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Identifying neurophysiological mechanisms of susceptibility to estradiol fluctuation and irritability symptoms in the menopause transition: An experimental approach

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Master Protocol Document

Title	Identifying neurophysiological mechanisms of susceptibility to estradiol fluctuation and irritability symptoms in the menopause transition: An experimental approach
Sub-Title	Determining the neurophysiologic basis of susceptibility to estradiol fluctuations and irritability
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Research Sites	UNC Chapel Hill
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I have read, understood, and approved this version of the protocol.

Principal Investigator:  Date: February 4, 2022

Statistical Co-investigator:  Date: February 4, 2022

Table of Version Changes

Previous Version No.	Affected Sections	Summary of the Changes to the Protocol	Reason for Changes
1.0	**5.2 & 8.1 & 9.0**	**REVISED ELIGIBILITY REQUIREMENTS** **ADDED SENSITIVITY ANALYSIS TO STATISTICAL ANALYSIS** **ADDED REFERENCES TO POWER ANALYSIS**	**RESPONSE TO REVIEW**
2.0	**4.3 & 5.2**	**REVISED ELIGIBILITY REQUIREMENTS** **CHANGED SCID-V TO MINI** **CHANGED WEEKLY IDAS TO CES-D**	**REFLECT MODIFICATIONS MADE SINCE INITIAL APPROVAL**
3.0	**4.3 & 7.1 & 7.4**	**REVISED STUDY DESIGN** **ADDED GREEN CLIMACTERIC SCALE AND PSQI TO BASELINE WEEK 4 LAB VISIT**	**REFLECT MODIFICATIONS MADE SINCE INITIAL APPROVAL**

Table of Contents

Statement of Compliance

This study will be conducted as specified in the protocol and in accordance with the *International Conference on Harmonization Guidelines for Good Clinical Practice* (ICH E6) and the *Code of Federal Regulations on the Protection of Human Subjects* (45 CFR Part 46).

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the *Institutional Review Board* (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

If required by the IRB, the master protocol document, informed consent form(s), recruitment materials, and all participant materials will be submitted to the *Scientific Review Committee* (SRC) prior to IRB review (research.unc.edu/clinical-trials/src).

The statistical analysis plans will be consistent with guidance in CONSORT Statement [1] or STROBE Statement [2], ICMJE recommendations [3], the 2016 and 2019 statements of the American Statistical Association [4,5], and recommendations in Nature [6,7].*

All personnel involved in the conduct of this study have completed human subjects protection training.

* [1] www.consort-statement.org
[2] www.strobe-statement.org
[3] www.icmje.org
[4] Wasserstein RL, et al. (2016), The ASA's Statement on p-Values, ***The American Statistician***, 70:2, 129-133
[5] Wasserstein RL, et al. (2019), Moving to a World Beyond p < 0.05, ***The American Statistician***, 73:sup1, 1-19
[6] Amrhein, et al. (2019) Scientists rise up against statistical significance, ***Nature*** 567, 305-307
[7] Editorial (2019) It's time to talk about ditching statistical significance: Looking beyond a much used and abused measure would make science harder, but better. ***Nature*** 567, 283-283.

Table of Abbreviations

AE / SAE	Adverse event / serious adverse event
BIS/BAS	Behavioral inhibition scale/behavioral activation scale
BMI	Body mass index
BP	Blood pressure
CES-D	Center for Epidemiological Studies- Depression
CFR	U.S. Code of Federal Regulations (www.ecfr.gov)
CI	Confidence interval
CoC	Certificate of confidentiality
CONSORT	Consolidated Standards of Reporting Trials (www.consort-statement.org)
CRF	Case report form
CT.gov	Clinicaltrials.gov website
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DSMB	Data and safety monitoring board
DVT	Deep vein thrombosis
E1G	Estrone-3-glucuronide
E2	Estradiol
eCRF	Electronic case report form
eCTD	Electronic common technical document
EEG	Electroencephalography
ERT	Estrogen replacement therapy
FDA	U.S. Food and Drug Administration (www.fda.gov)
FFR	Federal financial report
FNR	Frustrative non-reward responsiveness
GCP	Good clinical practice
HIPAA	U.S. Health Insurance Portability and Accountability Act (www.hhs.gov/hipaa)
HPA	Hypothalamic-pituitary-adrenal
HRT	Hormone replacement therapy
Hx	History
ICH	International Council for Harmonization (www.ich.org)
ICMJE	International Committee of Medical Journal Editors (www.icmje.org)
IDAS	Inventory of Depression and Anxiety Symptoms
IDE	Investigational device exemption
IDS	UNC Investigational Drug Services (uncids.web.unc.edu)
IND	Investigational new drug application
IRB	Institutional review board
JNC	Joint national committee
MI	Myocardial infarction
MINI	Mini International Neuropsychiatric Interview
MPA	Medroxyprogesterone acetate
MPD	Master protocol document
MT	Menopause transition
N	Number of enrolled participants
NIH	National Institute of Health
OCT	UNC Office of Clinical Trials (research.unc.edu/clinical-trials)
OHRP	Office for Human Research Protections
P4	Progesterone
PDG	Pregnanediol-3-glucuronide
PFHQ	Personal and Family History Questionnaire
PI	Principal investigator
PRC	UNC Oncology Protocol Review Committee (unclineberger.org/protocolreview)
PSQI	Pittsburgh sleep quality index

QA	Quality assurance
RCT	Randomized controlled trial
RDoC	Research domain criteria
REDCap	Research Electronic Data Capture system
SBP	Systolic blood pressure
SD	Standard deviation
SE	Standard error
SOP	Standard operating procedures
SRC	UNC Scientific Review Committee (research.unc.edu/clinical-trials/src)
STRAW	Stages of Reproductive Aging Workshop
STROBE	Strengthening Reporting of Observational Studies in Epidemiology (www.strobe-statement.org)
TE2	Transdermal estradiol
TraCS	N.C. Translational and Clinical Sciences Institute (tracs.unc.edu)
UNC	The University of North Carolina
UNCH	UNC Hospitals
VTE	Venous thromboembolism
WHI	Women's Health Initiative
Wk	Week

1. Protocol Synopsis

Title	Identifying neurophysiological mechanisms of susceptibility to estradiol fluctuation and irritability symptoms in the menopause transition: An experimental approach
Study Description	**The primary objective** of this research is to determine the neurophysiologic basis of susceptibility to estradiol (E2) fluctuations and *irritability* symptoms in perimenopausal women. **The rationale for examining E2 variability in** perimenopausal irritability stems from the evidence that: 1) the amygdala and other limbic regions are densely innervated with E2 receptors; and 2) E2 regulates limbic feedback control of the HPA stress response, dysregulation of which may increase stress vulnerability. Urinary estrone-3-glucuronide (E1G), a urinary metabolite of E2 that is highly correlated with serum E2, will be used as the indicator of serum E2. Using a within-subjects, cross-over design and transdermal E2 (TE2) to stabilize E2 fluctuations (and increase levels) we will test if neural dynamics (oscillatory activity in the theta and beta frequencies assessed via EEG) associated with key constructs of irritability (attentional bias to threat and frustration to non-reward) represent a biomarker target of irritability symptom response to TE2.
Specific Aims **(objectives)**	**Aim 1.** Define the baseline relationships between: (a) E1G variability and irritability symptom severity; (b) E1G variability and theta and beta oscillatory correlates of threat and frustration; (c) irritability symptoms and theta and beta oscillatory correlates of threat and frustration. **AIM 2:** Determine whether TE2, versus placebo, will: a) beneficially modify task-evoked theta and beta oscillations (i.e., reduce theta and increase beta activation) in response to threat and frustration; and b) reduce irritability symptom severity.
Target Population	**Inclusion Criteria:** 1. Medically healthy women, 45 – 59 years of age undergoing a natural menopause transition 2. Moderate to severe levels of irritability symptoms 3. Normotensive blood pressure (< 160/100 mmHg) 4. Meet Stages of Reproductive Aging Workshop criteria for early menopause transition (STRAW+10, stage -2) or late menopause transition (STRAW +10, stage -1) based on bleeding patterns 5. Have experienced 1+ very stressful life event in previous six months 6. Report a significant increase in irritability concurrent with onset of menstrual cycle changes 7. Normal screening mammogram within two years of enrollment. **Exclusion Criteria:** 1. A history of cardiovascular disease 2. A history of type I diabetes 3. A recent history of migraine with aura 4. A history of thromboembolic disorders

	5. A history of E2 dependent neoplasia 6. A history of liver dysfunction 7. A personal or family history suggesting elevated risk for E2-related cancer or thrombotic events 8. Use of psychotropics or hormonal agents 9. A history of psychosis or bipolar disorder 10. Suicidality with intent 11. Current mood disorders of severity to require treatment
Numbers of Enrollees	**A total of N = 50 eligible individuals will be enrolled.** Based on our prior studies of TE2, of the estimated 50 women who pass our phone screening and come to an in-person enrollment, we predict that 30% (n=15) will not meet eligibility for TE2 after personal and family history review or will not meet reproductive staging criteria based on further detailed questions about their menstrual bleeding patterns. We anticipate that up to an additional 15% (n =5) may withdraw after enrollment, yielding **30 women who we anticipate will complete all aspects of the protocol and will have complete data.** **Up to 135 individuals will be recruited and screened.** 40% will fail the screening for TE2 eligibility, and an additional 20% will not be interested in participating.
Interventions or Exposures/Conditions	Using a within-subjects, cross-over, placebo-controlled, double-blind design, participants will be randomized (1:1) to either three weeks of transdermal E2 (0.1 mg/day) or to three weeks of transdermal placebo as their first condition (Condition 1). Weekly mood ratings and daily ill temper (irritability) ratings will be collected across the three weeks. In week 3 of each Condition, daily E1G collections will commence (days 15-21) and the EEG session with Dot-Probe and Affective Posner Paradigm tasks will be administered (day 21). A three-week washout period will follow. Condition 2 (3 weeks) will follow the washout (placebo E2 if Condition 1 was active E2 or active E2 if Condition 1 was placebo) with identical assessments and timeline as for Condition 1. To prevent TE2-induced endometrial hyperplasia, 200 mg micronized progesterone/day for 10 days will be given to all women at study completion (Progesterone follow-up phase).
Outcome Measures	**For Aim 1.** Over the four-week baseline period, irritability symptoms will be measured daily using the Ill temper subscale of the Inventory of Depression and Anxiety Symptoms (IDAS). Urinary measures of E1G will be collected every other day (14 samples total) and assayed using an enzyme immunoassay (Arbor Assays, Ann Arbor, MI), with sensitivity at < 22.5 pg/mL to calculate within subject fluctuation in E1G (indexed by the standard deviation of E1G over the four weeks). At the end of baseline week 4, Theta and Beta oscillatory frequencies will be assessed at rest and in response to the Dot Probe task which measures attention bias to angry faces (threat attention bias) and in response to the Affective Posner Paradigm that measures relational aggression (approach behavior) in response to frustration. **For Aim 2.** The outcomes are identical to those in Aim 1. The primary comparison will be within subject – comparing the active TE2 condition

	to the placebo TE2 condition. Irritability symptoms will be measured daily using the Ill temper subscale of the Inventory of Depression and Anxiety Symptoms. Theta and Beta oscillatory frequencies will be assessed at rest and in response to the Dot Probe task and the Affective Posner Paradigm administered at the last day of each condition. Urinary measures of E1G will be collected every day during the last week of each condition and measured using an enzyme immunoassay (Arbor Assays, Ann Arbor, MI), with sensitivity at < 22.5 pg/mL to generate to within subject variability in E1G (indexed by the standard deviation of E1G).
Statistical Analysis Plans for Each Aim	**Aim 1 Plans.** Linear regression models will predict irritability symptoms and theta and beta frequencies in response to tasks from E1G variability (based on the standard deviation of seven daily E1G measurements) and predict irritability symptoms from theta and beta task-related frequencies. **Aim 2 Plans.** The two repeated post-treatment EEG measures (theta and beta task-related frequencies) will be modeled as a function of fixed factors including baseline EEG measures (continuous), Condition status (TE2 or placebo; binary), and order of Condition (binary) in a linear mixed effects model with a random intercept accounting for the within-subject variance. The two-sided Wald test will test the significance of TE2 effects. **2b)** Similarly, daily irritability symptoms (3 weeks/Condition) will be modeled as a function of fixed factors including baseline irritability symptoms (continuous), Condition, and order of Condition in a linear mixed effects model with a random intercept accounting for within-subject variance.
Study Duration	Four years
Participation Duration	Sixteen weeks
Enrollment Duration	Two years

2. Introduction

2.1. Background Information

Women in the menopause transition (perimenopause) have a 2-4-fold increase in major depression risk. Among the 30% of perimenopausal women with affective impairment, most report irritability, not "depression", is their primary source of impairment and distress. Irritability, the predisposition towards anger, is a dimensional construct evident across disorders, including depression, anxiety, and psychosis. Two inter-connected constructs underlie irritability: 1) dysfunctional threat processing, including aberrant approach responses to threat (attention bias to threatening stimuli), and 2) dysfunctional reward processing, including aberrant approach responses (frustration/anger) to non-reward. These aberrant responses are similarly mediated by dysregulation in affective limbic networks. Affective processing of emotional stimuli relies on synchronous neural oscillations in theta (4-8Hz) and beta (13-30Hz) frequencies for the functional coupling of frontal-limbic neural networks. Greater theta relative to beta frequencies is consistent with deficient frontal top-down control of limbic-mediated affective processing.

The menopause transition (MT) is a reproductive stage with substantial day-to-day variability in estradiol, within a trend toward hypoestrogenism. While most women are exposed to erratic hormone flux in the MT, about 40% are susceptible to the emergence of affective symptoms tied to *changes in estrogen*.[1] While the neurobiological basis of this susceptibility to hormonal change remains unknown, predictors of that susceptibility include: 1) a history of menstrual or postpartum mood disorders,[2–4] in which experimental studies show that symptoms are induced by *changes* in ovarian hormones;[5,6]2) recent stressful life events, which we showed predicts the emergence of depressive symptoms in perimenopausal women who have greater E2 variability;[7] and 3) early menopause transition phase based on results of our RCT showing that transdermal E2 (vs. placebo) prevented the emergence of depressive symptoms and clinical depressive episodes over 12 months – effects that were predicted by both menopausal stage and stressful life events (greater benefit in women in the early transition phase and with more stressful events).

Estradiol (E2) modulates neural systems implicated in affective illness; has anti-depressant/anti-anxiety effects; and regulates limbic networks important for affective and reward processing. Thus, the menopause transition may provide an endocrine context of vulnerability for the emergence of affective impairment and an ideal model in which to study the neurophysiologic basis to the susceptibility to E2 fluctuations and the emergence of irritability. However, the research to date has focused on only gross measures of perimenopausal "depression" (e.g., total CES-D score), limiting the ability to identify the neurobiological basis of affective susceptibility to E2 flux. **We will address this gap by taking a dimensional approach** and study *irritability* symptom severity because it is associated with the greatest impairment/distress in the menopause transition, and we will study women who are likely to be susceptible to E2 flux (based on stressful life events, early transition stage, and emergence of irritability concurrent with entering the menopause transition). This will increase our ability to, for the first time, **determine if fronto-limbic neurophysiology is a mechanism of hormone susceptibility and irritability** in perimenopausal women which represents the second gap that our research will address.

