The Menopause Transition: Estrogen Variability, HPA Axis and Affective Symptoms

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UNC Changes Study

The Menopause Transition: Estrogen Variability, HPA Axis and Affective Symptoms

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

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.]

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title: The Menopause Transition: Estrogen Variability, HPA axis and Affective Symptoms

Study Description: Women in the menopause transition ('perimenopause') are exposed to extreme hormone variability, tend to experience a unique set of severe stressors (e.g., divorce, death of loved ones), and are also at substantially elevated risk to suffer from mood and anxiety disorders. The purpose of this research is to understand the mechanisms by which variability in estradiol (E2) is associated with the symptoms of anxiety and anhedonia (loss of interest and pleasure - a common symptom of depression). By stabilizing E2 variability with a hormonal manipulation, this research will determine the degree to which the E2 variability (or E2 levels) plays a causal role in perimenopausal anxiety and anhedonia symptoms and whether it does so by affecting biological responses to stress.

Objectives:

Primary Objectives:

1. Change over time in anxiety score from State-Trait Anxiety Inventory [Time Frame: Baseline (Week 8), Weeks 16, 20 and 24]
2. Change over time in anhedonia score from the Snaith-Hamilton Pleasure Scale [Time Frame: Baseline (Week 8), Weeks 16, 20 and 24]

Secondary Objectives:

1. Change over time in AUC cortisol stress response [Time Frame: Baseline (Week 8), Weeks 16, 20 and 24]
2. Change over time in threat reactivity from Dot Probe behavioral task [Time Frame: Baseline (Week 8), Weeks 16, 20 and 24]

3. Change over time in measure of effort for motivation and anhedonia from Effort Expenditure for Reward Task [Time Frame: Baseline (Week 8), Weeks 16, 20 and 24]

Endpoints:	Primary Endpoints: 1. Change in STAI score [Time Frame: Baseline (Week 8), Weeks 16, 20 and 24] 2. Change in SHAPS score [Time Frame: Baseline (Week 8), Weeks 16, 20 and 24] Secondary Endpoints: 1. Change in AUC cortisol response [Time Frame: Baseline (Week 8), Weeks 16, 20 and 24] 2. Change in "threat bias" score from Dot Probe task [Time Frame: Baseline (Week 8), Weeks 16, 20 and 24] 3. Change in "hard choice" score from EEfRT [Time Frame: Baseline (Week 8), Weeks 16, 20 and 24]
Study Population:	250 medically healthy female volunteers aged 45-60 undergoing natural menopause transition who meet STRAW+10 criteria for early or late menopause transition and are candidates for transdermal E2 and have had a normal mammogram in the previous 12 months.
Phase:	N/A
Description of Sites/Facilities Enrolling Participants:	Subjects are recruited from the area surrounding Chapel Hill, North Carolina and will be enrolled at the UNC-CH laboratory site located at 2218 Nelson Highway, Suite 1, Chapel Hill, NC 27517. Medical screening, including the gynecological exam and screening mammogram will occur at the UNC Medical Center on the UNC-CH campus. This study is not intended to include sites outside of the United States.
Description of Study Intervention:	Subjects randomized to the active treatment arm will wear a transdermal estradiol patch (0.1mg) every day for 16 weeks, changing the patch every 7 days. They will also take 200mg of micronized progesterone oral capsules every day for 12 days 8 weeks after randomization to estradiol and again at the end of the 16th week of randomization to estradiol. Subjects randomized to the placebo arm will follow the same regimen using matching placebo transdermal patches and placebo capsules.
Study Duration:	Enrollment will occur for approximately 54 months and data analysis will occur for 6 months following the close of enrollment. Estimated total study duration is 60 months.
Participant Duration:	Following medical screening, individual participation study duration is 6 months (24 weeks). Randomization to active/placebo treatment arm occurs at week 8, and subjects will be in the intervention phase for a total duration of 16 weeks.

1.2 SCHEMA

Week 0

Medical Screening and Enrollment

- Obtain informed consent
- Screen potential participants by inclusion and exclusion criteria with online, phone, and medical screening via gynecological exam and mammogram
- Obtain history, document

Week 8

Lab Session- Baseline Assessments

- Administer STAI and SHAPS questionnaires
- Administer Dot Probe and EEfRT behavioral tasks
- Administer Trier Social Stress Test (TSST) and collect serum to measure cortisol at the end of baseline rest and at minutes 20, 30, and 45 of recovery post-TSST
- Urine HCG test to confirm subject is not pregnant prior to randomization

Randomization

- Active Intervention Group (0.10mg transdermal estradiol (E2))
- Placebo (matching inactive patch)
- Administer initial dose of study drug intervention

Week 16

Lab Session- Study Intervention, Side Effect & Compliance Checks

- Administer STAI and SHAPS questionnaires
- Administer Dot Probe and EEfRT behavioral tasks
- Administer Trier Social Stress Test (TSST) and collect serum to measure cortisol at the end of baseline rest and at minutes 20, 30, and 45 of recovery post-TSST
- Administer oral micronized progesterone (200mg daily)/matching placebo capsules for subjects to take for twelve days starting on Week 17 for the same treatment arms as E2/placebo randomization

Week 20

Lab Session- Study Intervention, Side Effect & Compliance Checks

- Administer STAI and SHAPS questionnaires
- Administer Dot Probe and EEfRT behavioral tasks
- Administer Trier Social Stress Test (TSST) and collect serum to measure cortisol at the end of baseline rest and at minutes 20, 30, and 45 of recovery post-TSST
- Continued administration of transdermal E2/placebo patch

Week 24

Final Lab Session- Study Intervention, Side Effect & Compliance Checks

- Administer STAI and SHAPS questionnaires
- Administer Dot Probe and EEfRT behavioral tasks
- Administer Trier Social Stress Test (TSST) and collect serum to measure cortisol at the end of baseline rest and at minutes 20, 30, and 45 of recovery post-TSST
- Administer oral micronized progesterone (200mg daily)/matching placebo capsules for subjects to take for twelve days starting on Week 25 for the same treatment arms as E2/placebo randomization
- Discontinue use of transdermal E2/placebo patch

1.3 SCHEDULE OF ACTIVITIES (SOA)

Procedures	Medical Screening and Enrollment Week 0	Lab Session- Baseline Assessments & Randomization Week 8	Lab Session- Study Intervention, Side Effects, and Compliance Checks Week 16	Lab Session- Study Intervention, Side Effects, and Compliance Checks Week 20	Final Lab Session- Study Intervention, Side Effects, and Compliance Checks Week 24
Informed consent	X				
Demographics	X				
Medical history	X				
Physical and gynecological exam (including height and weight)	X				
Screening mammogram	X				
Pregnancy test[a]		X			
Randomization		X			
Administer transdermal estradiol/placebo		X	X	X	
Administer micronized progesterone/placebo			X		X
Discontinue transdermal estradiol/placebo					X
Administer STAI and SHAPS questionnaires		X	X	X	X
Administer Dot Probe and EEfRT behavioral tasks		X	X	X	X
Administer Trier Social Stress Test (TSST) and collect serum to measure cortisol[b]		X	X	X	X
Adverse event review and evaluation		X	X	X	X
a: urine pregnancy test (women of childbearing potential) b: serum collected at the end of baseline rest and at minutes 20, 30, and 45 of recovery post-TSST					

2 INTRODUCTION

2.1 STUDY RATIONALE

Vulnerability to the deleterious mood effects of normal changes in reproductive hormones is a likely underpinning to reproductive mood disorders. The menopause transition (MT) is associated with pronounced hormonal variability (within the context of relative E2 deprivation) and substantially increased risk for clinically impairing anxiety and anhedonia. Anxiety is characterized by cognitive bias to

interpret ambiguous stimuli in a threat-related manner. Anhedonia can be defined by decreased motivation to approach rewards. The neurobiologically-based constructs of 'threat reactivity' and 'approach motivation' provide a framework for studying the pathophysiology of the clinical impairment experienced by 25% of women in the MT. Although the causes of affective symptoms in the MT remain unknown, severe life stress proximate to the MT is a strong predictor. Framed within a diathesis-stress model, the primary objective of this research is to determine the pathophysiological mechanisms of estradiol (E2) in the clinical anxiety and anhedonia seen in the MT. Specifically whether E2 variability or E2 levels predict exaggerated hypothalamic-pituitary-adrenal (HPA) axis reactivity and impaired recovery to stress and, in turn, deficits in behavioral indices of threat responsivity and approach motivation and symptoms of anxiety and anhedonia. The secondary objective of the research is to use a hormonal manipulation as a mechanistic probe to stabilize E2 variability in premenopausal ranges and determine if HPA axis reactivity/recovery represents a biomarker of behavioral and symptom responses to E2 stabilization.

