after the birth. Exercise adherence (HRM and diary for SE; HRM, RPE and diary for WE) will be recorded weekly.

Self-Report Measures

Demographic (age, race/ethnicity, occupation, marital status, and education), health (pregnancy history, medical history, medication, family medical history), and lifestyle (smoking) information will be collected during the run-in.

Pregnancy Physical Activity Questionnaire (PPAQ): Participants will be asked to fill out the PPAQ It has been validated against ActiGraph[43](the intraclass correlation coefficients for reproducibility were 0.78 for total activity, 0.82 for moderate activity, and 0.81 for vigorous activity) and has been used for diverse pregnant populations.[44-47] This questionnaire assesses duration (time spent), frequency, and intensity of daily PA during the *previous 4 weeks.* The PPAQ has 33 items about activities of daily living in 6 domains: household, caring, sedentary non-occupational, occupational, sports/exercise, and transportation. It estimates daily energy expenditure related to physical activity.

Anxiety will be measured because it may *affect HRV* [48], adherence to exercise, and overall daily physical activity.[49]The State-Trait Anxiety Inventory (STAI) includes self-report scales measuring state and trait anxiety (STAI not included due to copyright restrictions). The state anxiety scale includes

statements about the intensity of feeling at a particular moment, while the trait anxiety scale includes statements about the frequency of feelings. The total score for each ranges from 20 to 80, with higher scores indicating more anxiety. The STAI is reliable and valid and has been used with pregnant women.[50,51] Cronbach's alphas are > 0.92 for both state and trait anxiety.[52,53]

Medical Record Extraction
A trained research team member blinded to intervention assignment will extract medical records for outcomes, including preeclampsia, eclampsia, gestational hypertension, gestational diabetes, elective or emergency caesarean section, small for gestational age, large for gestational age, intrauterine growth restriction, and preterm birth.

Monitoring Daily Physical Activity
Physical activity will be measured using **ActiGraph GT3** accelerometers worn at the right side of the waist during waking hours for 7 consecutive days at 3 time points. Accelerometry data are the gold standard for measurement of overall activity when worn for a minimum of 3 days and for at least 10 hours a day.[54,55] Accelerometry can be used to categorize movement as sedentary (<100 counts per minute), light (100–1,951 counts per minute), and moderate to vigorous (1,952 counts per minute).[56] We will report minutes per day and percent of total wear time in sedentary, light, and moderate/vigorous physical activity. We will follow the methods reported by our consultant Evenson and colleague.[57]

Physiological Measures Taken in Laboratory
Physiological measures will be assessed at all three timepoints. Participants will be asked to avoid caffeine, food, and vigorous exercise for two hours before testing. If participants do not comply, the assessment will be rescheduled. After arrival, participants will change into a hospital gown without removing undergarments and be given the opportunity to void. **Height** and **weight** will be measured and recorded, and participants will be asked to rest quietly without a pillow, in a slightly tilted supine position to avoid orthostatic hypotension due to the growing uterus. To achieve correct positioning, a rolled towel or small cushion will be placed under the left hip.[58] While the participant is resting, electrodes will be affixed to the chest for lead II configuration for heart rate variability (HRV) and impedance cardiography (ICG) wave form. Proximal and distal measurement for pulse wave verbosity (PWV) will be taken following an established protocol. Blood pressure will be taken. Participants will quietly rest with normal breathing for 10 minutes while cf-PWV, HRV, and ICG procedures are being prepared and completed.

Blood pressure (BP) and resting heart rate (HR). Brachial BP will be measured by a DINAMAP monitor. Because incorrect cuff size causes error in BP measurement,[59] the mid-section circumference of the dominant upper arm will be measured to select the proper-sized cuff. BP and HR will be measured twice in the dominant arm with a 2-minute rest between measurements; then the two measures will be averaged.[60]