2.2. Scientific Rationale

The rationale for investigating E2 fluctuations in susceptibility to develop irritability in the MT stems from evidence that: 1) limbic regions are densely innervated with estrogen receptors;[8,9] 2) E2 is essential in regulating limbic (amygdala and hippocampus) feedback control HPA stress reactivity,[10,11] dysregulation of which may increase vulnerability to stress; 3) E2 regulates limbic networks important for threat and reward processing,[8,9]4) E2 variability predicts emergence of depression in the MT, especially in women with recent stressful events; and 5) E2 "beneficially" modulates systems implicated in affective illness,[39–42] to exert anti-depressant/anxiety effects.[10]

Additionally, our work indicates that acute psychosocial stress increases the frontal theta response to emotional stimuli,[16] suggesting that recent stress exposure in combination with E2 flux during the menopause transition may interfere with frontal-limbic networks underlying irritability symptoms. Elucidating the oscillatory correlates of

aberrant fronto-limbic mediated threat and frustrative non-reward processing will provide ***mechanistic insight into the neurophysiology of susceptibility to E2 fluctuations and irritability symptoms in the menopause transition: a key objective of this research.***

Rationale for the design, preparation/dose of hormones, and duration of conditions: We have selected a within subjects, double-blind, **cross-over design** because of the greater statistical power over a between subjects RCT and to *minimize bias*. This allows optimal ability to detect a "signal" in: 1) the relationship of E1G variability to irritability and to behavior and neurophysiology (Aim 1); and 2) the ability of TE2 to beneficially modify theta and beta frequency oscillations in response to threat and frustrative non-reward, and reduce irritability. By avoiding the first-pass metabolic effects of oral estrogen, ***transdermal E2 (TE2)*** creates more stable, premenopausal follicular phase E2 levels than oral estrogens; and a physiologic profile of E2 relative to its metabolites.[17–19]TE2 also has a superior safety profile than oral estrogen.[20–22] A 7-day transdermal system provides more stable levels and better compliance than twice weekly patches.[23]The adhesive layer of the matrix in Climara® patches continuously releases E2, which is transported across skin leading to sustained E2 levels.[18]Progesterone**:** To prevent E2-induced endometrial hyperplasia, 200 mg micronized progesterone/day x 10 days will be taken at study completion. A progestin every 2-3 months is sufficient for endometrial protection,[24] particularly in women in the early MT who still menstruate. We will give progesterone in a discontinuous fashion instead of using Climara Pro® (delivers both E2 and a progestin) because progestins antagonize E2 effects.[25,26]

Duration of Conditions: We have chosen 3-week duration for each Condition based on: 1) The results of co-Investigator, Dr. Schiller, showing maximal effect of TE2 on irritability symptoms within one week; 2) placebo-controlled studies showing TE2 reduces perimenopausal depression symptoms with maximal effects seen in one to three weeks;[27,28] 3) withdrawal from TE2 increases depressive symptoms within one week in perimenopausal women with histories of perimenopausal depression;[29] and 4) clinical experience that maximal efficacy of TE2 for treating perimenopausal depression is evident within two weeks (personal communication, David Rubinow M.D.). Thus, the beneficial mood effects of TE2 are rapid and detectable within 3 weeks. With the mean half-life of TE2 equal to 161 mins,[30] pharmacokinetic clearance is complete in 13 hours (five half-lives). We will employ a **three-week washout** window to minimize, if not eliminate, even lagged carryover effects[31]

3. Specific Aims

Alignment of the Specific Aims with Measures and Aim-Specific Statistical Analysis Plans

Specific Aim	Outcome Measures	Estimands	Statistical Estimators
(1) ***Determine baseline relationships:***			
a) E1G variability and irritability symptoms	-Every-other-day baseline E1G measurements -weekly irritability score	-Standard deviation of 14 E1G measurements -average of 4 weekly irritability (IDAS) scores	Model-based estimates, all with 95% C.I., regression coefficients
b) E1G variability and neural oscillations	-E1G standard deviation -frontal theta (dB) during threat, parietal beta (dB) during frustration	-E1G standard deviation -average event-related theta (dB) during Dot Probe Paradigm/ average event-related beta (dB) during Affective Posner Paradigm	Model-based estimates, all with 95% C.I., regression coefficients
c) irritability symptoms and neural oscillations	-weekly irritability score -frontal theta, parietal beta	-average of 4 weekly irritability scores -frontal theta (dot probe), parietal beta (Affective Posner Paradigm)	Model-based estimates, all with 95% C.I., regression coefficients

(2) Determine effect of TE2 administration on:			
a) neural oscillations	-Event-related frontal theta and parietal beta	- Frontal theta during dot probe/Parietal beta during Affective Posner Paradigm	Model-based estimates, all with 95% C.I.
b) irritability symptoms	-irritability symptoms	-IDAS irritability symptoms	Model based estimates

3.1. Aim 1

Define the baseline relationships between: (a) E1G variability and irritability symptom severity; (b) E1G variability and theta and beta oscillatory correlates of threat and frustration; (c) irritability symptoms and theta and beta oscillatory correlates of threat and frustration. The estimands of interest are the correlation coefficients between a) E1G variability (standard deviation (SD) of E1G over the four-week baseline phase) and irritability symptoms b) E1G variability (SD) and theta and beta oscillatory activation during threat and frustration tasks; and c) irritability symptoms and theta and beta oscillatory activation during threat and frustration tasks.

3.2. Aim 2

Determine whether TE2, versus placebo, will: a) beneficially modify task-evoked theta and beta oscillations (i.e., reduce theta and increase beta activation) in response to threat and frustration tasks; and b) reduce irritability symptom severity. The estimands of interest are a) the mean difference (and direction of difference) between the active TE2 and placebo TE2 conditions in theta and beta activation to threat and frustration tasks and b) the mean difference (and direction of difference) between the active TE2 and placebo TE2 conditions in mean irritability symptom severity.

4. Study Design

This single site, interventional study employs a within-subjects, cross-over, placebo-controlled, double-blind design.

Stage 1: A four-week baseline assessment involving: 1) at home urine collections of E1G; 2) mood ratings (general mood and irritability specific ratings); and 3) in-lab EEG session to assess theta and beta oscillations in response to behavioral tasks designed to elicit threat reactivity (Dot Probe task) and frustration to non-reward (Affective Posner Paradigm). Four weeks was chosen to allow for a sufficient number of E1G collections (n=14) to calculate a reliable standard deviation score for each participant.

Stage 2: Participants are randomized (1:1) to either three weeks of transdermal E2 (0.1 mg/day) or to three weeks of transdermal placebo as their first condition (Condition 1), followed by a three-week washout period and then exposed to Condition 2 (three weeks transdermal placebo if Condition 1 was active TE2 or three weeks of was active TE2 if Condition 1 was transdermal placebo). Daily irritability ratings and weekly irritability and general mood ratings are collected throughout all conditions in Stage 2. Daily urinary E1G is sampled in the last week of Condition 1 and Condition 2. Laboratory EEG sessions occur at the end of Condition 1 and Condition 2 to assess theta and beta oscillatory activity in response to behavioral tasks designed to elicit threat reactivity (Dot Probe task) and

frustration to non-reward (Affective Posner Paradigm).

We have chosen 3 week duration for each Condition based on: 1) Preliminary results of Dr. Schiller showing maximal effect of TE2 on irritability symptoms within one week; 2) placebo-controlled studies showing TE2 reduces perimenopausal depression symptoms with maximal effects seen in one to three weeks;[27,28] 3) withdrawal from TE2 increases depressive symptoms within one week in perimenopausal women with histories of perimenopausal depression;[29] and 4) clinical experience that maximal efficacy of TE2 for treating perimenopausal depression is evident within two weeks (personal communication, David Rubinow M.D.). Thus, the beneficial mood effects of TE2 are rapid and detectable within 3-weeks. With the mean half-life of TE2 equal to 161 mins,[30] pharmacokinetic clearance is complete in 13 hours (five half-lives). We will employ a three-week washout window to minimize, if not eliminate, even lagged carryover effects[31]

Stage 3: Participants will be given, in an unblinded fashion, 200 mg Prometrium for ten days to induce a menstrual bleed in order to prevent thickening of the endometrium in response to TE2. Participants will be followed for 3-4 weeks to document onset, duration, and magnitude of the menstrual bleed. A progestin every 2-3 months is sufficient for endometrial protection,[24] particularly in women in the early menopause transition who are still menstruating, albeit irregularly.

4.1. Treatment Design

We have selected transdermal estradiol because it avoids the first-pass metabolic effects of oral estrogen, and thereby creates more stable, premenopausal follicular phase E2 levels than oral and a physiologic profile of E2 relative to its metabolites.[17–19]TE2 also has a superior safety profile than oral estrogen.[20–22] We have selected the 0.1 mg/day dose because that is the dose that prior RCT research has shown to be effective in treating perimenopausal women with current depression[27] and the dose we have used in our previous RCTs involving TE2 in which we found it to be both safe as well as effective in reducing depressive symptoms and in preventing the emergence of depressive episodes in perimenopausal women, particularly in those with recent life stress.[7]

Additionally, we chose a 7-day system because a 7-day transdermal system provides more stable levels and better compliance than twice weekly patches.[23]The adhesive layer of the matrix in Climara® patches continuously releases E2, which is transported across skin leading to sustained E2 levels.[18]

4.2. Experimental Design

We have selected a within subjects, double-blind, cross-over design because of the greater statistical power over a between subjects RCT and to minimize bias.

Participants are randomized (1:1), without stratification, to order of TE2 versus transdermal placebo. Participants will receive either three weeks of transdermal E2 (0.1 mg/day) or three weeks of transdermal placebo as their first condition (Condition 1) and the other treatment (active E2 or placebo) as their second condition.

4.3. Measurement Design

Table 1. Variables of interest: their occasions of evaluation, their uses for the aims, their roles in the study

Variables within Domains	Scale[1]	Visits[2]	Aims	Main Roles
Identifiers				
Participant ID	nominal	all	all	identifier
Screening Profile				
systolic blood pressure (SBP)	mmHg	0, 5-7, 11-13	N/A	screening and safety
diastolic blood pressure (DBP)	mmHg	0, 5-7, 11–13	N/A	screening and safety

body mass index (BMI)	kg/cm^2	0, 7, 13	N/A	screening and safety
age	decimal yrs	0	N/A	screening
Life Events Survey	binary	0	N/A	screening
Psychiatric history	categorical	0	N/A	screening
Medical and cancer history	binary	0	N/A	screening
Reproductive Stage	binary	0	all	screening
Research Lab Assays				
Urinary E1G	pg/ml	1-4, 7, 13	Aim 1	predictor
EEG (theta and beta) oscillatory activation	dB	4, 7, 13	all	primary outcome
Patient-Reported Outcomes				
Daily self-reported irritability	continuous	1 – 13	all	primary outcome
Behavioral inhibition/Activation Scale (BIS/BAS)	continuous	4, 7, 13	Aim 2	secondary outcome
Frustration to non-Reward Scale	continuous	4, 7, 13	Aim 2	secondary outcome
Green Climacteric Scale	continuous	0, 4, 7, 13	all	covariate
Pittsburgh Sleep Quality Index	continuous	0, 4, 7,13	all	covariate
Safety Monitoring				
AEs and SAEs documentation	events	0, 4-13	N/A	safety monitoring

[1] Units of measurement or the scale.
[2] Study Visits: 0 = enrollment; 1-4 = baseline phase; 5-7 = Condition 1; 8-10 = washout phase; 11 - 13 = Condition 2; 14 = Prometrium follow-up

Screening Measures:

Blood pressure will be taken at baseline to confirm eligibility (< 160/100 mmHg) based on a minimum of three stethoscopic blood pressures taken by a trained research staff member. Participants will be seated comfortably for a minimum of five minutes prior to the first reading with arm supported on a table. Assessments will be at least one minute apart. A mean systolic and diastolic pressure (SBP and DBP) will be derived based on the three readings. Participants must have a resting SBP <160 and a resting DBP <100 mmHg to continue in the research. The same BP protocol will be used at study visit 5-7 and 11-13 to ensure the participant remains normotensive.

Body Mass Index will be derived based on height and weight measured by a research assistant in the laboratory using the Health-o-meter (Professional) scale. BMI will be calculated using the formula: weight in kg/height in m^2

Age will be determined by self-report.

Reproductive Stage will be initially determined by phone and confirmed in the enrollment session based on self-report of bleeding patterns.

Psychiatric History will be determined by in-person interview using the Mini International Neuropsychiatric Interview (MINI) screens. Research staff will be trained by Dr. Schiller to conduct the interview using the MINI training protocol that is used in Dr. Girdler's laboratory. All MINIS will be recorded, and Dr. Schiller will review each MINI with the research staff member who conducted the interview to determine a consensus diagnosis. The audio recordings will be reviewed when needed to arrive at the diagnosis.

The Life Events Survey[32,33]empirically modified to assess only those events that are moderate to severely stressful ("very stressful"),[34–36] will be used to confirm very stressful life events in the preceding 6 months. These include: divorce/separation, serious illness/death of close family member/friend, major financial problems, assault, life threat,

personal or close relative's arrest for a serious crime. We will require 1+ very stressful life event (60% of our prior perimenopause samples). Other stressful life events (e.g., new job, major move, disagreements) are also assessed. We will administer this scale at the enrollment visit to confirm the presence of 1+ recent very stressful life event.

Outcome Variables:

The following questionnaires will be administered using the REDCap platform and associated participant MyCap app.

We will administer the **Inventory of Depression and Anxiety Symptoms (IDAS)**[37] at enrollment. This 64-item survey assesses ill temper (i.e., irritability), dysphoria, panic, social anxiety, appetite, lassitude, suicidality, well-being, traumatic intrusions, and insomnia. The IDAS has excellent psychometric properties and is validated in reproductive mood disorders.[37] Symptoms items for each scale are rated 1 (not at all) to 5 (extremely). The total IDAS score may range from 64 – 320 reflecting general level of psychological distress. Clinical cut points with diagnostic predictability have been established for Major Depressive Disorder (score ≥32.5), Panic Disorder (score ≥37.5), Post Traumatic Stress Disorder (score ≥377.50), and Generalized Anxiety Disorder (score ≥ 35.5).

We will administer the **Center for Epidemiologic Studies Depression Scale (CES-D)** weekly throughout the study. The total CES-D score may range from 0–60 reflecting level of depressive symptoms. A clinical cutpoint for Major Depressive Disorder has been established (score ≥16). We will monitor the weekly CES-D submissions and use scores at these levels to warrant follow-up by Dr. Schiller or Dr. Girdler to assess psychological distress and determine if the participant is able to continue in the study.

The **5-item ill temper scale of the IDAS will be the primary measure of irritability** and will be used at enrollment to ensure at least moderate levels of irritability, and daily to examine associations with E1G variability and EEG measures. **We will require an ill temper score of >10** (range 5-25), consistent with Dr. Schiller's preliminary data showing perimenopausal depressed women with levels of 11 on this scale are responsive to TE2.

The **Behavioral Inhibition/Behavioral Activation Systems (BIS/BAS)**[38] (RDoC Matrix) is comprised of 20 items. Respondents answer with a 1 – 4 scale (the statement is 1 = very true for me vs. 4 = very false for me). Lower scores reflect more agreement with the statement. This survey has five subscales that assess:

1) Behavioral activation (BAS, impulsivity) – five item subscale with scores ranging from 5 – 20
2) Reward Responsiveness – five item subscale with scores ranging from 5 - 20
3) Fun Seeking – four item subscale with scores ranging from 4 - 16
4) Behavioral inhibition (BIS, anxiety) – seven item subscale with scores ranging from 7 - 28.