A total of 250 women in the early or late MT who are eligible for the hormonal probe will be recruited to reflect the full continuum of anxiety and anhedonia symptoms based on self-report to the State-Trait Anxiety Inventory and the Snaith-Hamilton Pleasure Scale, respectively. At baseline week 8, HPA axis (plasma cortisol) response to the Trier Social Stress Test and behavioral measures of threat responsivity (via Dot-Probe task) and approach motivation (Effort Expenditure for Rewards Task 'EEfRT') will be determined. Using transdermal E2 as a pharmacological probe to stabilize variability of E2 in premenopausal ranges, women will then be randomized to transdermal E2 (0.10 mg) or placebo for 16 weeks. This is not a clinical efficacy trial. We will use an RCT design with a hormonal manipulation to investigate the pathophysiologic role of E2 variability (or E2 levels) in HPA axis dysregulation and, in turn, threat responsivity and approach motivation. HPA axis reactivity to stress and behavioral responses to the Dot-Probe and EEfRT tasks, as well as self-report symptoms on the STAI and SHAPS measures will be assessed at weeks 8, 12, and 16 during the 16-week probe.

2.2 BACKGROUND

Clinical, basic, and epidemiologic research indicates that reproductive events provide an endocrine context of vulnerability for the emergence of mood and anxiety disorders and impairment in women[1]. Though the causes of affective disorders tied to reproductive events are unknown, studies in menstrual mood disorders and postpartum depression suggest that a vulnerability to normal changes in ovarian steroid hormones is etiologic [2,3]. The menopause transition ('perimenopause') is a reproductive stage associated with pronounced hormone variability and risk for psychiatric illness. The menopause transition (MT) is an ideal model for studying the etiologic role of ovarian hormone changes in affective illness.

During the MT (average duration = 5 years), the risk of major depressive disorder (MDD) increases two to three-fold [4], in addition to the two to four-fold increase in risk of clinically significant depressive symptoms, affecting 26-33% of women in the MT [5-8]. A parallel increase in suicide ideation and attempts emerges in perimenopausal compared with pre- or postmenopausal women or age-matched men. While the causes of perimenopausal affective disorders are unknown, stressful life events are a strong predictor of the development of depression in the MT [4-6].

The Phenotype of Perimenopausal Depression: Women in the MT with impairing mental health symptoms typically present with an anxious and anhedonic phenotype [9-11]. Common to all anxiety

disorders is an exaggerated neurobiological sensitivity to threat, indexed by cognitive biases to regard ambiguous stimuli as threatening [12,13]. The brain regions involved in processing threat information (e.g., hippocampus) activate stress-responsive systems, including the hypothalamic-pituitary-adrenal (HPA) axis. Perceived threat is a feature of stress that elicits cortisol increases[14]. Anhedonia (loss of pleasure or interest) has emerged as an endophenotype in depressive disorders[15]. Patients with anhedonia exhibit diminished sensitivity and attentional bias towards positively valanced stimuli and reduced behavioral and neurobiological responsiveness to reward cues[15]. Anhedonia can be defined as reduced motivation to approach rewards (reward "wanting").

This research will employ a more fine-grained approach to defining impairment in perimenopausal women by focusing on anxiety and anhedonia symptoms. This will allow for more precise integration of a particular psychopathology with underlying biological mechanisms (E2 regulation of HPA axis reactivity) and fundamental constructs (threat responsivity and approach motivation), and therefore advance our knowledge of the causes of the clinical impairment experienced by 25% of women in the MT.

Endocrinology of the Menopause Transition: While there are individual differences in the hormonal trajectory during the MT [16,17], most women experience the following changes beginning in the early and progressing to the late MT [18,19]. First, cycle length becomes increasingly variable, with long cycles becoming more common as the transition progresses. While short cycles are due to early follicular recruitment by intermittently high FSH, long cycles can be due to anovulation or a delayed ovarian response to FSH stimulation, resulting in an extended (low-E2) early follicular phase. Second, luteal progesterone (P4) also declines, resulting from declining dominant follicle quality. Third, appearing in the early and continuing into the late MT are cycles in which E2 levels are elevated compared to premenopausal levels, due to high FSH and ovarian hyperstimulation [20]. The late MT is marked by more anovulatory cycles, characterized by low P4 and erratic E2. In sum, most women in the MT are exposed to erratic hormonal flux. Anovulatory cycles results in fewer increases in P4, but variable FSH causes periods of both hypo- and hyper-estrogenism [21].

The Hormone Milieu and Affective Symptoms in the Menopause Transition: While women in the MT are exposed to periods of relative hyper-estrogenism, mean levels of E2 are lower than in premenopausal women [22,23], especially in late MT. Whether the clinical symptomatology experienced during the MT is consequent to E2 deprivation or E2 variability has not been empirically tested. Estradiol: 1) "beneficially" modulates systems implicated in depressive illness (e.g., neurotransmitters, neuroplasticity)[24-27]; 2) displays anti-depressant effects in animals [28-31]; 3) regulates brain circuits important for emotional processing[32] and fear conditioning[33] and protects against fear and anxiety[34]. While the planned research will test whether alterations in the neurobiologically based constructs of threat reactivity and approach motivation underlying anxiety and anhedonia is best explained by E2 variability or E2 levels, most evidence supports the variability hypothesis [35-45]. Schmidt et al.,[46] placed euthymic perimenopausal women (CES-D = 3) on three weeks of transdermal E2 (100 ug/day) then blindly and abruptly withdrew half of the women. Those with a history of perimenopausal depression (PMD) had an increase in depressive symptoms (CESD = 12) within 1-3 weeks, an effect not seen in unaffected controls or in those maintained on E2. The specific symptoms that increased with abrupt E2 withdrawal were only: anxiety, excessive worry, anhedonia, motivation, and social avoidance – supporting an etiologic role for E2 in the clinical anxiety and anhedonia experienced in the MT. However, that study did not measure E2, limiting conclusions about the mechanism underlying emergence of symptoms (i.e., withdrawal from E2 stabilization or induction of E2 deprivation). Our prior research strongly implicates E2 variability, not levels[47].

E2 regulation of the HPA axis as a Candidate Mechanism in the Psychopathology of the MT: Impairment in HPA axis regulation is implicated in human affective illness[48]. Gonadal steroid hormones play a vital role in modulating HPA axis function. Estrogens stimulate the HPA-axis through an interaction of estrogen receptors (ERs) in the promoter region of the human CRH gene [49]. ERs are expressed throughout the brain [50], including the paraventricular nucleus of the hypothalamus. The genomic action of E2 is mediated by ERα and ERß. E2 also has rapid cellular effects by inducing changes in second messenger pathways and intracellular Ca+ [51,52]. Although ERs are abundant in regions involved in affect regulation[53, 54,55], E2 regulatory control of the HPA axis may be most relevant to understanding anxiety and anhedonia in the MT, particularly in the context of stressful life events.