Sympatho-vagal balance: Sympathetic (SNS) activity will be assessed by measuring pre-ejection period (PEP) using impedance cardiography (ICG). PEP is the interval between onset of left ventricular depolarization and opening of the aortic valve.[34] PEP has been extensively used to measure sympathetic activity in studies that examined effect of exercise,[40, 41]behaviors,[42] and prenatal adversities,[15] on autonomic function. PEP reflects cardiac contractility, which is primarily controlled by the beta-adrenergic system.[34] Studies have provided evidence that increase SNS activity decrease PEP [35-37], while beta-adrenergic blockage[35] or anxiety[38] increase PEP.[39] While the ECG is being recorded, the ICG wave form (dZ/dt) will be derived from the raw impedance cardiogram (Z) by the BioNex Amplifier for Impedance Cardiography[61]. Although PEP is generally taken as the time between ECG Q wave onset and B point on the dZ/dt waveform (corresponding to opening of aortic valve), the ECG R wave onset is

recommended as a more reliable and valid point. In this study, PEP will be the time (in milliseconds) from the ECG R point (left ventricular depolarization) and B-point on the dZ/dt wave form (opening of aortic valve), and will be analyzed by Impedance Cardiography Analysis Software.[61]

Parasympathetic (PNS) activity will be assessed by measuring high frequency (HF) of HRV. HRV is a non-invasive measure of the variation in beat-to-beat intervals to assess the sympatho-vagal balance at the sinoatrial node of the heart. The standard measures of HRV include time domain methods and frequency domain methods. More knowledge exists on physiological interpretation of the frequency domain methods (than time domain methods) and short-term HRV measure (than long-term HRV measure). For the frequency domain methods of HRV, the oscillations of the heart rate signal are decomposed at different frequencies (the low frequency band [LF], the high frequency band [HF])[62] HF of HRV is a well-known marker of PSNS tone.[63] In investigating short-term HRV, two 5-minute recording segments (a total of 10 minutes) are preferred.[62] Since respiration influences HRV, subjects will be asked to breathe at their normal rate (14-16 per minute). While electrocardiography (ECG) wave form are being recorded, respiration frequency will also be recorded by the respiration transducer and BioNex data acquisition system.[61]ECG recordings that are free of body movements and ectopic beats will be divided into 5-min segment(s). Using the Mindware HRV software,[61]RR-intervals (RRI) will be generated, and respiration rate and frequency will also be determined with data where respiration frequency is within the respiration sinus arrhythmia (RSA) band (0.15-0.4hz). HF power in absolute and normalized units will be calculated and averaged. Electrocardiogram (ECG) and ICG wave form (dZ/dt) for HF and PEP will be obtained over a 10-minute period. Subjects will be instructed to rest quietly, keeping as still as possible with normal breathing. After 10 minutes, data acquisition will be terminated, and the reports of HF and PEP will be generated.

Arterial stiffness will be measured by carotid femoral pulse wave velocity (cf-PWV) using the SphygmoCor system (AtCor Medical, Itasca, IL). cf-PWV is considered the "gold-standard" measurement of arterial stiffness. Each carotid and femoral waveform will be acquired by applying a pressure sensitive transducer (tonometer) on the carotid and femoral sites. The transit time of the pulse from the left ventricle to the carotid artery (t1) and the transit time of the pulse from the left ventricle to the femoral artery (t2) will be calculated by the system software on the basis of the ECG, using the foot-to-foot method. The straight distance from the suprasternal notch to the carotid artery site (d_carotid) and the distance from the suprasternal notch to the femoral artery site (d_femoral) will be measured using *a pelvimeter, which allows measuring a distance without interference of the bulging abdominal surface due to pregnancy*. cf-PWV (?D[meters] /?t[seconds]) is the difference in the distances from suprasternal notch to two arterial sites (?D = d_femoral – d_carotid) divided by the mean time difference (?t = t2-t1). [64,65] *Pulse Wave Velocity (cf-PWV)* will be measured by applying a tonometer to the carotid and femoral pulses, and will be visually displayed. The tonometer may need to be angled slightly, optimizing wave forms until uniform and free of artifact. Once five consecutive and uniform waves are displayed, data will be captured. Pressure waves of insufficient quality will automatically be rejected by the SphygmoCor device and will generate an error message.