We also include the 5-item **Frustrative Non-reward Responsiveness Subscale**[39] (RDoC Matrix) to quantify approach motivation following FNR. This subscale is imbedded in the BIS/BAS instrument with lower scores reflecting greater frustration to non-reward. The scores range from 5 – 20.

We will administer these scales immediately before the two EEG tasks designed to activate threat/anxiety (the Dot Probe task) and frustration to non-reward (the Affective Posner Paradigm) at baseline Visit 4 and again at end of Condition 1 (Visit 7) and Condition 2 (Visit 13).

EEG Data Acquisition and Processing. A 32-channel actiCAP will be positioned according to the 10-20 system, with a Brain Products actiCHamp Plus amplifier. Preprocessing and analysis of EEG data will be performed using custom MATLAB scripts and the EEGLAB processing toolbox. EEG will be digitally re-referenced to the average reference, high pass filtered at 1.0 Hz, and epoched from -1 to 2 seconds to accommodate time-frequency analyses. EEG epochs containing amplitudes >100 μV at any scalp electrode with abnormally distributed data will be rejected. All cleaned, epoched data will be loaded into an EEGLAB STUDY mechanism to compute the time-frequency transform using the 'newtimef' 3-cycle wavelet function to yield Time X Frequency spectrograms. Theta (4-8Hz) and beta (13-30Hz) will be extracted from the pre-computed matrices at frontal (Fz) and parietal (Pz) midline electrodes.

E1G Assessments. We will use the same E1G assay and methods as Gordon et al,[1] who showed that variability in urinary E1G reliably identified perimenopausal women for whom *changes* in E1G predicted depression symptom severity. Week-to-week changes in E1G also differentiated women based on the direction of E1G change (i.e., increasing vs. decreasing levels) and symptom severity. The first morning voided urine will be used for measuring E1G, reflecting an integrated measure of overall E1G level from the previous day, and correlating very highly with serum E2 (r= 0.93).[40] At the end of each collection week, research staff will retrieve samples (stored in home freezer) and bring to our lab for storage at -80°C. Urinary E1G is not affected by the repeated freezing and thawing of specimens,[40] E1G will be assayed using an enzyme immunoassay (Arbor Assays, Ann Arbor, MI), with sensitivity at < 22.5 pg/mL. The specificity is high, with £ 0.1% cross-reactivity with similarly structured compounds, though cross-reactivity with E2 is higher at 5%. Since this research is focused on within person variation and we are using E1G as an indicator of E2 serum levels, any cross reactivity with E2 will not affect interpretability of our findings. The intra-assay variability = 4-6% and the inter-assay variability = 4-7%. Although there is little evidence to date that sensitivity to progesterone is a risk factor for affective impairment in the MT,[1] pregnanediol-3-glucuronide (PDG; a metabolite of progesterone) is assessed simultaneously with E1G and can be used as a covariate in analyses.

Control Variables:

The Pittsburgh Sleep Quality Index (PSQI)[41] is comprised of 19 self-rated items and five questions rated by the bed partner if one is available. Only self-rated questions are included in the scoring. The 19 self-rated items are combined to form seven "component" scores, each of which has a range of 0 – 3 points. In all cases, a score of 0 indicates "no difficulty", while a score of 3 indicates "severe difficulty" The seven component scores are then added to yield a global scores with a range of 0 – 21, 0 indicating 'no difficulty' and 21 indicating severe difficulties in all areas.

The components are: Subjective Sleep Quality, Sleep Latency, Sleep Duration, Habitual Sleep Efficiency, Sleep Disturbances, and Use of Sleep Medication.[41]

This survey will be measured at enrollment (Visit 0) and again at the end of Baseline (Visit 4) and each Condition (Visits 7 and 11) to be used as covariates in the model to confirm (or refute) that beneficial changes in outcomes due to TE2 cannot be explained by changes in perceived sleep quality.

The Greene Climacteric Scale[42] is a 21 item survey comprised of six subscales. Respondents indicate the degree to which they are bothered currently by symptoms on a 0 (not at all) to 3 (extremely) scale. The subscales are: measuring general psychological ii items), Anxiety (six items) , Depression (5 items, score ranging from 0 – 15), somatic symptoms (7 items, score ranging from 0 – 21), vasomotor symptoms (2 items, score ranging from 0 – 6), sexual interest (1 item, score ranging 0 – 3)[43–45]

This survey will be administered at enrollment (Visit 0) and again at the end of Baseline (Visit 4) and each Condition (Visits 7 and 11) to be used as covariates in the model to confirm (or refute) that beneficial changes in outcomes due to TE2 cannot be explained by changes in perceived sleep quality or menopausal symptoms (subscales somatic and vasomotor).

5. Study Participants

5.1. Numbers of Participants

Number to be screened: N £ 135

Number to be enrolled: N = 50

Up to 135 individuals will be screened. Based on our prior experience with perimenopausal women and intervention studies involving transdermal E2, we estimate that 40% will fail the medical screening for TE2 eligibility, and an additional 20% will not be interested in participating.

It will be necessary to enroll 50 perimenopausal women because our prior experience also suggests that 30% (n=15) will not meet eligibility for TE2 upon further screening, yielding about 35 eligible for the randomized, cross-over study. We conservatively account for 15% (n =5) withdrawal following randomization, yielding 30 women who complete the protocol

Since this is a cross-over study design, all enrolled women will receive both the active TE2 condition and the placebo TE2 condition.

Recruitment: Participants will be recruited through the Menopause Clinics at the UNC Hospitals, and through the UNC Center for Women's Mood Disorders. Additionally, participants will be respondents to advertisements in local newspapers, social media, and mass email listservs seeking volunteers for research. To increase minority applicants, physicians who are African American or of other minority ethnic groups will be included as referral resources, and certain ads will particularly request minority volunteers.

5.2. Eligibility Criteria

5.2.1. Inclusion Criteria

To be eligible to participate in this study, an individual must meet all of the following criteria:

- Biologically female and 45 – 59 years of age based on self-report
- STRAW+10 reproductive staging criteria[59] for being in the early or late menopause transition based on self-reported bleeding patterns – specifically menstrual periods of variable lengths and longer or shorter than premenopausal menstrual cycle lengths by seven or more days but no more than 12 months apart
- Negative screening mammogram within two years of enrollment based on screening mammogram results released to study personnel
- Body mass index between 18 – 45 kg/m2 measured by trained staff with professional scale in the laboratory during enrollment visit
- Moderate to severe irritability symptoms defined as a score of >10 on the ill temper scale of the Inventory of Depression and Anxiety Symptoms (IDAS)
- Emergence of, or increase in, irritability symptomatology concurrent with entering the menopause transition (defined by irregular bleeding patterns as defined above) based on self-report
- Experienced at least one very stressful life event (e.g., divorce, death of family member) within six months of enrollment based on self-report on the Life Experiences Scale.

5.2.2. Exclusion Criteria

Any individual who meets one or more of the following criteria will be excluded from participation:

- Use of psychotropic agents or hormonal preparations based on self-report.
- Use of herbal supplements (other than multivitamins) that are believed to affect mood or menopausal symptoms, such as St. John's Wort or black cohosh.
- Current suicidal intent or recent history of suicide attempts (within past 10 years) based on Mini International Neuropsychiatric Interview (MINI).
- A history of psychosis, bipolar disorder or substance dependence or active psychological symptoms severe enough to require treatment based on the MINI and a consensus diagnosis with co-Investigator, Dr. Schiller (licensed Clinical Psychologist)
- A personal history of thrombophlebitis or thromboembolic disorders based on self-report with the Personal and Family History questionnaire (PFHQ)

- A personal or family history of cancer indicative of more than average risk (i.e., risk seen in the female population at large) for breast, ovarian or endometrial cancers based on self-report with The Personal and Family Cancer History Questionnaire. Specifically:
    - A personal history of breast, ovarian or endometrial cancer
    - Known BRCA mutation in self or first or second degree relative
    - A first degree relative with premenopausal breast cancer
    - A first degree relative with ovarian cancer
    - A first degree relative with endometrial cancer
    - A history of an abnormal breast biopsy indicative of precancerous cells
- Personal history of migraine with aura based on self-report and defined as
    - Focal neurological symptoms – nerve-related functions or sensations, visual motor, or sensory
        - Changes in vision
            - 5-60 minutes before the pain begins
            - Vision blurred by flickering lights, spots, or lines
        - Unusual sensations
            - Pins and needles in one area of the body
            - Numbness in one area of the body
        - Other neurological
            - Difficulty talking
            - Sensitivity to light or sound

- According to JNC criteria, higher than stage 2 hypertension (systolic blood pressure ≥ 160 mmHg or diastolic blood pressure ≥ 100 mmHg) based on three stethoscopic blood pressures during quiet rest in the laboratory taken by trained personnel following the Girdler laboratory blood pressure measurement protocol. Women whose blood pressure is being managed with a stable blood pressure regiment and meet BP inclusion criteria (Stage 2 hypertension or lower blood pressure (140-159 or 90-99 mmHg)) will be included.
- Liver dysfunction or disease based on self-report with the PFHQ
- Undiagnosed abnormal genital bleeding based on self-report with the Side effects checklist (administered at baseline and at interim points in the protocol)
- A personal history of any cardiovascular disease including coronary artery disease, arteriosclerosis, heart attack, stroke based on self-report with the PFHQ
- Type I diabetes based on self-report with the PFHQ
- Known sensitivities to the matrix patch system in Climara® or allergy to peanut oil used in Prometrium® based on self-report with the PFHQ

5.3. Enrollment/Selection Strategies

5.3.1. Prospective Recruitment -or- Retrospective Selection

We have extensive experience recruiting women in the menopause transition into our clinical research studies involving transdermal estradiol interventions (three prior R01 projects). That experience tells us that Facebook is our most effective strategy for this demographic in terms of number of potentially eligible candidates (number who contact us). The UNC informational listserv is also highly effective as are flyers posted in the community (e.g., coffee shops, libraries, grocery stores). We will also advertise in periodicals or magazines that target this demographic such as *Carolina Woman*. Finally, we will notify our colleagues in the UNC Center for Women's Mood Disorders and the UNC Department of Obstetrics and Gynecology about our study and ask them to refer appropriate candidates.

5.3.2. Screen Failures

Of the estimated 50 women who pass our phone screening and come to an in-person enrollment, based on prior experience we predict that 30% (n=15) will not meet eligibility for TE2 after personal and family history review during enrollment or will not meet reproductive staging criteria based on further detailed questions about their menstrual bleeding patterns. For those women in the latter category, if it appears they are too early in the menopause transition (not yet meeting the variance in cycle length of +/- 7 days) we will obtain verbal consent to follow up with them and ask them to contact us if bleeding patterns change at which time, they will be re-screened. We also estimate that up to 15% (n =5) will decide to withdraw after enrollment. The primary reason, based on our past experience, is time commitment.

5.4. Strategies for Retention

We will rely on our successful retention strategies in our prior studies in perimenopausal women that randomized women to TE2 or placebo and followed them for up to 12 months successfully. The much shorter duration of involvement in this study (16 weeks) will naturally have a positive impact on retention. Other strategies that we have used and plan to employ include:

- Enrolling only fully informed participants using an infographic that summarizes events and timeline of the protocol and asking participants to restate to us risks and potential side effects of TE2 and progesterone during the enrollment session.
- An emphasis during staff training on the importance of effective communication strategies and establishing rapport with participants.
- Maintaining frequent communication with study visit reminders and 'check-ins'.
- Convenient free parking outside of Carolina Crossings B, Suite 1 where all study visits will occur.
- Appropriate, non-coercive monetary incentive for mid-life women to acknowledge their time. Each participant who completes the entire protocol will receive $650.
- Whenever possible, involving staff from underrepresented groups to help recruit and retain participants from underrepresented groups

5.5. Matching and Stratification

Not applicable.

5.6. Randomization and Concealment

Using a within-subjects, cross-over, placebo-controlled, double-blind design, participants will be randomized (1:1) to either three weeks of transdermal E2 (0.1 mg/day) or to three weeks of transdermal placebo as their first condition (Condition 1) and will have the other treatment (active TE2 or placebo) as Condition 2. Randomization to order of conditions will not be stratified.

Dr. Kai Xia, statistical collaborator on the project and Director of the Psychiatry Biostatistics Core, will develop the randomization table and the UNC Hospitals Investigational Drug Services will manage the randomization.

5.7. Blinding

The transdermal patches will be blinded by the UNC Hospitals Investigational Drug Services who will package active and placebo preparations into blinded, identical pouches. All study staff, investigators, and research participants will remain blind to subjects' order of active vs. placebo intervention conditions until final data collection is complete.

In the event of an adverse event that could be potentially related to the pharmacologic intervention, the gynecology collaborator, Dr. Maria Munoz will be unblinded to treatment to determine if the subject needs to be withdrawn. In that event, only Dr. Girdler will be informed who will, with Dr. Munoz, determine the course of action for the participant (e.g., follow-up with their doctor, ultrasound if suspected DVT, etc.). Dr. Girdler will not

be performing any assessments with research participants so her unblinding should not create bias in data collection, analyses, or interactions with other participants. Staff will not be informed as to the reason any particular subject is withdrawn.

6. Treatment Design: Procedures

Description

Using a within-subjects, cross-over, placebo-controlled, double-blind design, participants will be randomized (1:1) to either three weeks of transdermal E2 (0.1 mg/day) or to three weeks of transdermal placebo as their first condition (Condition 1). A three-week washout period will follow, and Condition 2 will follow the washout (placebo E2 if Condition 1 was active and active TE2 if Condition 1 was placebo). At the end of a subject's participation, each will be given Prometrium® (200 mg/day for 10 days).

Acquisition Investigational Drug Services will order the commercially available Climara® transdermal patches and the commercially available Prometrium® progesterone.

Storage Investigational drug services will store the Climara® and Prometrium® at a controlled room temperature of 77° stored away from heat, moisture, and direct light. Prometrium® will be dispensed in tight, light resistant containers. Climara® and the placebo patch will be dispensed in light resistant foil pouches. Participants will be instructed to store their study medications under the same temperature and light controlled conditions. Given the short duration of the intervention, there will not be a risk that the medications will expire.

Preparation and Administration By avoiding the first-pass metabolic effects of oral estrogen, ***transdermal E2 (TE2)*** creates more stable, premenopausal follicular phase E2 levels than oral; and a physiologic profile of E2 relative to its metabolites.[17–19] TE2 also has a superior safety profile than oral estrogen.[20–22] A 7-day transdermal system provides more stable levels and better compliance than twice weekly patches.[23]The adhesive layer of the matrix in Climara® patches continuously releases E2, which is transported across skin leading to sustained E2 levels.[18] Climara®, estradiol transdermal system, is designed to release estradiol continuously upon application to intact skin. The period of use is 7 days/patch. Progesterone: To prevent E2-induced endometrial hyperplasia, 200 mg micronized progesterone/day x 10 days will be taken at study completion. A progestin every 2-3 months is sufficient for endometrial protection,[24] particularly in women in the early MT who still menstruate. We will give progesterone in a discontinuous fashion instead of using Climara Pro® (delivers both E2 and a progestin) because progestins antagonize E2 effects.[25,26]

Once the IDS has prepared the blinded patches or the progesterone, for each participant, that participant's medications will be stored at a controlled room temperature of 77° F in a locked file cabinet in Dr. Girdler's laboratory away from heat and direct light until dispensed to the participant. If there is a delay in dispensing to a participant (due to illness or other need to reschedule), this will be documented in the participant's file and in our medication dispensing log, but we will continue to hold that participant's medication for up to four weeks since both TE2 and Prometrium are stable.