In rodents, ovariectomy reduces stress-induced corticosterone (CORT) and ACTH secretion, an effect reversed by systemic E2 treatment [56, 57,58]. Acute stress increases production of E2 in the PVN[59] and its presence is associated with enhanced responsiveness of the HPA axis to stress, mediated by E2-induced impairment in glucocorticoid negative feedback[57]. The effect of E2 depends on the ER signaling pathways[60] since stimulation of ERα and ERß, exerts opposite effects on the CORT and ACTH stress response [56,59-61]. Consequently, alternations between hyper- and hypo-estrogenism, characteristic of the MT, could exert a destabilizing effect on the regulatory control of the HPA axis. Testing HPA stress reactivity as a mediator in the relationship of E2 variability (or levels) to threat responsivity and approach motivation, and clinical symptoms, in perimenopausal women represents a novel contribution of this research.

The significance and impact of the proposed research is in delineating the role of E2 variability versus E2 levels in the clinical symptoms seen in a substantial proportion of women in the MT and testing a candidate physiologic mediating mechanism (HPA axis) of E2. This research will advance a diathesis-stress model of affective state change involving E2 (variability or levels) as the diathesis for HPA axis dysregulation and affective symptoms, in the context of severe life stress.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

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 associated with estradiol use 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 use 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 associated with progesterone use include:

- vaginal discharge
- chest pain
- abdominal bloating

Rare yet Serious Risks Associated with Perimenopausal Hormone Use:

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), 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. Two years later, although there appeared to be reduced risk of cardiovascular disease (CVD) among 50–59-year-old women who were on ERT, 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 and the Endocrine Society (representing the work of 30 leading experts and four levels of review) as well as reports of subgroup analyses and one surrogate endpoint study from the WHI 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.

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. 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 16 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-old subjects (estimated excess events 9-10/1,000 for HRT and 3-4/1000 for ERT). Notably, the risk for VTE is significantly reduced using transdermal E2 in case control studies (HR 0.9 compared with HR 4.2 in those taking oral estrogen).

Stroke. It is possible that ERT increased the risk of stroke, but this is age dependent. ERT increased risk of stroke in the Women' Health Initiative study in 50 - 59-year-old women by an additional 1 case per 5000 women.

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". 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). Given our plans to administer transdermal E2 to women: 1) for only 16 weeks, 2) no older than 60 years of age, 3) who are within 1 year of their final menstrual period, and 4) who are at no greater than average risk for CVD, breast cancer or VTE, the risk of serious adverse events are exceedingly low.

Endometrial Cancer - Exposure to estrogen by itself increases the risk of endometrial cancer two-fold. However, use of a progesterone prevents the increased risk of endometrial cancer. Although giving a small dose of progesterone every day or giving a larger dose for 12 days every month are the most common clinical practices to protect against endometrial cancer when using estrogen, there have been no research studies specifically designed to determine which is the most protective type of progesterone and which is the most protective frequency of administration. In the current research study, we plan to give a progesterone (200 mg/day) for 12 days every two months. In a prior study of Dr. Girdler's, that used the same 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. Of the 86 women who received the active estrogen and this regimen of progesterone, one woman (1% of the women) developed endometrial hyperplasia. 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. Only 1.6% of patients diagnosed with hyperplasia without atypical cells develop endometrial cancer, while 22% of patients

who have the atypical cells go on to develop endometrial cancer. This research participant was withdrawn from the study and treated clinically with progesterone which was effective in eliminating the endometrial hyperplasia.

Risks Associated with Discontinuation of Hormones. For women who were experiencing menopausal vasomotor symptoms and/or vaginal dryness and are randomized to the active estradiol, there is the risk that these menopausal symptoms may return once hormones are discontinued. Over a sixteen-week interval of treatment, we would rate this as a likely possibility in those who were experiencing symptoms at baseline. These risks are minimal as it simply returns the woman to the pre-randomized hormone state. There is no evidence in the literature or clinical practice that abruptly discontinuing hormone replacement therapy is associated with negative clinically meaningful sequelae.

Risks Associated with the Vaginal Exam and Endometrial Biopsy: There are no significant risks associated with the vaginal ultrasound of the uterus, though the insertion of the ultrasound probe and speculum to be associated with a sensation of increased pressure but immediately terminated when the probe and speculum are removed. Endometrial biopsies may cause cramping both during the procedure and afterwards. There may also be temporary spotting. Rare complications include excessive uterine bleeding and uterine perforation (0.1-1.3% risk).

Risks Associated with Mammography: The amount of radiation to the breast from one mammogram is 43 mrem which is equivalent to the radiation exposure that everyone receives in 52 days from natural background radiation. There is the risk that a mammogram will falsely indicate an abnormality that could cause unnecessary anxiety and/or extra procedures to be performed. There may be some discomfort by the compression of the breast tissue.

Other Risks: Venipuncture (the needle stick) may result in syncope (light headedness), discomfort, nausea, and bruising.

2.3.2 KNOWN POTENTIAL BENEFITS

The benefit to subjects includes study-related medical evaluations, including a gynecological exam and screening mammogram. Subjects may also benefit by knowing that they are contributing to research aimed at enhancing our understanding of the causes of clinically significant anxiety and anhedonia symptoms in women in the menopause transition.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

Minimizing these risks. The risk are minimized as much as possible by the following: 1) young age (45-60 years of age); 2) careful medical evaluations, including normal mammogram and endometrial exam, to ensure no greater risk for these side effects than the average population of women 45 - 60 years of age; 3) the use of intermittent micronized progesterone (200 mg/day for 12 days every 2 months) and close monitoring of bleeding patterns will substantially minimize the likelihood of developing endometrial hyperplasia and virtually eliminate any increased risk of endometrial cancer; 4) exposure to estradiol for only 16 weeks since short-term exposure (< 3 years) has not been shown to be associated with an increased risk of breast cancer; 5) the frequent assessment of side effects; 6) the provision of educational information so that participants are fully aware of signs and symptoms that they should

report to the study personnel; 7) endometrial biopsies (if needed) performed in a UNC gynecology clinic by a board certified gynecologist; 8)The level of radiation used by modern mammography systems does not significantly increase the risk for breast cancer. Scientific data has shown that doses 100-1000 times greater than those used for mammography are required to show any statistical increase in breast cancer frequency. There is no significant risk of radiation damage to breast tissue from mammography; and 9) there is an independent safety board made up of a cardiologist, gynecologist, psychiatrist, and statistician that will be closely monitoring this study for side effects and risks.

Special Considerations for Cancer Risk. To minimize any possible risks associated with exposure to E2, we will obtain a comprehensive family history of cancer in each of our subjects to ensure that women enrolled into the present study would meet clinical criteria for average risk (i.e., risk seen in the female population at large) for breast and ovarian cancer. This is the approach Dr. Girdler has used in her most recent study using transdermal E2 in perimenopausal women. A review of the accuracy of self-reported patient history found that for breast cancer specifically, the comparison of patient reports with first degree relative medical records or first degree relative self-report yielded 95% sensitivity and 97% specificity. Thus, patient self-report of family medical history is a valid, reliable approach and continues to be the approach recommended for determining the clinical management not only of breast cancer risk, but of all common diseases. Moreover, as in our eligibility criteria for subjects to have 'average cancer risk profile', each will also be required to have a negative mammogram within one year of enrollment.