STATISTICAL CONSIDERATIONS

5.1 Primary Endpoint

Statistical analyses will be conducted using SAS, and all hypothesis tests will use a two-sided alpha of 0.05. Missing visits and measures of physical activity or physiology will not be imputed, but missing items on psychosocial measures may be imputed according to the scoring rules for the particular measure. **Aim 1**(primary outcomes): We expect that, compared to eUC, participants in the SE group will have lower blood pressure (systolic and diastolic), sympatho-vagal balance (PEP, HF), and arterial stiffness (cf-PWV). We will fit a linear mixed-effects model to each of these outcomes at 32 and 37 gw. We will control for the 27 gw value of the outcome and covariates though to be related to the outcomes

(anxiety, age, race/ethnicity. BMI and smoking) and any other covariates that are found to differ between the SE and eUC groups.

5.2 Secondary Endpoint

Aim 2(secondary outcomes): The use of pre-specified composite outcomes is common in clinical trials studies of pregnancy outcomes [66,67] and has been recommended in the evaluation of lifestyle interventions in pregnancy.[14] The composite outcome is dichotomization of several negative outcomes- "yes" if one or more outcomes was experienced, and "no" otherwise. In addition to serving as general measures of pregnancy wellness, composite outcomes overcome the large sample sizes needed to identify intervention effects on rare events. We propose maternal and fetal/neonatal composite outcomes based on Yeo et al.'s work.[14] We will fit a logistic regression model predicting each composite outcome from intervention assignment, controlling for covariates (anxiety, age, race/ethnicity. BMI and smoking) and any other covariates that are found to differ between the SE and control group. We will repeat this model for each individual component of the composite outcomes, though power is low to detect a difference for outcomes with low prevalence.

3

Aim 3: a. Using a t-test, we will compare overall adherence (percent of recommended sessions and percent of recommended time) in the SE and eUC arms. Within each arm, we will look at the association between adherence to the intervention and the primary and secondary outcomes using correlation coefficients and t-tests. Additionally, we will add interactions between adherence and exercise type to the models for Aims 2 and 3 to explore adherence as a potential effect modifier of the relationship between exercise type and outcome. This model will be exploratory (i.e. looking for trends), as we are only powered to detect very large effect modification. b. Using a t-test, we will compare overall physical activity as measured by the accelerometer (percent time spent in light and moderate/vigorous physical activity) in the SE and eUC arms. We will use correlations and t-tests to examine the relationships between physical activity and the outcomes. If there is evidence of associations (i.e., potential confounding), we will re-fit the models for Aims 2 and 3 using physical activity level as a covariate.

5.3 Sample Size and Power

Power calculations for the continuous dependent variables in **Aim 1**were performed with POWERLIB20 SAS/IML modules that incorporate methods for hypothesis tests in repeated measures linear models.[68] Diastolic blood pressure was measured at clinic visits 32 and 37/38 weeks in our stretching vs. walking pilot study (N=61 at 32 weeks, 57 at 37/38 weeks), and effect sizes (Cohen's d) were 0.34 at 32 weeks and 0.33 at 37/38 weeks. These effects correspond to ~3 mmHg. Though the pilot did not measure sympatho-vagal balance or arterial stiffness, the effect on resting heart rate was similar (d=.37). Assuming a within-participant correlation of 0.37 (also from the pilot study), our sample size of 153 per group (after dropout) yields 80% power for d=0.35 or higher, which is similar to what was seen in the pilot study. The components of the composite outcomes in **Aim 2** have the following estimated prevalence rates in our population: Maternal- pre-eclampsia/gestational hypertension, 10% [69,70], gestational diabetes 5% [71], preterm delivery 7%[72], elective or emergency c-section, 25%; fetal/neonatal-intrauterine death, 1%[73]; large for gestational age, 9%[74]; small for gestational age, 11%[74]; and NICU admission, 8%[75]. We conservatively estimate the overall prevalence of the composite outcome in the eUC group to be 25% for the maternal outcome and 15% for the fetal/neonatal outcome. With N=306 (153 per group), we have at least 80% power to detect a difference in the eUC group vs. SE group of 25% vs. 13% for the maternal outcome and 15% vs. 6% for the fetal/neonatal outcome. The detectable difference is smaller than the reduction in preeclampsia rates seen in preliminary study 1. Power is low to detect differences in individual components of the composite outcomes, but we will examine these for the purposes of understanding which changed outcomes are driving changes in the composite outcome. The analysis in **Aim 3** consists of t-tests and correlations in the entire sample (N=306) and within each arm (N=153). With an alpha level of 0.05, we have 80% power to detect d=0.32 and a correlation of

0.16 in the entire sample; and d=0.46 and a correlation of 0.22 in a single arm. These are adequate for the exploration of the association of adherence and overall physical activity with treatment arm and primary/secondary outcomes.