Accountability For each subject, a medication dispensing log will be kept in their study file. At weekly study visits, the research staff will record number of patches and pills dispensed and the research participant will sign the log indicating receipt of the next supply. Participants will return all used and unused patches at each weekly study visit. Number of returns will be recorded in the same medication dispensing log. Returns (used and unused patches) will be returned to Investigational Drug Services for destruction.

Rescue Procedures/Medications Not applicable. Adverse events resulting from transdermal E2 would warrant discontinuation of the medication as determined by the Dr. Munoz. Discontinuation of estradiol does not require a tapering schedule and there would be no 'rescue' medication.

Adherence Monitoring/Evaluations Subjects will: **1)** be educated on the importance of replacing patches every 7 days and we will develop an individualized behavioral strategy to enhance compliance; **2)** record date of patch

application on their calendars and receive text/call reminders; **3)** bring used and unused patches to each visit; and **4)** be given extra replacement patches. Patch counts (both used and unused) will be conducted at each weekly visit as the 'real time' assessment of compliance. Retrospectively, we can evaluate compliance by comparing urinary E1G levels under each of the two treatment conditions (transdermal E2 and placebo). If compliant, mean E1G levels should be significantly higher under the active TE2 condition.

Concomitant Therapies All psychotropic and hormonal therapies are prohibited as would insulin use. Stable thyroid hormone supplementation is allowed as is stable blood pressure medication as long as the medication is effective at maintaining blood pressure below 160/100 mmHg. Medication use will be assessed by self-report during screening and again at enrollment via the Personal and Family Health History Questionnaire.

7. Schedule of Activities and Procedures

7.1. Table of Events

Study Visit	Enrollment	Baseline				Condition 1			Wash-out			Condition 2			P4 daily administration
Visit Number:	0	wk1 1	Wk2 2	Wk3 3	Wk4 4	Wk1 5	Wk2 6	Wk3 7	Wk 1 8	Wk2 9	Wk3 10	Wk 1 11	Wk2 12	Wk3 13	14
Informed consent	X														
Medical & cancer Hx	X														
Side effects	X				X	X	X	X	X	X	X	X	X	X	X
Vitals and Weight	X														
MINI	X														
Stressful Events	X														
IDAS	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Daily Irritability		X	X	X	X	X	X	X	X	X	X	X	X	X	
Urinary E1G		X	X	X	X			X							
Tasks (EEG)															
Dot Probe					X			X						X	
Affective Posner Paradigm					X			X						X	
BIS-BAS and FNR					X			X						X	
Green Climacteric Scale	X				X			X						X	
PSQI	X				X			X						X	
P4															X

MINI – Mini International Neuropsychiatric Interview

Stressful Events – assessed via the Life Events Survey will assess moderate to severe events in the preceding six months

IDAS – Inventory of Depression and Anxiety Symptoms

Daily Irritability – assessed using the irritability subscale of the IDAS

Urinary E1G – assessed every other day over the baseline period and the last week of each condition

Dot Probe task – assesses threat

Affective Posner Paradigm – assesses frustration to non-reward

BIS-BAS and FNR – Inhibition/Behavioral Activation Scales

FNR – Frustrative non-reward Responsiveness Subscale

PSQI – Pittsburgh Sleep Quality Index

7.2. Screening

Participants will undergo an initial pre-screening within 30 days of enrollment (days -30 through day -1). We will obtain verbal consent over the phone for this initial pre-screening. A negative screening mammogram will be acquired during the 30-day prescreening window as part of pre-screening. Additional pre-screening by phone will include assessing medication use, medical history, bleeding pattern history, and irritability symptoms. If the participant meets all pre-screening criteria, an enrollment visit will be scheduled.

Consistent with clinical practice as per consultation with Dr. Munoz, study gynecologist, and current opinion[46] we will not require a gynecological screening exam for our participants for the following reasons: 1) each is medically healthy with no chronic medical conditions; 2) each will have a normal screening mammogram within the preceding 24 months; 3) each is of normotensive blood pressure status; 4) each has no more than base rate risk for ovarian or endometrial cancer; and 5) each is in the early menopause transition and therefore having semi-regular menstrual periods (within +/- 7 days difference in cycle length from premenopausal cycle length). If, however, a woman reports at screening that her menstrual bleeding lasts longer than seven days or her bleeding is profuse ('flooding' or soak through protection), Dr. Munoz will perform a screening endometrial ultrasound to confirm eligibility based on thickness of the endometrium. Those participants with any evidence of endometrial hyperplasia will be withdrawn and referred for gynecological care.

7.3. Enrollment

During the enrollment session (Visit 0) the following sequence of events will occur:

1. Obtaining informed written consent will involve a six-step process: 1) Participant reads consent form; 2) Research staff answer questions; 3) Research staff asks participant to summarize risks associated with the research; 4) Research Staff asks if participant wishes to speak the PIs; 5) participant signs consent form witnessed by research staff member; and 6) participant receives a copy of the signed consent form.
2. Participants complete the Personal and Family Medical History Questionnaire (PFHQ) which includes the Cancer History Questionnaire. These will be reviewed in real time. The side effects checklist will be administered that includes detailed questions on bleeding patterns, indicators of deep vein thrombosis, and mood in order to obtain baseline levels of these indicators. Weight and vitals will also be obtained. If ineligible, based on any of these assessments, the enrollment session will be concluded and the participant withdrawn.
3. Baseline questionnaires will be administered to assess menopausal symptoms (Green Climacteric Scale) and Sleep Quality (PSQI).

7.4. Study Visits

Visit 0: Enrollment session. During the enrollment session (Day 0) the following sequence of events will occur:

1. Obtain informed written consent using a six-step process: 1) Participant reads consent form; 2) Research staff answer questions; 3) Research staff asks participant to summarize risks associated with the research; 4) Research Staff asks if participant wishes to speak to the PIs; 5) participants signs

consent form witnessed by research staff member; and 6) participant receives a copy of the signed consent form.

2. Participants complete the Personal and Family Medical History Questionnaire and the Cancer History Questionnaire that will be reviewed in real time. If ineligible, based on medical history or concomitant medication use, the enrollment session will be concluded and the participant withdrawn.
3. The MINI interview is conducted by a trained staff member.
4. Baseline questionnaires will be administered to assess menopausal symptoms (Green Climacteric Scale) and Sleep Quality (PSQI).

Visits 1 - 4 (weeks 1 – 4 of the baseline phase).

1. Weekly assessment of mood using the CES-D questionnaire – participants will be prompted to complete this questionnaire at home using the HIPAA compliant REDCap software
2. Daily assessment of irritability symptoms using the irritability subscale of the IDAS questionnaire - participants will be prompted to complete this questionnaire at home using the HIPAA compliant Redcap software
3. Participants collect E1G using urine strips every other day during weeks 1 – 4 of baseline. Research staff visit the home to retrieve samples at the end of each week
4. Side effects assessed in person at week 4 of baseline during home visit to collect urine samples in order to provide an additional comparator of baseline, pre-intervention symptoms
5. Participants visit the laboratory at the end of baseline week 4 (+/- 2 days from day 28 of the baseline phase) and undergo EEG testing with Dot Probe and the Affective Posner Paradigm

Visits 5-7 (weeks 1 – 3 of Condition 1)

1. Side effects will be assessed at the end of each week in Condition 1 either via phone (weeks 1 and 2) or in person during the home visit to collect the urine strips (week 3).
2. Weekly assessment of mood using the IDAS questionnaire – participants will be prompted to complete this questionnaire at home using the HIPAA compliant Redcap software
3. Daily assessment of irritability symptoms using the irritability subscale of the IDAS questionnaire - participants will be prompted to complete this questionnaire at home using the HIPAA compliant Redcap software
4. At the end of week 3 of Condition 1, participants visit the lab in person at the end of baseline week 4 (+/- 2 days from day 21 of the Condition 1) and undergo EEG testing with Dot Probe and the Affective Posner Paradigm tasks

Visits 8-10 (weeks 1 – 3 of washout phase)

1. Side effects will be assessed via MyCap survey at the end of each week of the washout phase
2. Weekly assessment of mood using the CES-D questionnaire – participants will be prompted to complete this questionnaire at home using the HIPAA compliant Redcap software
3. Daily assessment of irritability symptoms using the irritability subscale of the IDAS questionnaire - participants will be prompted to complete this questionnaire at home using the HIPAA compliant Redcap software

Visits 11 - 13 (weeks 1 – 3 of Condition 2)

1. Side effects will be assessed by phone at the end of weeks 1-2 or in person at the end of week 3 during the home visit to collect urine strips.
2. Weekly assessment of mood using the CES-D questionnaire – participants will be prompted to complete this questionnaire at home using the HIPAA compliant Redcap software
3. Daily assessment of irritability symptoms using the irritability subscale of the IDAS questionnaire - participants will be prompted to complete this questionnaire at home using the HIPAA compliant Redcap software

4. At the end of week 3 of Condition 2, participants visit the lab in at the end of baseline week 4 (+/- 2 days from day 21 of the Condition 2) and undergo EEG testing with Dot Probe and the Affective Posner Paradigm tasks

Visit 14 (Progesterone Follow-up Phase)

1. Research staff will follow participants for up to 28 days or until bleeding ceases to document onset, duration and magnitude (degree of flow) of the progesterone-induced menstrual bleed

7.5. Final Visit

Visit 14 (Prometrium phase)

At the end of the lab session for Condition 2, participants will be given their 10 day Prometrium (P4) supply with instructions for storage and schedule for administration (once per day, prior to bed). Research staff will follow participants until menstrual bleeding ceases with weekly phone calls to assess onset, duration and magnitude of the menstrual bleed (degree of flow from light to profuse). It is anticipated that menstrual bleeding with onset and cease within a 28 day window though we will continue to follow until it ceases.

7.6. Phone Contacts

Phone calls will be used to collect initial screening and eligibility data (prior to study Day 0). Throughout the study (Visits 1 – 14) phone calls will be used to remind participants of the timing for replacing their patches, to schedule home visit times to collect urine and assess AEs and vitals, to schedule lab visits, and to monitor menstrual bleeding following P4-administration.

7.7. Follow-Up Contact

As described above, we will follow the participant until menstrual bleeding ceases following the progesterone administration. We will document the onset, duration, and magnitude of menstrual bleeding. Dr. Munoz (gynecologist collaborator) will be contacted to follow up with the participant should bleeding persist for longer than 14 days or should it be prolonged (defined as bleeding or spotting that lasts more than 14 days consecutively or profuse (defined as more than one tampon or pad per hour on any one day or more than 3 tampons or pads per day for more than 3 days).

7.8. Early Discontinuations

Data to be Collected

If a participant chooses to withdraw consent or discontinue with the study interventions no medical records data will be collected at any point, however the following procedures will employed:

1. We will ask participant the reason for their withdrawal and record that in in their file and in the study database
2. We will attempt to meet with the participant for one last visit to collect used/unused patches to determine compliance up to that point, assess and document vitals, and collect any E1G stored or unused urine strips.
3. If the participant wishes to withdraw from the intervention conditions, we will ask if they remain willing to complete the self-report surveys, particularly the irritability symptom scale on a daily basis, throughout the remainder of time that would correspond to their 14 study visits as irritability symptomatology is the primary outcome variable.
4. If the participant withdraws during or after at least one of the Conditions (TE2 or Placebo), the study gynecologist, Dr. Maria Munoz will be unblinded and determine if the participant needs the progesterone intervention for uterine protection. If that is determined to be the case, in order to

maintain the blind for research staff and the PI, Dr. Munoz will be the one to follow the participant and document menses onset, duration, and magnitude associated with the Prometrium.

Criteria for Intervention Discontinuation

An individual will be withdrawn from the study by the investigators under any of the following conditions:

1. Inability to tolerate the adhesive in the patches
2. If there is indication of psychological distress that impairs function and would warrant treatment. We will monitor the CES-D in real time, on a weekly basis. If the clinical cut point with diagnostic specificity for Major Depressive Disorder (score ≥ 16), Dr. Schiller will follow-up to assess psychological distress and determine if the participant is able to continue in the study. Dr. Schiller will determine the next course of action (i.e., participant may continue or will be withdrawn and referred to the UNC Center for Women's Mood Disorders)
3. Emergent suicide ideation with intent or suicide behaviors. We will monitor the suicide questions (#s 14, 15) on the CES-D in real time, on a weekly basis. #14: I thought about my own death; #15: I thought about hurting myself. Question #14: I wished I were dead; #15: I wanted to hurt myself. Although endorsing these questions in and of themselves does not indicate imminent risk for suicide behaviors, a response of 4 on a 1 – 5 scale on any of these items (0 = not at all or less than 1 day; 2 = 1-2 days; 3 = 3-4 days; 4 = 5-7 days; 5 = nearly every day for the past week) will trigger a review by Dr. Girdler, who holds a master's in counseling psychology, or Dr. Schiller. They will use their clinical judgement based on the number of other suicide risk items endorsed and the magnitude of thoughts or experiences (scores of 4 – 5) to determine if a follow-up assessment is warranted to ascertain more detail about these thoughts, including suicide intent and access to means. Someone with suicide ideation with intent or who has engaged in suicide behaviors will be withdrawn and referred for treatment.
4. Start of an exclusionary medication. At each study visit we will ask if the participant has started or stopped any medication or hormonal agent. The research staff will compare with the list of allowable and exclusionary medications.
5. Failure or inability to comply with study procedures as determined by the staff and ultimately by Drs. Girdler or Andersen.
6. Development of an exclusionary condition such as migraine with aura, deep vein thrombosis, diabetes.

7.9. Enrollees May Drop Out

Participants may voluntarily withdraw from participation at any time, for any reason, with no penalty or loss of rights. The reasons for drop-out and missing data will be documented in/with the database. If a participant wishes to withdraw from the intervention conditions, we will ask if they remain willing to complete the self-report surveys, particularly the irritability symptom scale on a daily basis, throughout the remainder of time that would correspond to their 14 study visits as irritability symptoms is the primary outcome variable.

8. Statistical Analysis Plans

8.1. Strategies that Apply to all the Aims

The statistical analysis plan was developed in collaboration with Dr. Kai Xia, biostatistician for the Department of Psychiatry. Dr. Andersen will perform the analyses for this protocol under the supervision of Dr. Xia. Participant flow will be tracked and reported using the CONSORT flow diagram and recommendations for cross-over study designs. The following baseline predictors will be calculated to model outcome variables: baseline irritability (sum of 5-items on the Ill Temper scale of the IDAS), E1G variability (standard deviation of 14 every-other-day E1G collections), and neurophysiological (EEG) correlates of threat and frustration (e.g., theta and beta oscillatory activity, event-related potentials (ERPs)) during the Dot Probe and Affective Posner Paradigm EEG tasks. Statistical estimates of population parameters will be estimated and tabulated using the statistical models

described in statistical plan of specific aims, along with corresponding confidence intervals and standard errors to indicate precision. All hypothesis tests yielding large p-values will be reported as being inconclusive. All the p-values will be reported with 3 decimal places to indicate the accuracy. The outcome of the proposed study will be discussed within the study team and outcome-dependent exploratory analysis will be designed and performed to generate new hypothesis if it is deemed necessary by the team. We acknowledge that no p-value can reveal the plausibility, presence, truth, or importance of an association or effect. All the response outcomes will be visualized using either boxplots by treatment or scatterplots between predictors and outcomes.

We will conduct a sensitivity analysis with and without missing data by assessing the following: 1) point estimates from complete cases, and 2) mean imputed values based on the observed data.