Risks of loss of confidentiality will be minimized using subject codes linked to all data and by maintaining the file linking the subject code to the name in separate secured locations. All databases will be stored on a secure, HIPAA compliant network. The server is backed up nightly. The backup tapes reside in a separate location from the server off site. The database is also protected by logins and passwords required to open the system. Different levels of privileges are granted to different users. This study will use a web-based, secure, password protected access to the database. Passwords will be changed every 90 days.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
The primary objective of this study is use transdermal estradiol to determine the pathophysiologic role of estradiol in clinically significant symptoms of anxiety and anhedonia in the menopausal transition.	*The primary endpoints are changes in mean levels from week 8 (baseline) to weeks 16, 20, and 24 in validated questionnaires for symptoms of anxiety and anhedonia. Specifically, the validated State-Trait Anxiety Inventory and the Snaith-Hamilton Pleasure Scale (SHAPS) will be assessed.*	*The goal is to establish whether there is a causal role of estradiol in the clinically relevant anxiety and anhedonia symptoms seen in perimenopausal women.*
Secondary		
The secondary objectives of this study are to use transdermal estradiol to determine i) the role of	*The secondary endpoints are changes from week 8 (baseline) to weeks 16, 20, and 24 in i) mean levels of*	*The goal is to establish whether there is a causal role of estradiol*

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
estradiol in anxiety and anhedonia related behaviors and ii) if HPA stress activation is a biomarker of estradiol-related behavior and symptomatology.	*behavioral measures of anxiety (Dot Probe task) and anhedonia (EEfRT task) and ii) in serum cortisol stress-reactivity, as a marker of HPA stress activation*	*in the clinically relevant anxiety and anhedonia related behaviors and stress physiology in perimenopausal women.*

4 STUDY DESIGN

4.1 OVERALL DESIGN

- Hypothesis: Our central hypothesis is that stabilization of E2 variability, not increasing E2 levels, will modify HPA axis stress activation and in turn, beneficially modify cognitive bias towards perceived threat and approach motivation, thereby decreasing anxiety and anhedonia.
- Phase: N/A
- Design of trial: randomized, placebo-controlled, double-blinded parallel design.
- Study arms: 2 (active and placebo)
- Single site study
- Methods to minimize bias: Under double-blind procedures, subjects will be randomized to receive transdermal E2 or placebo for 16 weeks. The study biostatistician will create the randomization scheme and UNC Investigational Drug Services will manage the randomization and dispensing of hormones in blinded form.
- Intervention and Dosing: Subjects randomized to active treatment arm will wear a patch deliver 0.10mg of E2 over 24 hours and will take oral micronized progesterone (200mg/daily) for 12 days at two points in the intervention. Subjects randomized to placebo arm will wear placebo patches and take placebo pills matching in appearance.
- Planned Interim Analysis: Data and Safety Monitoring Board will monitor the safety of the study every six months. The DSMB will evaluate issues of participant safety as well as enrollment information and the adequacy and integrity of accumulating data

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

THIS IS NOT A CLINICAL EFFICACY TRIAL. However, we will employ an RCT design to study the pathophysiologic role of E2 variability [or levels] in the clinical symptoms of women in the MT. Under double-blind procedures, subjects will be randomized to receive 17β- E2 or placebo for 16 weeks. The study biostatistician will create the randomization scheme and UNC Investigational Drug Services will manage the randomization and dispensing of hormones in blinded form. For those randomized to transdermal E2 (n=60), each will wear a patch (generic for Climara®) delivering 0.10 mg E2/24 hours. Those randomized to placebo (n=60), will wear a patch identical in appearance (3M Pharmaceuticals).

4.3 JUSTIFICATION FOR DOSE

By avoiding the first-pass metabolic effects of oral estrogen, transdermal E2 creates stable, premenopausal follicular phase E2 blood levels; and a physiologic profile of E2 relative to its metabolites estrone and estriol [136-138]. Transdermal E2 also has a superior thromboembolic and metabolic safety profile than oral estrogen. [139-141] A 7-day transdermal system provides significantly more stable concentrations and better patch adherence than twice weekly patches.[142] We have chosen (generic version) Climara® 7 day patches because the adhesive layer of the matrix distributes E2 molecules to continuously release E2, which is transported across skin leading to sustained levels of E2.136 Because of the dynamic changes in the reproductive axis during the MT and the possibility that, over 24 weeks, some women may transition from one STRAW stage to another, placebo will control for effects of reproductive aging, per se, on measures of interest and for habituation effects to repeat testing. Progesterone Administration: To prevent E2-induced endometrial hyperplasia, women randomized to active E2 will take 200 mg micronized progesterone/day x 12 days during weeks 17-18 (after 2 months of drug intervention) and at the end of the study (weeks 25-26; after 4 months of drug intervention). Exposure to a progestin every 2 months is sufficient for endometrial protection.[143] Under double-blind procedures, women randomized to placebo-E2 will take placebo P4; those randomized to active E2 will take active P4. We have chosen to give P4 in a discontinuous fashion instead of using Climara Pro® (a transdermal system that delivers both E2 and a progestin) because progestins antagonize E2 effects. [144,145]

4.4 END OF STUDY DEFINITION

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the Schedule of Activities (SoA), Section 1.3.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

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

1. Provision of signed and dated informed consent form.
2. Stated willingness to comply with all study procedures and availability for the duration of the study.
3. Female aged 45-60
4. In good general health as evidenced by medical history
5. Ability to take oral medication and be willing to adhere to the study intervention regimen.
6. Negative HCG urine pregnancy test results administered at Baseline lab session prior to randomization.
7. Normal screening mammogram results within 12 months of enrollment
8. Meet STRAW+10 criteria for early or late MT.

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Any history of CVD, including coronary artery disease, arteriosclerosis, heart attack, or stroke.
2. Type I or II diabetes
3. Stage I or greater hypertension based on 3 stethoscopic measures at enrollment.
4. Personal or family history suggesting elevated risk for E2-related cancer or thrombotic events.
5. Sensitivities to any ingredient in transdermal E2 or micronized P4
6. Use of psychotropics, statins, or hormonal preparations.
7. Use of herbal supplements that may affect mood or menopause symptoms (e.g., Black Cohosh or St. John's Wort)
8. Nicotine use of greater than 10 cigarettes per day.
9. BMI of greater than or equal to 36
10. History of migraine headaches in current smokers
11. History of migraine with aura within the last 3 years
12. History of psychotic disorders, bipolar disorder, or severe substance use disorder (within 10 years of enrollment)
13. History of suicide attempts, or current suicide ideation with intent
14. History gall bladder disease, liver dysfunction, or other disorders for which E2 or P4 is contraindicated
15. History or signs of endometrial disorder (based on gynecological exam)
16. Pregnancy or lactation
17. Known allergic reactions to components of the <study intervention>, <specify components/allergens>

5.3 LIFESTYLE CONSIDERATIONS

During this study, prior to laboratory stress testing sessions, participants are asked to:

- Refrain from consumption of alcohol for 24 hours prior to session
- Refrain from marijuana use for 48 ours prior to session
- Limit caffeine consumption to 1 cup (8 oz.) before 9AM on the day of session
- Refrain from consumption of anti-inflammatory agents (e.g., ibuprofen) 24 hours prior to session

5.4 SCREEN FAILURES

Participants who are consented to participate in the clinical trial, who do not meet one or more criteria required for participation in the trial during the screening procedures, are considered screen failures. Screen failures will be withdrawn from participation and offered referrals to the UNC Women's Mood Disorder Clinic upon request.

Individuals who do not meet criteria for participation (screen failure) because they are too earlier in the menopause transitions (i.e., pre-menopausal; experiencing regular menstrual cycles) may be rescreened when menstrual cycles become irregular. Rescreened participants will be assigned the same participant number as for initial screening.

5.5 STRATEGIES FOR RECRUITMENT AND RETENTION

In carrying out this recruitment we will partner with UNC's the North Carolina Translational

and Clinical Science Institute (NC TraCS). Established in 2008 by UNC's Clinical and Translational Science Award (CTSA) from the NIH, NC TraCS includes the following programs that we will utilize: 1) The Research Recruitment Office (RRO), which will assist the project in developing subject recruitment plans, identifying enrollment barriers specific to the targeted population, and monitoring recruitment statistics; and 2) The Community Engagement Core, which will assist the project in building community relationships, using research navigators – UNC faculty leaders in clinical and translational research who have dedicated time to work with research projects such as this one.