5.4 Interim Analysis

The project coordinator will generate semi-annual interim analysis reports on data obtained during the lab visits, weekly phone calls, and medical record for birth outcomes to understand issues related to the measurements, adherence, usability, and fidelity of this project protocols. We will evaluate the screening and enrollment procedures, barriers to participation and retention, acceptability, technology problems encountered if any, and user feedback from the participants and providers. This information gained from this structured process will be used to both guide the refinement of the current protocol. Stopping rules with regard to benefit--The rate of preeclampsia and other birth outcomes. Stopping rules are planned when the effect of stretching exercise is statistically undeniable. Stopping rules with regard to statistical power and adverse events--The DSMC will review subject accrual and adverse events for compelling evidence of futility or harm and will address these using appropriate measures.

6 STUDY INTERVENTION ADMINISTRATION (IF APPLICABLE)

After baseline data collection, we will randomly assign participants to intervention or control groups using a 1:1 ratio with a block size 5-10, which will be randomly sequenced. The statistician (JC) will generate the allocation sequence before the study starts, using a computerized randomization program for pre-sequence generation and allocation concealment. The coordinator will reveal group assignment to participants sequentially. Participants are not blinded to arm assignment, but will be blind to the study hypotheses. Dr. Caughey, who will be unaware of participants' group assignment, will assess cardiovascular function. Group assignments will not be included on data in the computer systems until final analysis.

7. SAFETY MANAGEMENT

The PI will responsible for ensuring that all AE and SAEs are reported to the NIH/NINR and UNC-CH's IRB in compliance with their requirements. Any AE will be reported by the PI or PC to the Adverse Event Monitoring Committee within one week. An incident report will be created and sent by electronic email by the PI or PC to Drs. Thorp, Wilbur, Mottola, Caughey, Logan, Hinderliter, Evenson, Crandell (Study Statistician), and Blumenthal. The PI will follow-up with the participant and will prepare a report. The AEs will be reviewed every six months and a report prepared for the Human Participants Committee and Dr. Yoon at NIH/NINR. Process notes will be kept concerning any decisions.

The safety of participants in this study will be ensured through a local and impartial Data Safety and Monitoring Committee (DSMC). DSMC will consist of 3 voting faculty members: Dr. Andrea K. Knittel, MD, PhD is a board-certified Obstetrician and Gynecologist, and Assistant Professor at UNC-CH School of Medicine, Department of General Onstetrics. She received her medical scientist training program for MD/PhD at the Department of Health Behavior and Health Education at the University of Michigan Medical School and School of Public Health. Dr. Todd Schwartz, PhD is a Biostatistician Research Associate Professor at the UNC-CH School of Nursing and Gillings School of Global Public Health. He is a Methodology Core Co-Director and Principal Investigator at National Institute of Arthritis, Musculoskeletal and Skin Diseases' Multidisciplinary Clinical Research Center in the Thurston Arthritis Research Center. He will assume the Chair of DSMC. Dr. Carmen Samuel-Hodge is Research Assistant Professor of Public Health and the Center for Health Promotion and Disease Prevention. She served a role of reviewer for UNC-CH IRB from 2007 till 2017. All three members are not associated with this research project and thus works independently of the PI. They are not any key personnel involved in this

project. They are qualified to review the participant safety data generated by this study because of their expertise in human research.

8. DATA COLLECTION AND MANAGEMENT

The team of investigators will convene monthly meetings to review the activities of the study including management, personnel, recruitment, performance, and any emerging problems. All data from participants screened for the study will be entered into the REDCap electronic study database. Designated research staff will collect, gather, and enter required data (written informed consent, Health Insurance Portability and Accountability Act (HIPAA) authorization, medical history and demographics) onto study data forms. Screened patients who do not meet study eligibility will have specific screening data entered into the study database. The collected data will be helpful in examining the patient population and feasibility of enrollment criteria and will include gender, age, race and reason for exclusion. All dates will be shifted and other Personal Health Information (PHI) will be removed from the study database upon study completion. All data obtained from this study will be used for research purposes only and will comply with Federal HIPAA regulations.