8.2. Sample Description

A combination of graphical representation (e.g., boxplot), and tabulated summary statistics (e.g., sample size, sample mean, standard deviation, and range of values) will be used to describe baseline sample characteristics such as demographic, baseline measures, etc. for each treatment sequence. For outcome responses, both the graphical visualization and summary statistics will also be generated according to the treatments they received.

8.3. Aim-Specific Plans

Plans for Aim 1. The objective of Aim 1 is to define baseline relationships between E1G variability, irritability symptom severity and neurophysiological correlates of threat and frustration (e.g., theta, beta, and associated ERPs (continuous)). Linear regression models will predict irritability symptoms and EEG variables from E1G variability (3) and predict irritability symptoms from EEG measures (2).

Plans for Aim 2. The objective of Aim 2 is to determine whether TE2, versus placebo, will beneficially modify EEG variables and reduce irritability symptom severity. Normality assumption will be accessed using studentized residual and extreme outliers will be assessed individually.

Aim 2a) The two repeated post-treatment EEG measures (theta, beta, ERPs) will be modeled as a function of fixed factors including baseline EEG measures (continuous), Condition status (TE2 or placebo; binary), and order of Condition (binary) in a linear mixed effects model with a random intercept accounting for the within-subject variance. The two-sided Wald test will test the significance of TE2 effects. **2b)** Similarly, weekly irritability symptoms (3 weeks/Condition) will be modeled as a function of fixed factors including baseline irritability symptoms (continuous), Condition, and order of Condition in a linear mixed effects model with a random intercept accounting for within-subject variance. Although we hypothesize that E1G *variability* will predict outcomes in Aim 1 and Condition effects in Aim 2, we will examine E1G levels if analyses do not support variability. In the statistical models described above, all the estimates of treatment effect, along with 95% confidence interval will be reported in the tabulated forms. Whenever multiple testing is used in the analysis described, Benjamini-Hochberg Procedure will be used to adjust for multiple comparison. All the hypothesis tests that yield large p-values will be reported as being inconclusive. To evaluate the robustness of the proposed study, we will perform sensitivity analysis for all the important findings. More specifically, we will repeat all the statistical analysis by excluding some potential outliers that could be influential points. We expect the sensitivity analysis will preserve the direction of original point estimates from full samples. Based on the study design, there will be potential missing data points caused by subject drop-out, and we assume all the missingness will most like to be missing at random (MAR). Since the linear mixed effects model is capable of handling missing values, the analysis plan will not be affected because of missing data.

8.4. Planned Interim Analyses

We will review safety data (adverse events) continuously, however, no formal interim analyses are planned.

9. Sample Size Rationale

We will enroll 50 perimenopausal women, 45 – 59 years of age, who have high probability for affective susceptibility to *changes* in E2 by recruiting women who: 1) report the emergence of irritability concurrent with entering the menopause transition; 2) are in the early menopause transition stage; and 3) have had 1+ recent severe stressful life event. However, as described earlier, we anticipate that 30 will meet all eligibility criteria and complete the study.

Based on large effects in our prior work of ovarian hormone changes on daily symptoms of depression, anxiety, and impulsivity (average *f*=0.69),[31] and other studies showing strong correlations between E2 levels and EEG theta and beta power in women (*f*=0.79; *d*=1.58),[47] we expect to find large effects of E1G variability in **Aim 1**. With *N* = 30, we will have > 95% power to detect large effects of E1G variability on irritability symptoms (*f*=0.69), and we maintain 85% power to detect medium effects (*f*=0.25). For **Aim 2**, with N = 30, we will have over 95% power to detect large effects of TE2 on irritability symptoms consistent with the magnitude of reduction seen in irritability symptoms in a study using TE2 to treat depression (*f*=0.55; *d*=1.11).[27] With N = 30, we will maintain 85% power to detect a medium effect of TE2 on irritability (*f* =0.25). While we considered powering to detect small effects, *detecting small effects would be unlikely to have the same degree of clinical relevance and unlikely to provide a strong scientific premise to pursue our R21 project results further.*

10. Data Capture and Database Management

10.2. Software for Data Capture

The study data will be entered into a REDCap database developed by the study personnel. REDCap is a 21 CFR Part 11-compliant data capture system provided by the NC TraCS Institute at UNC. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Study data will be entered directly from the source documents or synchronized from the MyCap participant app to the database.

The database structure consists of a table of participants enrolled in the study, a table of study visits, a table for each study assessment, and several tables to log database activity. Each field in the forms will be displayed as a text box or drop-down box. Missing data fields will be tagged, require a comment explaining the missing field, and will be listed as a query until resolved. The database will be supervised and managed by the PI (Andersen), and a data monitor will also participate in the verification process to assure data accuracy and integrity. The database will be stored on a secure, HIPAA compliant network on the Psychiatry server, and will be password protected. Different levels of privileges will be granted to different users.

10.3. Responsibilities for Data Capture and Database Management

Investigators (Girdler, Andersen) will be primarily involved in monitoring participant safety, adherence to the protocol and the adequacy and integrity of accumulating data. Drs. Girdler and Andersen will review study progress including enrollment and retention data at bi-weekly lab meetings, or more frequently if necessary. All

adverse events will be discussed and reviewed, and investigators will work with the medical monitor (Dr. Nathan) to determine if any study procedures should be altered or stopped in the event of an indication of harm to participants.

On-site quality assurance. The Study Coordinator will check all questionnaires for accuracy and completeness prior to the termination of each session if possible and prior to data entry. Principal Investigators (Girdler, Andersen) will monitor adherence to the protocol and verify informed consent has been obtained from all participants.

Protocol fidelity monitoring. The staff member obtaining the informed consent must log the date of consent, acknowledge that the signature has been witnessed, and acknowledge that the participant has received a copy of the informed consent document and follow the other steps of the consent process as described above. All these steps must be completed prior to entering any information about the participant other than the phone screen. Additionally, adherence to inclusion/exclusion criteria, completion of appropriate measures at each assessment point, appropriateness of withdrawal and termination procedures and maintenance of training and certification for all research personnel will be monitored by the research assistant and Principal Investigators (Girdler, Andersen).

Report generation. Monthly reports will be generated from the database and distributed to monitor study progress. Recruitment rates will be provided, along with demographic breakdowns of study participants. Frequency distributions and graphical displays of key variables will be provided on an ongoing basis to monitor data integrity. Reports will be provided to the PIs (Girdler, Andersen) by the research assistant every month that address recruitment and data quality and missing assessments. Reports will also include information about any adverse events and follow-up procedures with both the PIs and Dr. Schiller for psychological events (study licensed clinical psychologist) and Dr. Munoz for medical events.

10.4. Study Records Retention

This is an NIH-funded study. Therefore, we will retain all records for a minimum of three years from the date of the final Federal Financial Report (FFR) submission. No permission is required prior to destruction of records.

11. Collection and Management of Tissue Specimens

11.2. Use in Current and Future Studies

E1G collections every other day: Participants will collect a sample of their first-morning urine to test estrone-3-glucuronide -E1G (a urinary metabolite of estradiol), pregnanediol glucuronide -PDG (a metabolite of progesterone) and luteinizing hormone (LH) every-other-day during the baseline (4-weeks) and every day for 1 week at the end of each condition. E1G reflects an integrated measure of the overall hormone levels from the previous day and correlates highly with serum E2 (rs=0.93-0.97). Dried urine testing offers superior hormone stability, simple processing, and easy storage over other methods, including liquid urine, serum and saliva. Samples will be sent to ZRT laboratories (OR) and will be tested using enzyme immunoassay technique. This combination of technologies provides the most cost effective and yet still sensitive enough method for the perimenopausal population.

11.3. Sample Preparation

After the collection card is completely dried, participants will store the dried urine cards in their home freezer until the end of the study duration, at which point a research assistant will retrieve the samples and transfer them to a -80C freezer. Each card will be labeled with an in-house produced barcode identifying the study name, participant ID, Collection Timepoint.

Short Term Storage:

DRIED URINE SPOT:

Samples should be dried completely (overnight is ideal) before being stored in a sealed Ziploc type bag with desiccant to prevent exposure to moisture and stored in a freezer at - 20° C if not shipping within 7 days of collection.

Long Term Storage: At the completion of a participant's collection period, all samples should be gathered and stored as directed above in a -80° C, Ultra-Low Temp freezer. Dried urine cards can be stored indefinitely at -80 deg. C as long as there is a desiccant and the bag is sealed.

Shipping Preparation: All samples should be clearly labeled with the participant ID, collection time and date if relevant. All samples should be documented in a shipping log using Microsoft Excel. The shipping log may be used as the template for the results spreadsheet and should include collection information. This document should be emailed to Genevieve Neyland at gmneyland@zrtlab.com prior to shipment as notification that the package should be expected. ZRT Laboratory has templates available if needed. All samples should be organized in the order of the shipping log. If participants have multiple samples they should be bundled together and clearly labeled with the collection times & dates.

Packaging and Shipment: Governing bodies have specific requirements for the transport of biological specimens. The samples mentioned in this document are considered Biological Substance, Category B. (UN3373) They are non-hazardous, "Exempt Human Specimens" and are not subject to regulation as hazardous materials but must be triple packaged as follows:

1. The primary container (sample card).
2. The secondary packaging must be sift-proof (solids). Sealed Ziploc-type bags are acceptable.
3. The outer, third level of packaging must be a rigid container to protect the primary and secondary containers. The secondary container cannot serve as the outer shipping container. Cardboard covered, Styrofoam, shipping containers are ideal but any rigid shipping container is acceptable.

Ship to: ZRT Laboratory
ATTN: Darren Konidakis
8605 SW Creekside Pl. Beaverton, OR 97008
(503) 466-2445

11.4. Record Keeping and Monitoring

The barcode information will be scanned into a spreadsheet which correlates all information regarding the collection date/time, sample type, intended assays. No additional samples will be collected as undesignated archives. Once the dried urine card has been assayed, there is no more potential for additional data to be gained and the sample is discarded. Each step in the collection, storage and assay will be captured, updated, and maintained in the central electronic worksheet by the Study Coordinator.

11.5. Storage and Security

The Laboratory and accompanying freezer space are housed in a controlled access environment. Only personnel with authorized card access can enter the suite, and this information can be monitored by Campus Police in the event of a breach. The internal function of the ultra-low (–80 degree C) freezers are continually and remotely monitored by the Laboratory Manager through an app on multiple devices. A user defined matrix of parameters including Interruption in power, internet service, internal and ambient temperature are all continually pushed to the app via the internet from a collection appliance located in the suite. Since the service

uses the UNC network, UNC Information Technology department has layered its own cyber-security on top of the access control and data security build into the system by the service provider, Minus80 Monitoring, LLC. Sample chain of custody is maintained by noting shipment of samples to ZRT Laboratories on the master spreadsheet, which is only accessible by authorized personnel using dual verification of ID.

12. Safety Monitoring and Management

12.2. Risk / Benefit Assessment

Potential Risks: The most common side effects associated with transdermal estradiol (TE2) use include edema, breast tenderness, and changes in appetite and weight. Less frequent side effects include jaundice, nausea, abdominal cramps, increased blood pressure, headache and worsening of migraines or asthma, depression, nervousness, acne, cystitis-like syndrome, enlargement of uterine fibroids, intolerance to contact lenses.

The most common side effects associated with progesterone include breast tenderness, dizziness, fatigue, abdominal bloating, vaginal discharge, chest pain, and diarrhea. Less common side effects include headache, dizziness, breast pain, musculoskeletal pain, and viral infection.

More Rare yet Serious Risks:
The Women's Health Initiative (WHI) study results will be summarized here to provide a context for understanding risk and the current expert opinions about hormone administration to women in the menopausal transition. The WHI enrolled > 27,000 women aged 50-79 years old (mean=63 years). They were randomized to either oral Estrogen Replacement therapy (ERT, for those without a uterus), Hormone Replacement Therapy (HRT, conjugated equine estrogen + medroxy progesterone acetate (MPA)), or placebo. The HRT arm was terminated after 5 years due to an increased incidence of breast cancer; the results also suggested an increase in non-fatal MI and stroke.[20] Two years later, although there appeared to be reduced risk of cardiovascular disease (CVD) among 50–59 year old women who were on ERT,[48] the NIH also terminated the ERT arm based on overall risk/benefit ratios for all age groups. These findings appropriately ended the routine prescription of HRT for CVD prophylaxis, a practice formerly based on observational studies demonstrating a 50% reduction in CVD in women taking HRT.

Cardiovascular Risk. In the intervening years, two "white paper" reviews by the North American Menopause Society[49]and the Endocrine Society (representing the work of 30 leading experts and four levels of review);[50] as well as reports of subgroup analyses and one surrogate endpoint study from the WHI[20 5] all concluded that the effects of HRT on CVD are modified by the timing of its initiation, with beneficial or neutral effects on CVD in women who initiate therapy close to the menopause onset (during the fatty streak to uncomplicated plaque stage of atherosclerosis) and harmful effects in women who are older or initiate therapy with a long latency after onset of menopause (presumably in the stage of plaque necrosis and inflammation). These data are compatible with those from cynomolgus monkeys demonstrating that , E2's atheroprotective effects decrease with prolonged E2 deficiency; ERT or HRT reduces coronary atherosclerosis by 50-70% if initiated immediately after ovariectomy, while no benefit is observed if delayed for years.[25,26,51,52]

Breast Cancer. The increase in breast cancer observed in the WHI reflects the prior use of HRT in 25% of the study sample; the hazard ratio for breast cancer in the remaining 75% of the sample was 1.02.[53]Data from the Endocrine Society Scientific Statement report a worst-case scenario increased risk of breast cancer in a 50-54 year old woman with five years of unopposed estrogen from 13/1000 women (no ERT) to 14.94/1000 women. Thus, the risk of exposure to 3 weeks of E2, especially given our plans to screen out individuals at risk for heritable breast cancer, would be insubstantial.

Venous Thromboembolism (VTE). The risk of VTE is clearly increased with hormone use, with the risk influenced by age and hormone preparation. In the WHI, the risk of VTE was greater with combined estrogen + progestin (HRT) (HR 2.06) than with conjugated equine estrogen alone (HR 1.32), with both risk rates reduced in the 50-59 year olds (estimated excess events 9-10/1,000 for HRT and 3-4/1000 for ERT).[20,21] Notably, the risk

for VTE is significantly reduced by the use of transdermal E2 in case control studies (HR 0.9 compared with HR 4.2) in those taking oral estrogen.[54]

In sum, the Endocrine Society concludes that *"Data from the various Women's Health Initiative studies, which involved women of average age 63, cannot be appropriately applied to calculate risks and benefits of menopausal hormone therapy in women starting shortly after menopause."*[50]In fact, there is substantial evidence for the safety of E2, particularly if administered to women within 10 years of their final menstrual period and if administered as the transdermal form (e.g., lower risk of VTE).[50,54,55]**Given our plans to administer transdermal E2 to women: 1) for only 3 weeks, 2) no older than 55 years of age, 3) who are still menstruating, albeit with variable cycle lengths, and 4) who are at no greater than average risk for CVD, breast cancer or VTE, the risk of serious adverse events is exceedingly low.**

Psychological Risks: The structured interviews to assess current and lifetime psychiatric illness and suicide ideation and behavior and the Life Events survey may be associated with some psychological distress. Some items in the questionnaires may provoke some negative emotion in some individuals. There is also the risk a woman will experience the onset of a clinically significant affective episode, worsening of affective symptoms over the course of the 13-week protocol that would go undetected or untreated. There is the risk for emergent suicide ideation with intent or suicide attempts. However, importantly, there is no evidence that asking about suicidality increases suicidality—it actually tends to reduce it.[56]However, because we are excluding women with past suicide attempts (the strongest predictor of future attempts) and excluding those at enrollment with any current suicide ideation with intent, combined with the requirement that suicide ideation (without intent) at enrollment be of low frequency and duration, the chance that suicide intent or behavior will emerge over the course of the 13 week study is remote. One third of Americans endorse suicide ideation at some point in their lives,[57]yet less than 0.01% die by suicide each year. Because women in the MT are at increased risk for both ideation and attempts,[58]we will include women with current suicide ideation if it is of mild severity (no intent) and limited frequency and duration (≤ once/week; < 1 hour/episode) as it does not predict imminent risk for a suicide attempt (in the absence of a history of multiple attempts).[57,59]

Although measures are taken to protect the privacy of every participant, there is a remote risk of breach of confidentiality/loss of privacy.