We intend to make use of the UNC Health Care System's (UNCHCS's) enterprise-wide patient data base, the Carolina Data Warehouse for Health (CDW-H), to identify and enroll eligible subjects. The CDW-H is a central repository of clinical, research and administrative data on all patients seen since July 2004 by the UNCHCS. Updated every 24 – 48 hours from its source systems, this research portal is a user-friendly web-based tool that allows researchers to identify patients as potential research subjects and to estimate subject pools.

We will also recruit using advertisements on social media platforms (e.g., Facebook and Instagram), which will target females aged 45-60 within a specified geographical radius surrounding Chapel Hill. Interested subjects will click on a link within the advertisements to the study's website and Qualtrics screening survey.

Inclusion of Minorities: There is no evidence to suggest that estradiol variability would influence the mechanisms to be studied in this project (HPA axis activation, and threat responsivity and approach motivation) differently in different races or ethnic groups. Since the primary analyses involve within-subject change in E2 variability, minorities will be enrolled in numbers proportional to our regional distribution (African American = 23%; Hispanic = 5%; American Indian/Alaskan Native = 1.2%; Asian = 1%, or Native Hawaiian/Pacific Islander = 0.8%). African American race will be examined, however, in covariate analyses and controlled for if indicated.

Recruitment Plan: We realize, however, that specialized efforts will be needed to meet these goals. Dr. Girdler has a long-standing track record of successful recruitment of African Americans (our largest minority population) into clinical research studies. In our current study of perimenopausal women, of those enrolled, 30% identify as African American. We will employ the strategies that have proven successful in prior studies. The single most effective strategy involves the use of minority research staff in the recruitment and retention process. Other strategies involve the use of culturally sensitive language in advertisements, including assurances that the study involves no experimental drugs or devices. We will also advertise in periodicals targeting African American populations and work with community-based minority programs. Statistics will be kept throughout the study documenting recruitment strategy and enrollment rates, allowing the PIS to adjust initiatives accordingly. Should additional initiatives be necessary, we will work directly with leaders from the African American community.

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

Study drugs will be provided and stored at 36-46°F in Neurosciences IDS pharmacy refrigerator.

- Estradiol 0.1mg topical patch, 4 patches/box
- Progesterone 100mg capsules, 100 capsules/bottle
- Placebo capsules, 100 capsules/bottle (Capsugel Orange, Size AA, DB Capsules)
- Placebo patches (provided by study)

6.1.2 DOSING AND ADMINISTRATION

Under double-blind procedures, women randomized to placebo-E2 will take placebo P4; those randomized to active E2 will take active P4.

By avoiding the first-pass metabolic effects of oral estrogen, transdermal E2 creates stable, premenopausal follicular phase E2 blood levels; and a physiologic profile of E2 relative to its metabolites estrone and estriol. [35-37] Transdermal E2 also has a superior thromboembolic and metabolic safety profile than oral estrogen.[38-40] A 7-day transdermal system provides significantly more stable concentrations and better patch adherence than twice weekly patches.[41] We have chosen generic estradiol 7 day patches because the adhesive layer of the matrix distributes E2 molecules to continuously release E2, which is transported across skin leading to sustained levels of E2.[35] Because of the dynamic changes in the reproductive axis during the MT and the possibility that, over 24 weeks, some women may transition from one STRAW stage to another, placebo will control for effects of reproductive aging, per se, on measures of interest and for habituation effects to repeat testing.

Progesterone Administration: To reduce the risk of E2-induced endometrial hyperplasia, women randomized to active E2 will take 200 mg micronized progesterone/day x 12 days during weeks 17-18 (8 weeks after randomization) and again eight weeks later at the end of the study (weeks 25-26). Although in clinical settings progesterone tends to be given either every day or for 12 days once per month, the literature is inconclusive regarding the precise regimen that provides the best protection against endometrial hyperplasia or endometrial cancer.

We have chosen to give P4 in a discontinuous fashion instead of using a transdermal system that delivers both E2 and a progestin because progestins not only antagonize E2 effects [43,44] but because the metabolites of progesterone (e.g., allopregnanolone) are potent modulators of the HPA axis response to stress. Thus, this would introduce a confound and mitigate against our ability to examine the role of E2 variability on the HPA axis stress response.

6.2 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1 ACQUISITION AND ACCOUNTABILITY

The study coordinator will notify the IDS Pharmacy of a patient's enrollment by the presentation of a pre-printed prescription. The prescription will contain the patient's name, medical record number, patient number, study visit week, informed consent, allergies, quantity of drug to be dispensed, date, and signature of an authorized prescriber. Dispensed study drugs are labeled with Vestigo outpatient prescription labels. All study drug returns may be discarded following documentation.

6.2.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

Active: Alvogen brand estradiol 0.1mg topical patch and generic prometrium progesterone 100mg capsules
Control: Placebo patches supplied by PI. Placebo capsules from Capsugel, orange, size AA, DB.
All patches will be dispensed in individually wrapped packages without manufacturer branding. Pills will be contained in Capsugel capsules and active/control capsules will be identical in appearance.

6.2.3 PRODUCT STORAGE AND STABILITY

Drugs will be stored in the Neurosciences IDS Pharmacy refrigerator at 36-46°F.

6.2.4 PREPARATION

Addressed in 6.2.2 above.

6.3 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Under double-blind procedures, subjects will be randomized to receive E2 or placebo for 16 weeks. The study biostatistician will create the randomization scheme and UNC Investigational Drug Services will manage the randomization and dispensing of hormones in blinded form. For those randomized to transdermal E2 (n=60), each will wear a patch delivering 0.10 mg E2/24. Those randomized to placebo (n=60), will wear a patch similar in appearance.

6.4 STUDY INTERVENTION COMPLIANCE

Compliance checks will occur at every study visit. Subjects will:

1. Be educated on the importance of replacing their patches every 7 days and we will develop an individualized behavior strategy to enhance compliance.
2. Record date of patch application, reviewed at each visit
3. Bring used an unused patches to each visit.
4. Be giving extra patches for replacement if necessary.

6.5 CONCOMITANT THERAPY

Not applicable

6.5.1 RESCUE MEDICINE

Not applicable.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 DISCONTINUATION OF STUDY INTERVENTION

At six-month intervals, the Biostatistician on the study will test whether there are significant differences between those in the active E2 versus placebo E2 for moderate or severe AEs. The statistical analysis method will be a two-sided Fisher exact Test, with alpha = 0.05. These results will be provided to the Data Safety and Monitoring Board (DSMB) along with the summary report of enrollment and other safety data. The DSMB will consider stopping the study based on the results of the statistical tests and other safety data.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

1. In the case of mood deterioration, Dr. Dichter (licensed clinical psychologist) will determine the next course of action, including a reassessment more frequently or more urgently if deemed to be clinically required. Participants will be withdrawn and referred for treatment to the UNC Center for Women's Mood Disorders if, in the opinion of Dr. Dichter the mood disturbance warrants pharmacological or other intervention and is rated Grade III or higher.

2. Any participant with emergent suicide ideation with intent or suicide behaviors will be removed from the study. Based on the clinical assessment by Dr. Dichter, she will either be taken to the emergency room or referred for treatment.

3. Development of a VTE, breast carcinoma, Stage 2 hypertension, or other medical conditions for which E2 is contraindicated.

4. Development of endometrial hyperplasia.

5. Start of an exclusionary prescription medication or herbal supplement (e.g., psychotropics)

6. Failure or inability to comply with study procedures as determined by study staff and ultimately by Dr. Girdler.

7. For participants who report experiencing current migraines (with or without aura) at the initial enrollment, staff will incorporate additional protocol to monitor all headaches they experience throughout the study protocol. Staff will document this headache assessment during each weekly visit, both before and after randomization. Should a participant report experiencing migraine with aura prior to randomization, they will not be randomized and complete the baseline visits only. If a participant reports a migraine with aura post-randomization, they will discontinue use of HRT immediately and be withdrawn from the study. If a participant reports an increase in frequency or severity of migraine without aura post-randomization, they will be referred to Dr. Munoz for further assessment.