All proposed study specific case report forms (source documents) for data collection will be designed by the PI and PM in concert with the Study Statistician and transferred by the PC into electronic Case Report Forms (eCRFs) for use in the study's REDCap database. These study specific eCRFs source documents (study logs for correspondence, contacts, compensation and other forms such as pre-eligibility screens) will be coded by the participant's unique study identification number for all data collected including study instruments will be maintained in the participant research record that will be made accessible to study monitors. Completed instruments that require a signature on a paper CRF will be scanned and uploaded into the study database as well as maintained on file in accordance with UNC-CH policies and applicable Federal Regulations for the Conduct of Human Participant Research.

The PI and the Study Statistician (Dr. Crandell) will oversee and train the project coordinator to establish a system to ensure the verification of source data compliance. The source data will include original records necessary for the reconstruction and evaluation of the clinical trial. It will be clear who documented the data, documentation will be readable, signature identifiable, contemporaneous, original, copy accurate and consistent, long-lasting and durable, available and accessible, complete, credible, and corroborated. All data will be first checked by two separate Research Assistants (RAs) at different times. REDCap will be used as our Research Electronic Data Capture System.

The safety of participants in this study will be ensured through a local and impartial Data Safety and Monitoring Committee (DSMC). DSMC will consist of 3 voting faculty members. Study progress and safety will be reviewed monthly (and more frequently if needed). Progress reports, including participant recruitment, retention/attrition, and AEs will be provided to Dr. Schwartz (DSMC) every six months. An annual report will be compiled and will include a list and summary of AEs and SAEs.

9. RECRUITMENT STRATEGY

We have planned and will implement this study with the support of four UNC entities: the University of North Carolina at Chapel Hill (UNC) Women's Health Clinics, UNC research participant recruitment list, UNC School of Nursing Biobehavioral Lab (BBL), and UNC Communication for Health Applications & Interventions (CHAI CORE). Participants will be: 1) Identified at the UNC-Women's Health Clinics or by the recruitment media (CHAI CORE) and UNC Campus-wide email system; and 2) Measured for physiological data at the BBL.

The UNC-Women's Health clinics provide prenatal care each year to ~3,000 adult pregnant women through 8 sites in Orange, Durham, Wake, and Alamance counties and their vicinities. Of these, 56% (1,700 women) are overweight/obese. 103We thus estimate ~600 eligible potential participants (see Enrollment criteria below). From previous experience, we predict 40% enrollment, for availability of 240 per year. Our enrollment goal is 352 (allowing for 15% attrition), therefore we require 60 enrolled participants in year 1, 90 in each of years 2 - 4, and 22 in year 5. To ensure timely recruitment, we will supplement clinic recruitment by contacting the university-controlled research study participant recruitment list, which is an effective way to reach out to the demographically diverse campus community. 103Because women who can communicate in English or Spanish will be recruited, we anticipate lower than the population percentage among small groups who speak a language other than English or Spanish. Using and monitoring multiple recruitment processes, we will ensure that diversity is maintained to reflect the region's population of pregnant women.

10. CONSENT PROCESS

At clinics, trained RNs will approach eligible patients to provide fliers about the study and assess patients' interest, willingness of the study candidates and obtain informed consent after providing detailed study information. RNs will talk to eligible patients in a private area such as an exam room or a consultation room. Candidates will be contacted in the candidate preferred way. Before screening questions, the candidate will be guided to find a private area. Eligible candidates will have sufficient and multiple times for consenting. When eligible candidates are interested but are not in a position to consent, the project manager will email consent form to the patient and schedule a telephone consent conference with a research nurses or research assistants in order to screen the candidates and obtain informed consent after providing detailed study information.

11. PLANS FOR PUBLICATION

We will prepare and submit a presentation and manuscript focused on the design and protocol of the study. Graduate students will be encouraged to conduct secondary data analysis on the data for their dissertations and theses. We will deposit our de-identified data into the Biomedical Research Informatics Computing System at the National Institute of Nursing Research.

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