Potential Benefits:

There are no direct benefit to participants, though they may benefit from knowing that they are contributing to research aimed at enhancing our understanding of the causes of clinically significant irritability symptoms in women in the menopause transition.

This research may benefit society by advancing our understanding and identifying therapeutic targets of intervention not only in reproductive mood disorders, but it will have implications for understanding irritability state change – a common feature of many psychopathologies.

12.3. Assessment of Safety

- Side effects and AEs will be assessed at enrollment (in order to obtain a baseline levels of symptoms that may represent AEs in response to TE2) and again at baseline week 4 and weekly throughout the remainder of the study
- Vitals (blood pressure and weight) will be assessed at enrollment.
- Suicidality will be assessed during the enrollment with the MINI and weekly throughout the protocol using the suicide questions of the CES-D.
- Mood symptoms are assessed at enrollment with the IDAS and weekly with the CES-D throughout the protocol.
- Bleeding patterns are assessed at enrollment and again at weekly throughout the remainder of the study. At enrollment, if a participant reports experiencing prolonged menstrual periods (> 7 days) or profuse bleeding ('flooding' or bleeding through protection), Dr. Munoz will perform an ultrasound of the endometrium to

determine if there is evidence of endometrial hyperplasia. In the event this is determined, the participant will be withdrawn from the study and referred for gynecological care.

12.4. Unanticipated Problems, Adverse Events, Serious Adverse Events

Unanticipated Problems: We will classify an event as an unanticipated problem if it meets all three OHRP criteria (1) unexpected (in severity, specificity, frequency, or nature), (2) related or possibly related to the research, and (3) suggests the research places participants or others at greater risk than previously known or recognized.

Adverse Event (AE) Definitions: We will model our definition of an AE after that of the FDA and define it as "any untoward medical or psychological occurrence that could be associated with the research procedures". We will only count 'events' that our relevant to our outcomes or interventions of interest.

These include:

Psychological events such as a significant deterioration of psychological well-being, or emergence of suicide intent or behaviors, or visible emotional distress in response to any study procedure.

Medical AEs are those that could possibly be related to the TE2 or Prometrium treatments:

- Edema
- Breast tenderness
- Changes in appetite and weight,
- Nausea
- Increased blood pressure to ≥140/90 mmhg
- Increase in headache frequency or development of a migraine with aura,
- Intolerance to contact lenses,
- Dizziness
- Sleepiness
- Calf pain
- Development of a DVT
- Chest pain
- Shortness of breath
- Development of breast lump
- Diagnosis of breast cancer or other estrogen dependent neoplasia
- Profuse menstrual bleeding
- Cardiovascular event (stroke, heart attack)

Serious Adverse Events (SAE) Definition: Participant death.

Grading the Severity of Adverse Events and Events of 'Special Interest':

While the UNC IRB will have the authority to determine the grading scale for AEs in the present study, we present here the grading scale that we have used in our previous research involving RCT designs employing transdermal estradiol. Adverse events will be classified according to severity as either mild, moderate, or severe.

Grading of Adverse Events (AEs)

Adverse events will be classified according to the Common Terminology Criteria for Adverse Events (CTCAE), developed by the National Cancer Institute Cancer Therapy Evaluation Program (NCI CTEP).

Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated. This could include breast tenderness, mild skin irritation; or something of equal significance that requires no medical intervention and is of marginal clinical relevance.

Grade 2 Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living. This could include a worsening of migraines or headache that require bed rest, an increase in depression symptoms consistent with clinically significant depression or anxiety symptoms on the IDAS (subscale score for depression ≥ 32; Panic Disorder ≥ 37), Post Traumatic Stress Disorder ≥ 37), or Generalized Anxiety Disorder ≥ 35), excess sleepiness or fatigue that resolves with additional rest, or something of equal significance.

Grade 3 Severe or medically significant but not immediately life-threatening.

Grade 4 Life-threatening consequences; urgent intervention indicated. Examples of such events would include a thromboembolic event, breast carcinoma, an increase in blood pressure consistent with Stage 2 hypertension, any cardiovascular event, any event that is permanently disabling, any event requiring hospitalization or is life threatening, severe mood impairment or failed suicide attempt or something of equal significance.

Grade 5 Death related to AE.

Serious AE: Death.

Relatedness Definition: An assessment of relatedness will be performed for each AE/SAE. We will employ four categories: 1) Definitely related to the study procedures; 2) Probably related to the study procedures; 3) Unknown relation to the study procedures and 4) Not related to the study procedures. The relatedness assigned will depend on the evidence that TE2 is associated with that particular event. Based on the literature, clinical evidence, and our own prior experience with TE2 in perimenopausal women we will employ the following relatedness criteria for the most commonly reported AEs with this population under these conditions. See Table on next page.

12.5. Table of Relatedness Criteria

Adverse Event Classification	Related to Study Drug?	Severity
Acne	**probably**	
Bloating	unknown	
Breast Tenderness	**probably**	
Heavy bleeding to progesterone	**probably**	
Prolonged bleeding to progesterone	**probably**	
Changes in Vision	unknown	
Chest pain	unknown	
Change in Mood symptoms (IDAS)	unknown	
Dizziness on Prog	**probably**	
Edema (swelling in feet/ankles)	**probably**	
Fatigue	unknown	
GI symptoms (nausea)	**probably**	
Headache – not migraine	unknown	
Hot flashes	unknown	
Joint pain	unknown	
Leg/calf discomfort, swelling, or pain	unknown	
Menstrual cramps to progesterone	**probably**	
Migraine Headache – no aura	unknown	
Migraine Headache with aura	unknown	
Skin irritation at patch site	**definite**	
Sleep problems	unknown	
Spotting	unkown	
Stage ≥1 Hypertension	unknown	
Suicidality	unknown	
Vaginal Dryness	unknown	
Vaginal Discharge	unknown	
Virus / infection	**not related**	
Weight gain	unknown	
Yeast infection	unknown	

Expectedness Definition: An assessment of expectedness will be performed for each AE/SAE. For those AEs that the literature and our experience indicate that the AE is probably or definitely related to the study procedures we will deem that AE 'expected'. Other AEs will be defined as 'unexpected.'

AE and SAE Assessment, Follow-up Procedures: AEs will be assessed at baseline and again weekly beginning at week four of baseline and throughout the protocol, including the progesterone follow-up month. They will be assessed via interview using our Side Effects and Adverse Events form. Each AE will be re-evaluated at the next weekly visit for whether it is continuing or it has resolved. Grade 3 or greater AEs will warrant a follow-up with Dr. Schiller if it involves mood impairment/suicidality. Calf pain consistent with a potential DVT (swollen, warm to the touch, pain with movement) will prompt an immediate referral to the UNC Doppler ultrasound clinic.

Reporting and Documentation Procedures: The study coordinator will monitor side effects and AEs at all study visits. We will follow our AE Chain of Command Reporting Protocol (uploaded). Side effects consistent with Grade 3 (moderate) or greater will be immediately reported to Drs. Girdler and Andersen. Staff will be specifically trained to monitor the IDAS for the clinical cut-points for depression, PTSD, and Anxiety as well as the suicide subscale. In the case of Grade 3 or greater mood impairment of suicide ideology with intent, Dr. Schiller will determine the next course of action, including more frequent assessment of mood if deemed to be clinically required. Dr. Munoz will be the responsible physician to

determine the next course of action involving medical side effects (e.g., continue in protocol, exclude and refer for treatment).

The PIs Drs. Girdler and Andersen will review all protocol data at monthly meetings, including enrollment and retention statistics and aggregate reports of side effects/AEs. Dr. Girdler will be the responsible one to report any individual occurrence of a Grade 3 or greater AE to the IRB within one week and to the medical monitor, Dr. Nathan, in real time on an ongoing basis. Since we are employing a marketed pharmaceutical product (i.e., a non-IND study), unexpected Severe AEs (Grade 4) will be also be reported to the FDA Medwatch Program. The NIMH program officer will be notified of any study modifications or suspension imposed by the local IRB or recommended by the medical monitor, Dr. Nathan, in response to an AE. Dr.Munoz will receive the randomization code by subject ID from Investigational Drug Services. In the event that it is medically necessary to become unblinded to treatment assignment, Dr. Munoz will be able to do so very quickly.

Participant Notification of New Information: Should any new safety information arise regarding either TE2 or Prometrium, this information will be communicated to current and former participants. Current participants will be re-consented to continue in the study.

12.6. Safety Monitoring

Dr. Margo Nathan, a reproductive psychiatrist, will serve as the independent medical monitor for this project. Dr. Nathan will review any AEs of moderate grade or higher in real time (within 5-7 days). She will evaluate AEs in real time and on an ongoing basis. Dr. Nathan will raise any concerns or issues with the PIs, and make any recommendations regarding safety to the PIs, while protecting confidentiality of the trial data and the results of monitoring. The PIs will be responsible for reporting Dr. Nathan's reviews to the IRB, although Dr. Nathan has the right to report any concerns directly to the IRB herself, including any recommendation that the study be stopped. This will ensure the independent oversight of issues related to safety and adverse events.

Dr. Nathan will specify the tables and data she wishes to evaluate, including but not limited to, all side effects and symptoms recorded at the study visits and all interim phone calls initiated by research subjects to report AEs or concerns. Dr. Nathan will evaluate issues of participant safety as well as enrollment information and the adequacy and integrity of accumulating data. She will also identify if any study procedures should be altered or stopped in the event of an indication of harm to participants attributable to the study interventions.

12.7. Study Suspension / Early Termination of the Study

After consultation with the study statistician, Dr. Kai Xia, it has been determined that the sample size will not allow for interim analyses that would reliably inform either study suspension or early termination.

13. Regulatory, Ethical, and Study Oversight Specifications

13.2. Informed Consent Process

Consent/Assent and Documents Provided to Participants

Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention. The consent form is submitted with this protocol in Appendix 1.

Consent Procedures and Documentation

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Consent forms will be Institutional Review Board (IRB)-approved and the participant will be asked to read and review the document. The PIs or study coordinator will explain the research study to the participant and answer any questions that may arise. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. The participants will have the opportunity to discuss the study with their family or surrogates or medical providers and think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. Participants will be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice.

Obtaining informed written consent involves a six-step process: 1) Participant reads consent form; 2) Research staff answers questions; 3) Research staff asks participant to summarize risks associated with the research; 4) Research Staff asks if participant wishes to speak with the PIs; 5) participant signs consent form witnessed by research staff member; and 6) participant receives a copy of the signed consent form.

The informed consent process will be conducted and documented in the source document (submitted with this application), and the form signed, before the participant undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

13.3. Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, the IRB, and the NIMH (funder).

If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Examples of circumstances that may warrant termination or suspension of the study include:

- Detection of an unexpected unacceptable level of risk to participants
- Unexpected inability to recruit participants
- A recommendation by the Medical Monitor, Dr. Nathan, to the PIs and/or IRB that the study should be discontinued due to safety concerns resulting from an unexpected number of AEs of Grade 3 or greater.

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the regulatory oversight (e.g., DSMB, sponsor, IRB, FDA.]

13.4. Confidentiality and Privacy

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the

study, or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities and interviews will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), regulatory agencies or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the UNC Department of Psychiatry password protected drives (for electronic data) and Carolina Crossings B for paper forms maintained in secured, locked file cabinets. These data will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The linkage file will be maintained on a password protected drive to which only the PIs and the research staff working on this project will have access.

The study data entry and study management systems used by Dr. Girdler and Dr. Andersen's research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the Department of Psychiatry password protected drives.

13.3.1. Certificate of Confidentiality

To further protect the privacy of study participants, a Certificate of Confidentiality will be issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

13.5. Future Use of Stored Specimens and Data

Data collected for this study will be analyzed and stored at the Carolina Crossing B laboratory of Drs. Girdler and Andersen. After the study is completed, the de-identified, archived data will be transmitted to and stored at the NIMH Data Archive (NDA) for use by other researchers including those outside of the study.

Permission to transmit data to the NIMH NDA is included in the informed consent. When the study is completed, access to study data will be provided through the NIMH NDA.

Biological samples will not be stored for future use.

13.6. Key Roles and Study Governance

Principal Investigator
Susan Girdler, Ph.D., Professor
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Medical Monitor
Margo Nathan, MD, Assistant Professor
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13.7. Safety Oversight

Safety oversight will be under the direction of an Independent Medical Monitor. Dr. Nathan, a reproductive psychiatrist, will serve as the independent medical monitor for this project. Dr. Nathan will review any AEs of moderate grade or higher in real time (within 5-7 days). She will evaluate AEs in real time and on an ongoing basis. Dr. Nathan will raise any concerns or issues with the PIs, and make any recommendations regarding safety to the PIs, while protecting confidentiality of the trial data and the results of monitoring. The PIs will be responsible for reporting Dr. Nathan's reviews to the IRB, although Dr. Nathan has the right to report any concerns directly to the IRB herself, including any recommendation that the study be stopped. This will ensure the independent oversight of issues related to safety and adverse events.

Dr. Nathan will specify the tables and data she wishes to evaluate, including but not limited to, all side effects and symptoms recorded at the study visits and all interim phone calls initiated by research subjects to report AEs or concerns. Dr. Nathan will evaluate issues of participant safety as well as enrollment information and the adequacy and integrity of accumulating data. She will also identify if any study procedures should be altered or stopped in the event of an indication of harm to participants attributable to the study interventions.

13.8. Clinical Monitoring Plan (CMP)

Not applicable

13.9. Quality Assurance and Quality Control

Not applicable

13.10. Protocol Deviations

A protocol deviation will be defined as any noncompliance with the protocol on the part of the participant, the investigators, or the study site staff. As a result of deviations, corrective actions will be developed by the PIs and implemented promptly.

Continuous vigilance on the part of the investigators and research staff will be used to identify and report deviations within ten working days of identification of the protocol deviation, or within thirty working days of the scheduled protocol-required activity. All deviations will be recorded in study source documents, (see Protocol Deviation Tracking Log) and reported to National Institute of Mental Health Program Official. The Program Official will communicate to the PIs what deviations she is interested in being reported to her. Protocol deviations will be sent to the reviewing Institutional Review Board (IRB) per their policies. The PIs are responsible for knowing and adhering to the reviewing IRB requirements.

13.11. Publication and Data Sharing Policy

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this study may be requested from other researchers after publication or project end date, whichever comes first by contacting the NIMH Data Archive (NDA).

The Principal Investigators will serve as first or senior author on all publications, with co-authors included according to journal guidelines.

13.12. Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the National Institute of Mental Health has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

14. Additional Considerations

Not applicable.