7.3 LOST TO FOLLOW-UP

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within 7 days and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant. These contact attempts will be documented in the participant's medical record or study file.

- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

This is not a clinical efficacy trial.

8.2 SAFETY AND OTHER ASSESSMENTS

Safety Screening prior to randomization:

- **Physical examination**: height and weight will be measured to determine eligibility based on BMI. Gynecological exam by Investigator gynecologist will determine if subjects have any signs or history of endometrial disorder, abnormal uterine anatomy, or abnormal ovarian anatomy (confirmed by endometrial ultrasound and/or biopsy).
- **Vital signs** Three stethoscopic blood pressure readings will be measured at enrollment to rule out hypertension.
- **Radiographic assessments.** Subjects will be required to have normal results from a screening mammogram within 12 months prior to enrollment.

The Snaith-Hamilton Pleasure Scale (SHAPS): [104] assesses anhedonia. SHAPS scores range from 14-56, with higher scores corresponding to higher levels of anhedonia. This scale has shown adequate overall psychometric properties in clinical and student samples 105. The SHAPS has satisfactory test-retest validity in healthy participants (intraclass correlation coefficient = 0.70, $p < 0.001$ 106.

The State-Trait Anxiety Inventory (STAI): the state measure of anxiety from the STAI consists of items such as “I am worried” or “I feel calm” and participants complete the items as to how they currently feel. A higher score indicates higher anxiety. While there are no standard clinical cut-offs for the STAI, past research indicates scores ≥ 40 are clinically significant. [107,108] The scale has good median alpha reliability (.92). [109]

Dot-probe (DP) task (15 min): will measure attentional bias to threat (threat responsivity)[119]. An angry and a neutral face of the same individual are presented vertically simultaneously (trials with neutral faces are controls). Immediately following the face pair, a probe letter replaces either the angry face (threat congruent trial) or the neutral face (threat incongruent trial). Participants indicate the probe letter via button press. The task consists of 144 trials (48 threat congruent, 48 threat incongruent, 48 neutral). Trials are separated by a variable length inter-stimulus interval (900 - 1300 ms). Bias scores are calculated by subtracting the reaction time on threat congruent from threat incongruent trials. Positive scores reflect a bias towards threat and negative scores reflect a bias away from threat. Attention bias calculations exclude trials with incorrect responses, trials with reaction times <150 ms or >2000 ms or >2.5 SD outside the mean.

Effort Expenditure for Rewards Task (“EEfRT”) (20 min) will measure approach motivation that indexes the willingness to expend effort to obtain monetary rewards under varying conditions of reward probability and magnitude [120]. On each trial, participants choose between an “easy task” and a “hard

task" and are presented with information about the probability of winning (i.e., 12%, 50%, or 88%), and the magnitude of the potential reward if they complete the button-press task successfully (range:$1.24 - $4.12). Completion of the easy task requires 30 button presses in 7 seconds using the dominant index finger, whereas completion of the hard task requires 100 presses with the non-dominant "pinky" finger in 21 seconds. The % of hard task choices is the dependent measure. We have experience with this task in clinical studies of reward processing deficits [121].

The Trier Social Stress Test (TSST) involves social evaluation of speech and serial subtraction stress and induces large HPA axis responses 122-125. Baseline Rest (30 mins): The subject will rest for 30 minutes. Instructions: (5 min) Subjects are introduced to a 2-person committee. The subject will assume the role of a job applicant who is invited for a personnel interview with the committee. Anticipation: The subject prepares for 10 min. Speech: The committee asks the subject to deliver her talk for 5 mins. The committee responds with prepared questions to ensure that the talk lasts for 5 mins. Serial Subtraction: The subject is given a number to start from and asked to subtract a number from that original number as fast and as accurately as possible for 5 mins. For each mistake, the subject restarts at the original number. Stress Recovery: The subject rests alone for 45 mins. The characteristics of the TSST that elicit large HPA axis response include: 1) lack of controllability; 2) creation of a context of forced failure; and 3) social evaluative threat[28]. Thus, the TSST provides a physiologic unit of analysis for the construct of threat responsivity. To minimize habituation, we will use the second TSST at baseline week 8 as the comparison for all analyses (the first TSST will be used to eliminate the novelty effect). Modifications with each TSST will minimize habituation:[126] 1) a different speech topic, such as modifying the job; describing one's positive and negative personality attributes and impact on likeability[126]; 2) different numbers in the serial subtraction; 3) different, opposite sex committee members each time. With these modifications, some novelty is maintained even with repeat exposure, ensuring a significant HPA response.

Neuroendocrine Sampling during Stress Testing: Plasma cortisol will be taken at the end of baseline rest and again during Recovery at minutes 20, 30, and 45, wince HPA-axis reactivity is reliably seen 10-30 minutes after cessation of the TSST [22,26].

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

Adverse event means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

8.3.3.1 SEVERITY OF EVENT

The following guidelines will be used to describe severity of AEs.

- **Mild** –is defined as having no effect on activities of daily living such as transient light headedness or sweating with venipuncture; breast tenderness, mild skin irritation; or something of equal significance that requires no medical intervention and is of marginal clinical relevance.
- **Moderate** – would be associated with temporary (minutes to a few days) disruption in activities of daily living such as temporary loss of consciousness with venipuncture; a worsening of migraines or headache that require bed rest, an increase in anxiety or anhedonia symptoms consistent with clinically meaningful levels, emergent suicide intent without specific plan (severity level 4 on the C-SSRS), excess sleepiness or fatigue that resolves with additional rest, or something of equal significance.
- **Severe** – would cause serious disruption in daily activities of living and may or may not require hospitalization. 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 emergent suicide ideation with specific plan (severity level 5 on the C-SSRS) or any suicidal behavior (actual attempt, interrupted attempt, aborted attempt, or preparation towards imminent suicide attempt), or something of equal significance. The development of endometrial hyperplasia with or without atypia would also be consistent with a severe AE.

8.3.3.2 RELATIONSHIP TO STUDY INTERVENTION

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal. Rechallenge information is not required to fulfill this definition.
- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology.
- **Unknown** – based on the temporal relationship and clinical judgment of medical monitor, the relationship between the AE and study intervention cannot be determined.

8.3.3.3 EXPECTEDNESS

The Investigator gynecologist, PI, and study clinical psychologist will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the

nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

8.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The study coordinator will monitor side effects and AEs related to the pharmacological E2 probe at all study visits (on a weekly basis initially and then monthly for two months) and report all side effects as well as any significant deterioration in anxiety or anhedonic symptoms (see below) directly to Dr. Girdler following study visits. Side effects consistent with moderate or severe AEs or that are troublesome to the subject, or evidence of mood deterioration reported by telephone, will also be immediately reported to Dr. Girdler. Based on the clinical skill required not only to assess but to act on emergent suicidality, a licensed clinical psychologist or psychiatry resident will conduct the suicide assessments at each laboratory session (weeks 8, 16, 20 and 24) or more frequently if indicated (e.g., every two weeks for those with a history of suicide intent). Investigator licensed clinical psychologist, Dr. Dichter, will oversee the fellows and residents in this responsibility.

Bleeding patterns and severity will be assessed at every study visit. Moreover, subjects will be instructed to contact us whenever they start a menstrual bleed. Staff will follow up to determine the amount and duration of bleeding. For any woman with profuse bleeding (defined as more than 3 tampons or pads for more than 5 days OR more than 1 tampon or pad per hour on any given day), Dr. Munoz will be contacted, and she will decide whether to evaluate if clinically indicated. The timing of the bleeding with respect to the timing of the progesterone will contribute to that determination. Any unscheduled or prolonged bleeding (> 10 days) will be followed by an ultrasound or an endometrial biopsy or both. If ultrasound reveals the vaginal the stripe thickness to be more than 3 mm, then an endometrial biopsy will be performed. If the pathology report indicates hyperplasia, the subject will be withdrawn from the study, estrogen discontinued, and Dr. Munoz will prescribe a stronger progestin. If the subject has hyperplasia, a follow-up biopsy would be performed at 3 months to make sure it is cured. The research study will pay for all biopsies required, including the follow-up biopsy.