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16. Appendices

Appendix 1: Version 1.0 Adult Consent Form, Version Date 1/13/2022.

Appendix 2: Version 1.0 of Documentation of Informed Consent Process, Version Date 1/18/2022.

Appendix 3: Version 1.0 of Unanticipated/Serious Adverse Event Log, modified from UNC Office of Clinical Trials, Version Date 2/27/2019.

Appendix 4: Version 1.0 of Adverse Event Log, modified from UNC Office of Clinical Trials, Version Date 3/13/2019.

Appendix 5: Version 1.0 of Adverse Chain of Command for Reporting, Version Date 1/18/2022. Describes protocol for reporting adverse events of differing type and severity.

University of North Carolina at Chapel Hill
Consent to Participate in a Research Study
Adult Participants
Consent Form Version Date: 03/20/2023
IRB Study # 21-3395
Title of Study: Identifying neurophysiological mechanisms of susceptibility to estradiol fluctuation and irritability symptoms in the menopause transition: An experimental approach
Principal Investigators: Susan Girdler & Elizabeth Andersen
Principal Investigator Department: Psychiatry - Research
Principal Investigator Phone number: (919) 445-6808
Principal Investigator Email Addresses: Susan_Girdler@med.unc.edu, Elizabeth_Andersen@med.unc.edu
Funding Source and/or Sponsor: NIH National Institute of Mental Health (NIMH)

Concise Summary

Women in the menopause transition (perimenopause) have a significantly greater risk of developing depression. For most women with depressive symptoms during the menopause transition, irritability not "depression" is their primary source of impairment and distress. While most women are exposed to erratic hormone changes in the menopause transition, about 40% are susceptible to the emergence of mood symptoms tied to changes in estrogen. Estrogen modulates brain systems associated with depression; has anti-depressant/anti-anxiety effects ENREF_48 ENREF_48 ENREF_48; and regulates brain networks involved in depression. Thus, the purpose of this study is to understand the biological mechanisms underlying the relationship between estrogen and the emergence of irritability symptoms during the menopause transition. Study duration will last 16 weeks. Participation will include an enrollment session with questionnaires on your current mood and stress, frequent hormone measurements (urine) and mood assessments at your home, and three weeks of wearing both an estrogen patch (active) and placebo (no active drug) patch in a randomized order. You will also complete 3 laboratory sessions that involve recording your brain activity while you perform several computer tasks. There are no direct benefits to you for participating in this research study. Serious risks, such as blood clots, are minimized by using transdermal (skin) estrogen patches and limited duration of exposure.

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?

Women in the menopause transition (meaning women who have irregular or skipped menstrual periods but have not gone a full year without a menstrual period) are more likely to suffer from depression and anxiety not only compared with men but also compared with women who still have regular menstrual periods (premenopausal women) and women who have not had a menstrual period in more than one year

(postmenopausal women). The menopause transition is a time of extreme changes in sex hormone levels. One of the main female sex hormones is estrogen. Estrogen levels can change greatly from one day to the next during the menopause transition. In other research, changes in sex hormone levels have been shown to contribute to negative mood in women with severe premenstrual syndrome (PMS) and in women with postpartum depression. Estrogen has also been shown to influence brain areas that are important in the emergence of mood symptoms, including irritability. Although the causes of depression during the menopause transition are unknown, severe life stress close in time to the menopause transition is often associated with the onset of depression.

The purpose of this research study is to determine if changes in estrogen (specifically a urinary metabolite of estrogen, E1G) during the menopause transition are related to irritability symptoms and brain correlates of irritability, measured with electrodes on the scalp in response to computer tasks, the responses to which are correlated with anxiety and depression. We will also test whether administering estrogen for three weeks via a skin patch, will decrease irritability symptoms and beneficially modify brain circuitry associated with irritability symptoms.

What is known about hormone therapy, depression and health:

There are two general types of hormone therapy. Hormone therapy can involve either the use of estrogen alone (ERT; estrogen replacement therapy) or combined with another hormone called progesterone (HRT; estrogen plus a progesterone). For women who have not had a hysterectomy (still have a uterus), progesterone must be taken to prevent an overgrowth of cells in the uterine lining. There are several ways in which estrogen can be taken. It can be taken by mouth in the form of a pill, through the skin (in the form of a patch or a gel; this is called transdermal) or through a device placed in the uterus or vaginal ring. This study will use transdermal ERT (estrogen alone given through the skin) and oral progesterone given by mouth for 10 days at the end of your participation.

There have been a number of studies examining whether hormone therapy is effective in treating depression in women in the menopause transition or in postmenopausal women. While not all studies have found that ERT or HRT is effective in treating depression, the majority of studies do indicate that ERT or HRT is linked to a large reduction in depressive symptoms in women in the menopause transition and in postmenopausal women.

There have also been studies in postmenopausal women suggesting that in women who used ERT or HRT during and/or after the menopause transition, their risk of heart disease was cut in half. However, in the Women's Health Initiative (WHI) study, that enrolled more than 27,000 women, the HRT part of the study was stopped after 5 years due to an increase in rates of breast cancer; the results also suggested an increase in non-fatal heart attack and stroke in women using the HRT.

Since then, reviews by the North American Menopause Society as well as other analyses, all conclude that the timing of HRT use has an impact on the benefits and risks associated with HRT. HRT or ERT appears to be beneficial or have no effects one way or another on heart health in women who start hormone therapy younger and closer to the menopause onset, and it appears to be harmful in women who are older or start hormone therapy a long time after onset of menopause. For example, later reports from the same Women's Health Initiative study have shown that women who were in their 50s and received ERT (estrogen alone) had a reduction in their chances of heart attack and stroke compared with women on placebo (an inert substance like a sugar pill).

In addition to timing, the preparation of hormones (i.e., whether estrogen is given alone or with progesterone and/or whether the hormones are taken by mouth or through the skin) is important in terms of both breast cancer and heart disease risk. For example, the reduced chance for heart attack and stroke seen in the younger aged women in the Women's Health Initiative was seen only in the women taking ERT (estrogen alone), not in those taking HRT (estrogen with a continuous progesterone). For breast cancer, prior studies suggest that ERT is associated with four times less of a chance of developing breast cancer than HRT. Also, the Women's Health Initiative results showed that only women who had a history of prior use of HRT (reflecting greater lifetime exposure to HRT) had an increased chance of developing breast cancer while

there was no increase in chance of breast cancer in the women who had not used HRT prior to entering the study. Studies also indicate that duration of ERT or HRT use is an important factor in the chance for breast cancer.

In trying to estimate the chances of breast cancer associated with ERT, consider that in women 50-54 years of age not taking ERT or HRT, 13 out of every 1000 women will get breast cancer each year. In 50 – 54-year-old women who take ERT for 5 years or longer, 15 women out of every 1000 women will get breast cancer each year.

Other evidence that the preparation of hormones is important comes from evidence that use of transdermal estradiol (estradiol – the primary estrogen naturally produced in women - delivered through the skin), as we will do in this study, is associated with greater beneficial effects for a number of heart disease indicators than is the form of oral estrogen used in the Women's Health initiative (which was a modified horse estrogen). Transdermal estradiol is also more effective in treating depression than oral estrogen.

In summary, the Women's Health Initiative was not designed to examine the use of ERT in healthy women in the menopause transition – the group of women for whom there is a medically valid reason to use hormone replacement. For example, the Food and Drug Administration (FDA) has approved the use of estrogen to both treat menopausal symptoms (e.g., hot flushes, night sweats) and to prevent osteoporosis (bone loss) in women in the menopause transition.

The drugs used in this study (estrogen patch, progesterone) are approved by the FDA (as discussed previously) and are being used within that approval for this study.

You are being asked to be in the study because you are a healthy woman, 45 – 55 years of age, who is in the menopause transition defined by irregular periods.

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

You should not be in this study if you:

- have a history of heart disease, including coronary artery disease, arteriosclerosis, heart attack or stroke
- have high blood pressure that is not managed by medication (the researchers will measure your blood pressure)
- have a history of any estrogen-related cancer such as breast, ovarian, or uterine cancer or have a strong family history of estrogen-related cancer (the researchers will assess your personal and family history of cancer)
- have a history of thrombophlebitis or thromboembolic disorders (e.g., blood clots)
- are allergic to peanuts
- smoke more than 10 cigarettes per day
- have ever been diagnosed with bipolar disorder or a psychotic disorder
- are currently abusing or dependent on alcohol or drugs
- are currently taking antidepressant, antianxiety or other psychiatric medication
- have Type I diabetes
- use herbal supplements that are believed to affect mood or menopausal symptoms, such as St. John's Wort or black cohosh. If you use other herbal supplements, you should discuss them with the researchers who will determine if they are allowed
- have experienced migraine headaches with aura (aura means that you have a perceptual disturbance associated with the headache such as visual changes, a strange light, an unpleasant smell or confusing thoughts or experiences)
- have a Body Mass Index (BMI) of > 45 (this will be determined by the researchers)
- are pregnant or nursing

How many people will take part in this study?

Approximately 50 people at this institution will take part in this study.

How long will your part in this study last?

Your total participation will extend over approximately 16 weeks. There will be 1 in-person enrollment session, 3 in-person laboratory visits at a UNC site and will last approximately 1 hour, and the remainder of the study will take place remotely.

What will happen if you take part in the study?

1. Medical and Psychiatric Screening: The first phase of this study involves a phone screening for medical conditions, psychiatric history, and life events.

- You will then come into the lab to complete questionnaires or interviews about your medical history, your psychiatric history, your menstrual bleeding pattern, your mood, recent life events, your menopausal symptoms, and your sleep. You do not have to answer any questions that you do not want to.
- You will take a pregnancy test (urine test) to confirm that you are not pregnant before enrolling in the study.
- You will complete a psychiatric interview, during which you will be asked questions about your current and past mood symptoms. You do not have to answer any questions you do not want to. The interview may be audio recorded both for training purposes and for review to ensure a more accurate diagnosis of symptoms. We may refer to the audio recording to make diagnostic decisions after consulting with the study's clinician. Recordings will be stored with de-identified data on a HIPAA-compliant medical school drive for up to five (5) years after publication, at which point they will be deleted. If you are ineligible for the study, the recording will be deleted immediately. The audio recording is not required for participation. You may request that the audio recording be turned off.

  Please initial on the line that best matches your choice:

  _____ I AGREE to have my interview recorded

  _____ I DO NOT AGREE to have my interview recorded

2. Baseline hormone, mood and neurophysiological testing (4 weeks):

- Daily and weekly mood assessments:
  - Daily mood ratings using your personal cell phone: 10 questions on current mood and stress (~2 minutes to complete).
  - Weekly mood ratings on depression, irritability, and stress: 5-7 minutes to complete.
- Hormone fluctuations: Every-other-day urine collections (14 total): dried on collection card and placed in home freezer.
- Neurophysiological (EEG) testing session ~1 hour:
  - Electroencephalogram (EEG) recordings during:
    - rest (eyes open and fixated on cross, and eyes closed) – 5 minutes
    - emotional face task – 5 minutes
    - Two different computer tasks that are designed to assess behavioral indices of irritability. In one, you will look at pictures of faces and symbols and be asked to press a key in response to the symbols. This task lasts approximately 5 minutes. In the second task, you will be given a monetary award (up to $10 per laboratory session) depending on your performance.

- At the end of the baseline phase at the laboratory session, you will have the first patch placed. Each patch is good for one week. A study team member will visit you at your home to change your patch once per week and collect all used patches.
- You will be asked to wear your patch every day throughout both Condition 1 and Condition 2 (descried below). In one of the Conditions your patch will contain active estrogen (specifically 0.1 mg/day of estradiol) and in the other Condition your patch will be a placebo patch (containing an inert, non-active substance). Which Condition contains active estradiol versus placebo estradiol will be determined randomly (like flipping a coin). In other words, you have a 50-50 chance of Condition 1 being active estradiol or placebo and the same for Condition 2.

3. Condition 1 (3 weeks):

- Side effects will be assessed at the end of each week in Condition 1 during the home visits to collect urine strips.
- Daily and weekly mood assessments:
    a. Daily mood ratings using your personal cell phone: 10 items (~2 minutes to complete) include questions on current mood and stress.
    b. Weekly mood ratings on depression, irritability, and stress: 5-7 minutes to complete.
    c. Daily urine collection during the last seven days of each Condition
- At the end of week 3 of Condition 1, you will return to the lab to complete the neurophysiological testing session (as described above)

4. Washout phase (3 weeks):

- During this phase you will not wear any patches.
- Side effects will be assessed by phone at the end of each week of the washout phase
- Daily and weekly mood assessments:
    - Daily mood ratings using your personal cell phone: 10 items (~2 minutes to complete) include questions on current mood and stress.
    - Weekly mood ratings on depression, irritability, and stress: 5-7 minutes to complete.

5. Condition 2 (3 weeks):

- You will be given a new patch which will be changed out weekly by a study team member (as described above).
- Side effects will be assessed at the end of each week in Condition 2 during the home visits to collect urine strips.
- Daily and weekly mood assessments:
    a. Daily mood ratings using your personal cell phone: 10 items (~2 minutes to complete) include questions on current mood and stress.
    b. Weekly mood ratings on depression, irritability, and stress: 5-7 minutes to complete.
    c. Daily urine collection during the last week of the Condition
- At the end of week 3 of Condition 1, you will return to the lab to complete the neurophysiological testing session (as described above)

6. End of study and follow-up (3 weeks):

You will take two capsules per day for the last 10 days of the study, each containing 100 mg of micronized progesterone (total dose = 200 mg/day for 10 days). This is necessary to protect your endometrium (uterine

lining) from getting too thick due to estradiol. The progesterone will induce menstrual bleeding, which it is designed to do, so that your uterine lining can shed. The number of days that of bleeding and the amount of bleeding varies between women. Because you are still having menstrual periods, it is anticipated that the amount of bleeding will not be more than your usual premenopausal bleeding, but this cannot be guaranteed.

You will complete weekly mood assessments, and side effects and weight will continue to be monitored.

What are the possible benefits from being in this study?

Research is designed to benefit society by gaining new knowledge. While the transdermal estradiol may have a positive benefit on mood symptoms, the possibility also exists that 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 **most common risks** involve side effects associated with the use of reproductive hormones (estradiol or progesterone).

The most frequent side effects associated with estradiol use include:

- breast tenderness (occurs in 29% of patients)
- abdominal cramps (occurs in 16% of patients)
- headache (occurs in 13% of patients)
- edema (swelling) (occurs in 10% of patients)
- nausea (occurs in 6% of patients)
- depression, nervousness (occurs in 11% of patients)
- acne (occurs in 3 – 12% of patients)
- skin rash or irritation may also occur at site where the patch is placed (occurs in 3 - 12% of patients)

Less frequent side effects include:

- jaundice (yellowing of skin)
- increased blood pressure
- worsening of migraines or asthma
- enlargement of uterine fibroids
- intolerance to contact lenses
- dizziness
- changes in appetite and weight

The most common side effects associated with progesterone include:

- breast tenderness (occurs in 16% of patients)
- dizziness (occurs in 24% of patients)
- abdominal cramping (occurs in 20% of patients)
- headache (occurs in 16% of patients)
- viral infection (occurs in 12% of patients)
- joint pain (occurs in 12% of patients)
- diarrhea (occurs in 8% of patients)

- menstrual bleeding, sometimes consistent with a heavy menstrual period (occurs in 20-30% of patients)
- drowsiness (occurs in 9% of patients)

Less common side effects include:

- vaginal discharge
- chest pain
- abdominal bloating

More Rare yet Serious Risks:
Venous Thromboembolism (blood clots) – the risk is increased with hormone use, but the risk is influenced by age and the preparation or type of hormone therapy. In the Women's Health Initiative Trial, the risk of venous thromboembolism in 50 – 59-year-old women taking estrogen replacement alone (without a daily progesterone) increased by less than 1% (that is, an additional 3-4 women out of every 1000 women taking estrogen replacement experienced a venous thromboembolism). The risk is even less with the use of transdermal estradiol.

Breast Cancer - ERT increases the risk of breast cancer but the risk is influenced by the duration of use. The risk in 50 – 54-year-old women who take estradiol alone for 5 years would increase from 13 out of every 1000 women not taking ERT (about 1%) to 14.94 per every 1000 women (about 1.5%).
Ovarian cancer – Long term use of ERT alone increases the risk of ovarian cancer, with a risk of 0.7 women per every 1000 women per every 5 years of use.
Endometrial Cancer - Exposure to estrogen by itself increases the risk of endometrial cancer two-fold (the endometrium is the lining of the uterus). However, use of a progesterone prevents the increased risk of endometrial cancer. In the current research study, we plan to give a progesterone (200 mg/day) for 10 days at the end of your participation. In a prior study of Dr. Girdler's, that used the similar enrollment criteria as in this study (medically healthy women, 45 – 60 years of age in the menopause transition), progesterone was given at a dose of 200/mg day for 12 days every two or three months. Unlike this study, however, these women were treated with active estradiol (or placebo) for 12 months. Of the 86 women who received the active estrogen and this regimen of progesterone, one woman (1% of the women) developed endometrial hyperplasia which is a condition of excessive growth of the cells of the inner lining of the uterus. Endometrial hyperplasia is a risk factor for the development of endometrial cancer. However, in this case, the cells were not atypical, meaning that the cells were not characteristic of cancer cells. Because we are giving estradiol for only three weeks in the current study, the risk of developing endometrial hyperplasia, especially since you are still menstruating, is exceedingly low.
Stroke - It is possible that ERT increases the risk of stroke, but this is age dependent**.** ERT increased risk of stroke in the Women's Health Initiative study in 50 – 59-year-old women by an additional 1 case per 5000 women.
Risks Associated with Stopping the Hormones: There is a risk that stopping the hormones after the three weeks of estrogen or placebo will be associated with hot flashes, night sweats or vaginal dryness.
Minimizing these risks: The risk to you of these rare side effects are minimized as much as possible by the following: 1) your young age (45-55 years of age); 2) exposure to estradiol for only 3 weeks since short-term exposure (< 3 yrs.) has not been shown to be associated with an increased risk of breast cancer;
3) the use of micronized progesterone (200 mg/day for 10 days) in combination with the short exposure to estradiol and close monitoring of your bleeding patterns will substantially minimize the likelihood you develop endometrial hyperplasia and virtually eliminate any increased risk of endometrial cancer; 4) the frequent assessment of side effects; and 5) the provision of educational information so that you are fully aware of signs and symptoms that you should report to the study personnel.

Psychological Risks: The structured interviews to assess current and lifetime psychiatric illness may be associated with some psychological distress. Some items in the questionnaires may provoke some negative emotion in some individuals. There is also the risk you could experience the onset of a depression episode or have a worsening of depressive or anxious symptoms over the course of the study.
There may be uncommon or previously unknown risks. You should report any problems to the researcher.

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.

How will information about you be protected?
No subjects will be identified in any report or publication about this study. 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.
Your privacy and confidentiality will be protected using ID numbers only and by securing all study files in a locked room. An electronic, password protected file linking your study ID number to you name and other identifying information will be kept separate from any study data. Only the researchers will have access to your identifiable information.
Your information and data collected for this research study will not be used or distributed for future research studies even if identifiers are removed.

What is a Certificate of Confidentiality?

This research is covered by a Certificate of Confidentiality. With this Certificate, the researchers may not disclose or use information, documents or biospecimens that may identify you in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings in the United States, for example, if there is a court subpoena, unless you have consented for this use.

The Certificate cannot be used to refuse a request for information from personnel of a federal or state agency that is sponsoring the study for auditing or evaluation purposes or for information that must be disclosed in order to meet the requirements of the federal Food and Drug Administration (FDA).

The Certificate of Confidentiality will not be used to prevent disclosure as required by federal, state, or local law, such as mandatory reporting requirements for child abuse or neglect, disabled adult abuse or neglect, communicable diseases, injuries caused by suspected criminal violence, cancer diagnosis or benign brain or central nervous system tumors or other mandatory reporting requirement under applicable law. The Certificate of Confidentiality will not be used if disclosure is for other scientific research, as allowed by federal regulations protecting research subjects or for any purpose you have consented to in this informed consent document.

You should understand that a Certificate of Confidentiality does not prevent you from voluntarily releasing information about yourself or your involvement in this research. If an insurer, employer, or other person obtains your written consent to receive research information, then the researchers may not use the Certificate to withhold that information.

Data sharing protection

Data from this study may be submitted to the National Institute of Mental Health Data Archive (NDA). NDA is a data repository run by the National Institute of Mental Health (NIMH) that allows researchers studying mental illness to collect and share deidentified information with each other. A data repository is a large database where information from many studies is stored and managed. Deidentified information means that all personal information about research participants such as name, address, and phone number is removed and replaced with a code number. With an easier way to share, researchers hope to learn new and important things about mental illnesses more quickly than before.

During and after the study, the researchers will send deidentified information about your health and behavior to NDA. Other researchers nationwide can then file an application with the NIMH to obtain access to your deidentified study data for research purposes. Experts at the NIMH who know how to protect health and science information will look at every request carefully to minimize risks to your privacy.

You will not benefit directly from allowing your information to be shared with NDA. The information provided to NDA may help researchers around the world treat future children and adults with mental illnesses so that they have better outcomes. NIMH will also report to Congress and on its web site about the different studies that researchers are conducting using NDA data. However, you will not be contacted directly about the data you contributed to NDA.
You may decide now or later that you do not want to share your information using NDA. If so, contact the researchers who conducted this study, and they will tell NDA, which can stop sharing the research information. However, NDA cannot take back information that was shared before you changed your mind. If you would like more information about NDA, this is available on-line at http://data- archive.nimh.gov.

Please initial on the line that best matches your choice:

_____I AGREE to have research data entered into NDA and NIMH

_____I DO NOT AGREE to have research data entered into NDA and NIMH

If you agree to have research data entered into NDA and NIMH, please fill out the fields below, as they appear on your *most recent* birth certificate. This information will be used to generate the unique code number that will be associated with your data.

Name: First_____ Middle _____ Last _____

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 be receiving up to $650 for taking part in this study. Any payment provided for participation in this study may be subject to applicable tax withholding obligations.
$25 for the enrollment session
$50 for each neurophysiological testing sessions ($150 total)
-Up to $10/session for task performance ($30 total)
$10/week for daily mood ratings ($130 total)
$5/week for survey collections ($80 total)
$35/week of urine collections ($210 total)
$25 bonus for full compliance
Your name, phone number, address, and U.S. taxpayer identification number (SSN or ITIN) are required to process payments and/or to report taxable income to the IRS. You must complete a W-9 (for U.S. persons) or W-8BEN and the Foreign Vendor Withholding Assessment with supporting documents (for non-resident aliens) in order to receive payment for participation.

U.S. person participants must complete Form W-9 in order to receive payment for participation. If payment by UNC equals or exceeds $600 per calendar year for U.S. persons, UNC will report the amount to the Internal Revenue Service on Form 1099. Nonresident alien participants must complete Form W-8BEN and the Foreign Vendor Withholding Assessment with supporting documents in order to receive payment for participation. Payments to nonresident alien participants may be subject to tax withholding and are generally reported to the Internal Revenue Service on Form 1042-S. This information will not be linked to any of the study data and will only be used for payment purposes.
If you do not provide your SSN or ITIN, or complete the appropriate documentation noted above, we cannot issue you a payment for participation. However, you may still choose to participate in this study.

Will it cost you anything to be in this study?

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

Who is sponsoring this study?

This research is funded by the National Institutes of Health. This means that the research team is being paid by the sponsor for doing the study. The researchers do not, however, have a direct financial interest with the sponsor or in the final results of the study.

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.

A description of this clinical trial will be available on www.clinicaltrials.gov, as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

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

DOCUMENTATION OF INFORMED CONSENT PROCESS

Subject ID:_____ Consent Form Version Date:_____

	I have reviewed the study and content of the consent form with subject and given the subject an opportunity to ask questions
	I have given the subject opportunity to review the consent form and to discuss participation in the study with family members/others
	Offered opportunity for subject to speak to PI
	The subject accurately summarized the risks associated with this study
	I have answered the subject's questions/concerns. *Document below* The subject did not verbalize any questions/concerns today.
	The subject has agreed to participate in the study and signed/dated a valid consent form prior to the start of any procedures. The RA witnesses the signature and signs
	A copy of the signed and dated consent form was provided to the subject.
	A copy of the signed and dated consent form was filed with UNC WISE Study documentation in a secure location.

Ok to record MINI?

Yes

No

Ok for NDAR?

Yes

No

Signature_____ Date_____

UNANTICIPATED / SERIOUS ADVERSE EVENT LOG
The WISE Study

Protocol: _____ **PI:** _____

Sponsor: _____ **Site Number:** _____

Subject Identifier	Date Event Occurred	Date Study Team Notified of Event	Event	Date Reported to Sponsor	Date Reported to IRB	Study SAE Form Completed
						☐Yes ☐No ☐N/A
						☐Yes ☐No ☐N/A
						☐Yes ☐No ☐N/A
						☐Yes ☐No ☐N/A
						☐Yes ☐No ☐N/A
						☐Yes ☐No ☐N/A
						☐Yes ☐No ☐N/A
						☐Yes ☐No ☐N/A
						☐Yes ☐No ☐N/A
						☐Yes ☐No ☐N/A
						☐Yes ☐No ☐N/A
						☐Yes ☐No ☐N/A
						☐Yes ☐No ☐N/A

Any event that meets Unexpected **and** Related or Probably Related Adverse Events that are serious or have new or increased risk(s) to subjects must be reported to the IRB (See IRB SOP #1401 for further information on reporting). There may be additional reporting requirements to Sponsor or other regulatory agencies.

Protocol: _____ ADVERSE EVENT LOG PI: _____

Subject's Initials: _____ Subject's ID: _____

ADVERSE EVENT **Make a separate entry for:** ➢ **All new adverse events** ➢ **All AEs with increased severity** ➢ **All AEs with changes in study drug relationship** ➢ **All medical conditions present at study drug initiation which have worsened**	**√ if AE meets definition of serious*** **(Grades 3 – 5)**	**Grade / Intensity** **1. Mild Asymptomatic** **2. Moderate** **3. Severe or medically significant** **4. Life-threatening** **5. Death**	**Start Date**	**End Date**	**Relationship to study intervention** **Definitely** **Probably** **Unknown** **Not Related**	**Was Action Taken?** **(Circle One)**	**Action(s) Taken^:**	**Outcome:** **Recovered** **Not Recovered** **Recovered w/Sequelae** **Fatal** **Unknown**	**PI Initials / Date**
						Yes / No			
						Yes / No			
						Yes / No			
						Yes / No			
						Yes / No			
						Yes / No			
						Yes / No			
						Yes / No			
						Yes / No			
						Yes / No			
						Yes / No			
						Yes / No			
						Yes / No			
						Yes / No			
						Yes / No			

*SAE Criteria:
1 Death
2 Life-threatening
3 Requires or prolongs hospitalization
4 Persistent or significant disability/incapacity
5 Congenital Anomaly/Birth Defect
6 Other Medical Importance

^Examples of Actions Taken:
A. Study drug dosage adjusted
B. Study drug interrupted
C. Study drug permanently discontinued
D. Concomitant medication taken (record on Con Med page)
E. Non-drug therapy given (record on Non-Drug Therapy page)
F. Hospitalized/prolonged hospitalization*

Any event that meets SAE or Unexpected Adverse Event must be reported to the IRB (See IRB SOP #1401 for further information on reporting). There may be additional reporting requirements to Sponsor or other regulatory agencies.

Severity		When to Contact	Who to Contact	Adverse Event	Course of Action
Mild	• No effect on ADLs • Requires no medical intervention • Of marginal clinical significance	After study visit	RA → Coordinator → Drs. Girdler or Andersen	○ **Nausea**	Monitor weekly, then monthly
				○ **Non-migraine Headaches**	
				○ **Breast tenderness**	
				○ **Mild skin irritation**	
Moderate	• Temporary disruption of ADLs	Immediate	RA → Coordinator & Drs. Girdler or Andersen	○ **Increase in depression or anxiety symptoms > 50% above baseline levels**	○ Side effects consistent with moderate AEs or that are troublesome to the subject will be immediately reported to Drs. Girdler or Andersen ○ Follow up evaluation with subject within 1 week. ○ If condition is not resolved or has worsened, report to Dr.Munoz or Schiller
				○ **Worsening headache or migraine requiring bed rest**	
				○ **Excessive sleepiness or fatigue that resolves with extra rest**	
				○ **Diagnosis of liver disease or other medical exclusionary criterion**	○ Withdraw subject and provide referral information if not under the care of a physician
				○ **Stage I or greater hypertension (Average SBP >140mmHg or DBP > 90 mmHg)**	○ Re-evaluate BP with another RA or the following week. Inform Drs. Girdler or Andersen
			RA → Coordinator & Drs. Girdler or Andersen → Dr. Schiller or Munoz	○ **Clinically meaningful levels of depression and anxiety symptoms based on the IDAS clinical cut point scores**	○ Dr. Schiller will determine next course of action, including more frequent assessment or withdrawal and referral for treatment
				○ **Suicide intent without a specific plan**	

			RA → Coordinator & Drs. Girdler and Andersen→Dr. Munoz	○ **Profuse bleeding: More than 3 tampons/pads for more than 5 days OR More than 1 tampon/pad on any day**	○ Dr. Munoz will decide to evaluate profuse bleeding
				○ **Prolonged bleeding: More than 10 days**	○ Dr. Munoz will perform an ultrasound and/or order endometrial biopsy
Severe	• Serious disruption to ADLs •May or may not require hospitalization •May or may not be life-threatening •May or may not be permanently disabling •Will result in withdrawing subject from study	Immediate to PIs who will report within 24 hours to Dr. Munoz or Dr. Schiller	RA & Coordinator → Drs. Girdler & Andersen →Dr. Munoz (medical) or Dr. Schiller (psychological)	○ **Thromboembolic event (thrombophilia, DVT, etc.)**	○ Inform Drs. Girdler and Andersen ASAP and participant will be withdrawn from study
				○ **Breast carcinoma or abnormal mammogram**	
				○ **Increased BP to Stage II hypertension (S: ≥160 or D: ≥100)**	
				○ **Any cardiovascular event or diagnosis of any CVD**	
				○ **Become pregnant or concerned might be pregnant**	
				○ **Developed seizures**	
				○ **Diagnosis of diabetes**	
				○ **Severe mood impairment**	
				○ **Suicide ideation with specific plan**	○ If immediate treatment needed, escort her to UNC ED ○ Otherwise, Dr. Schiller will meet with subject to evaluate safety and refer to community provider for further evaluation and treatment
				○ **Suicide-related behavior (e.g. preparation or attempt)**	
				○ **Endometrial hyperplasia with or without atypia**	○ Dr. Munoz will prescribe a stronger progestin and a follow-up biopsy will be performed at 3 months