Dr. Girdler will ensure that Dr. Dichter or Dr. Munoz are consulted within 24-hours of any severe AE. Moderate AEs will prompt a follow-up evaluation of the subject within one week. If the condition has not resolved or has worsened, Dr. Dichter or Dr. Munoz will be contacted to determine the next course of action. Staff will be specifically trained to monitor the STAI-S and the SHAPS for > 50% increase in symptom severity from baseline and an increase in stratification (e.g., from a low symptom group to a medium group or from a medium group to a high symptom group). If the subject is already in a high anhedonia or anxiety strata, then any increase above 50% will be reviewed by Dr. Girdler. Staff will be specifically trained to monitor the STAI-S and the SHAPS for > 50% increase from baseline levels in symptom severity. In the case of elevations in anxiety and anhedonia symptoms Dr. Dichter will determine the next course of action, including more frequent assessment of mood if deemed to be clinically required. In the case of emergent suicide ideation with intent or any suicide-related behavior, the individual will be withdrawn from the study and the blind broken. If the participant appears to need immediate treatment, she will be escorted to the UNC emergency department. Otherwise, Dr. Dichter will meet with the participant to evaluate her safety and administer the C-SSRS. He will then determine the appropriate next steps, including referral to the UNC Center for Women's Mood Disorders or a community provider for further evaluation and treatment. 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).

8.3.5 ADVERSE EVENT REPORTING

The PI Dr. Girdler 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 an AE to the Chair of the DSMB according to the guidelines established at the initial DSMB meeting (e.g., the DSMB may require the reporting of any serious adverse event on an individual basis and in an on-going fashion). She will also report all severe AEs to the IRB and DSMB within 1 week. Since we are employing a marketed pharmaceutical product (i.e., a non-IND study), unexpected Severe AEs will also be reported to the FDA MedWatch Program. The NIMH program officer will be notified of any study modifications or suspension imposed by the DSMB or local IRB in response to an AE.

8.3.6 SERIOUS ADVERSE EVENT REPORTING

Dr. Girdler will also report all AEs graded as severe to Investigator Dr. Munoz. Dr. Munoz will maintain a secured, password protected file linking subject ID to subject name. Dr. Munoz will also receive the randomization code by subject ID from Investigational Drug Services. If it is medically necessary to become unblinded to treatment assignment, Dr. Munoz will be able to do so very quickly.

8.3.7 REPORTING EVENTS TO PARTICIPANTS

Not applicable.

8.3.8 EVENTS OF SPECIAL INTEREST

Not applicable.

8.3.9 REPORTING OF PREGNANCY

Since perimenopausal women may still become pregnant, especially those in the early menopause transition, we will assess pregnancy status by point of care urine pregnancy test for all participants under the age of 55. Pregnancy status will be tested via the use of urine hCG test (SA Scientific, San Antonio). Urine hCG is a rapid (results within minutes) qualitative test to detect the presence of hCG in urine to aid in the early detection of pregnancy. The SA urine hCG rapid test will be conducted in Dr. Girdler's lab using a small urine sample taken prior to the week 8 baseline laboratory session that precedes randomization to confirm that subjects are not pregnant. Women who are pregnant at this time will be withdrawn from the study. Dr. Girdler's lab has a CLIAA waiver certification from the NC Dept of Health to perform this assay at Carolina Crossing which expires September 02, 2021.

8.4 UNANTICIPATED PROBLEMS

8.4.1 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied.
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Corrective actions to be considered in response to an UP include:

- Modification of inclusion or exclusion criteria to mitigate the newly identified risks
- Implementation of additional safety monitoring procedures
- Suspension of enrollment of new participants or halting of study procedures for enrolled participants
- Modification of informed consent documents to include a description of newly recognized risks
- Provision of additional information about newly recognized risks to previously enrolled participants.

8.4.2 UNANTICIPATED PROBLEM REPORTING

The investigator will report unanticipated problems (UPs) to the reviewing Institutional Review Board (IRB) and to the Data Coordinating Center (DCC)/lead principal investigator (PI). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number.
- A detailed description of the event, incident, experience, or outcome.
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP.
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

- UPs that are serious adverse events (SAEs) will be reported to the IRB and to the DCC/study sponsor within 1 week of the investigator becoming aware of the event.
- Any other UP will be reported to the IRB and to the DCC/study sponsor within 1 week of the investigator becoming aware of the problem.
- All UPs should be reported to appropriate institutional officials (as required by an institution's written reporting procedures), the supporting agency head (or designee), and the Office for

Human Research Protections (OHRP) within the timeline in accordance with policy of the IRB's receipt of the report of the problem from the investigator.

8.4.3 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

Corrective actions to be considered in response to an UP include:

- Modification of inclusion or exclusion criteria to mitigate the newly identified risks
- Implementation of additional safety monitoring procedures
- Suspension of enrollment of new participants or halting of study procedures for enrolled participants
- Modification of informed consent documents to include a description of newly recognized risks
- Provision of additional information about newly recognized risks to previously enrolled participants.

9 STATISTICAL CONSIDERATIONS

NOTE: There will not be a formal Statistical Analysis Plan (SAP) attached to this protocol. Rather, details on all analysis plans are to be found in this section under the specific subheadings. We will test whether there are any differences between related population means (time effect, within-subject) and whether mean levels change across time based on the treatment group the participants were randomized to (time x treatment effect, within and between-subject).

9.1 STATISTICAL HYPOTHESES

- Primary Efficacy Endpoint(s):
    - 1. Change in State-Trait Anxiety Inventory score [Time Frame: Baseline (Week 8), Weeks 16, 20 and 24]
        - H0: The mean scores of the State-Trait Anxiety Inventory are the same at baseline (week 8), and weeks 16, 20, and 24, both, in those randomized to active treatment and in those randomized to placebo.
        - H1: The mean scores of the State-Trait Anxiety Inventory will significantly change from baseline (week 8) to weeks 16, 20, and 24 in those randomized to active treatment, but not in those randomized to placebo.
    - 2. Change in SHAPS score [Time Frame: Baseline (Week 8), Weeks 16, 20 and 24]
        - H0: The mean scores of the SHAPS will not change from baseline (week 8) to weeks 16, 20, and 24, both, in those randomized to active treatment and in those randomized to placebo.
        - H1: The mean scores in the SHAPS score will significantly change from baseline (week 8) to weeks 16, 20, and 24 in those randomized to active treatment, but not in those randomized to placebo.

- Secondary Efficacy Endpoint(s):
    - 1. Change in AUC cortisol response [Time Frame: Baseline (Week 8), Weeks 16, 20 and 24]

- H0: The cortisol AUC will not change from baseline (week 8) to weeks 16, 20, and 24, both, in those randomized to active treatment and in those randomized to placebo.
- H1: The cortisol AUC will significantly change from baseline (week 8) to weeks 16, 20, and 24 in those randomized to active treatment, but not in those randomized to placebo.

- 2. Change in "threat bias" score from Dot Probe task [Time Frame: Baseline (Week 8), Weeks 16, 20 and 24]
  - H0: The mean score in the Dot Probe task will not change from baseline (week 8) to weeks 16, 20, and 24, both, in those randomized to active treatment and in those randomized to placebo.
  - H1: The mean score in the Dot Probe task will significantly change from baseline (week 8) to weeks 16, 20, and 24 in those randomized to active treatment, but not in those randomized to placebo.
- 3. Change in "hard choice" score from EEfRT [Time Frame: Baseline (Week 8), Weeks 16, 20 and 24]
  - H0: The mean score in the EEfRT task will not change from baseline (week 8) to weeks 16, 20, and 24, both, in those randomized to active treatment and in those randomized to placebo.
  - H1: The mean score in the EEfRT task will significantly change from baseline (week 8) to weeks 16, 20, and 24 in those randomized to active treatment, but not in those randomized to placebo.

9.2 SAMPLE SIZE DETERMINATION

Sample size calculations were performed for the primary outcome measures. Correlation and effect size estimations were based on previously published literature [173,174.] With a sample size of N=120 and performing hypothesis tests at two-sided alpha=.05 and a correlation of >.25 among repeated measures, we have >90% power to detect a small effect size of .15.

The G*power software was used to determine the sample size [175], based on a repeated measures ANOVA with within-between interaction [173,174]. We conservatively account for 15% (n =18) withdrawal following randomization, yielding 138 women who complete the protocol (powered for 120). This sample size will provide sufficient power to address secondary endpoints. No exploratory analyses will be performed.

By using proc mixed (not GLM), we will apply an analysis which is relatively robust to missing data. Before applying proc mixed, we will check for the randomness of missing data.

9.3 POPULATIONS FOR ANALYSES

We will use an Intention-to-Treat (ITT) Analysis Dataset, including all randomized participants (either to transdermal estradiol patch and micronized progesterone pill, or placebo patch and placebo pill.

9.4 STATISTICAL ANALYSES

9.4.1 GENERAL APPROACH

Descriptive statistics of continuous data will be presented as adjusted means (least squared means) and standard errors. A two-tailed p-value of ≤ .05 will be considered as statistical significance. Examinations of residual normality, linearity, homogeneity of variance, and influential outliers will be performed using the SAS Macro Mixed DX [176]. Outliers at 2 SD's or more above or below the mean will be winsorized. For cortisol, the distribution of the values before and after every Trier Social Stress Test will be visually inspected and, in the case of skewness, a log-transformation will be performed.

We will use two-way repeated measures analyses with between-subject factors. Our models will test whether there is a mean value change in the primary and secondary outcomes from week 8 to weeks 16, 20, and 24 (within-subject analyses) and based on the treatment arm (between-subject analyses of transdermal estradiol vs. placebo treatment groups). We expect a significant change over time in the primary and secondary outcome measures in those randomized to active transdermal estradiol, but not in those randomized to placebo. Data will be analyzed using proc mixed in SAS. All models will use an auto-regressive covariance structure to account for the repeated assessments of primary and secondary outcomes in the same participants.

9.4.2 ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

For each primary endpoint:

1.Change in the Anxiety score from State-Trait Anxiety Inventory
State anxiety will be assessed using the validated State-Trait Anxiety Inventory. The State-Trait Anxiety Inventory consists of 20 questions on a 4-point force-choice Like-type response scale (scores 0 - 3). The 20 questions are summed together for a final score. The score can range from 0 to 60 with higher scores representing higher levels of anxiety. Continuous, interval scaled sum scores will be calculated and treated as a repeated measure (assessed at weeks 8, 16, 20, and 24). Change in State-Trait anxiety inventory will be analyzed using two-way repeated-measures analysis for a time and treatment effect (proc mixed in SAS). Models will use an auto-regressive covariance structure to account for the repeated assessments in the same participants. Outliers at 2 SD's or more above or below the mean will be winsorized. Missing data will not be imputed, as the statistical procedures we will apply (proc mixed) are robust to randomly distributed missing data. Before applying proc mixed, we will check for the randomness of missing data. Results of the statistical procedures will be presented as adjusted means (least squared means) with standard errors.

2. Change in anhedonia score from Snaith-Hamilton Pleasure Scale
Anhedonia will be assessed using the validated Snaith-Hamilton Pleasure Scale (SHAPS). Scores range from 0-14, with higher scores corresponding to higher levels of anhedonia. The questionnaire will be applied at weeks 8, 16, 20 and 24. Continuous, interval scaled sum scores will be calculated and treated as a repeated measure (assessed at weeks 8, 16, 20, and 24). Change in the SHAPS will be analyzed using two-way repeated-measures analysis for treatment x week (proc mixed in SAS). Models will use an auto-regressive covariance structure to account for the repeated assessments of primary and secondary outcomes in the same participants. Outliers at 2 SD's or more above or below the mean will be winsorized. Missing data will not be imputed, as the statistical procedures we will apply (proc mixed) are robust to randomly distributed missing data. Before applying proc mixed, we will check for the

randomness of missing data. Results of the statistical procedures will be presented as adjusted means (least squared means) with standard errors.

9.4.3 ANALYSIS OF THE SECONDARY ENDPOINT(S)

All analyses of the secondary endpoints are independent of the primary endpoints.

For each secondary endpoint:

1. Changes in Cortisol stress response

The cortisol response will be presented as Area Under the Curve (AUC) with respect to ground and calculated using the trapezoid formula described by Pruessner [177]. At weeks 8, 16, 20, and 24, cortisol was assessed around a standardized psychosocial stress test – the Trier Social Stress Test. Before the stress test, a baseline, pre-stress serum sample was taken. After the end of the stress test, reactivity serum samples were taken at 10-, 20-, 30-, and 45-minutes post-stress. The distribution of the values at every single timepoint, at every week will be visually inspected and, in case of skewness, log-transformed before calculating the AUC. All AUC's will be treated as continuous, interval scaled measures and treated as repeated measure (assessed at weeks 8, 16, 20, and 24). Results of the statistical procedures will be presented as adjusted means (least squared means) with standard errors. Outliers at 2 SD's or more above or below the mean will be winsorized. In case of missing values for one or more single timepoints around the stress task, the AUC will not be calculated and as a consequence treated as missing data. The statistical procedures we will apply (proc mixed) are robust to randomly distributed m missing data.

2.&3. Changes in the EEfRT and Dot Probe task

Continuous, interval scaled sum scores will be used for statistical models testing changes in the EEfRT and the Dot probe task. Both scores will be treated as repeated measures (assessed at weeks 8, 16, 20, and 24). Results of the statistical procedures will be presented as adjusted means (least squared means) with standard errors. Outliers at 2 SD's or more above or below the mean will be winsorized. Missing data will not be imputed, as the statistical procedures we will apply (proc mixed) are robust to randomly distributed missing data.

9.4.4 SAFETY ANALYSES

At six-month intervals, the Biostatistician on the study will test whether there are significant differences between those in the active E2 versus placebo E2 for moderate or severe AEs. The statistical analysis method will be a two-sided Fisher exact Test, with alpha = 0.05. These results will be provided to the Data Safety and Monitoring Board (DSMB) along with the summary report of enrollment and other safety data. The DSMB will consider stopping the study based on the results of the statistical tests and other safety data.

9.4.5 BASELINE DESCRIPTIVE STATISTICS

The two intervention arms will be compared on baseline (week 8) characteristics in the SHAPS score, the State-Trait Anxiety Inventory score, the EEfRT and Dot Probe scores, and the Cortisol AUC. Differences between the two groups will be compared using inferential statistics (student t-Tests).

9.4.6 PLANNED INTERIM ANALYSES

See section 9.4.4 for details on safety analysis.

9.4.7 SUB-GROUP ANALYSES

The study intervention (transdermal estradiol patch and micronized progesterone pill) is only for use in women. Therefore, no sub-group analyses will be performed.

9.4.8 TABULATION OF INDIVIDUAL PARTICIPANT DATA

Not applicable

9.4.9 EXPLORATORY ANALYSES

Not applicable

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 INFORMED CONSENT PROCESS

10.1.1.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Consent forms describing in detail the purpose of the study, 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.

10.1.1.2 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 investigator 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. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or 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 must be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records.

The informed consent process will be conducted and documented in the source document (including the date), 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.

10.1.2 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, investigator, funding agency, and regulatory authorities. 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.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or Food and Drug Administration (FDA).

10.1.3 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 and genetic tests 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 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 in the study database. This 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 study data entry and study management systems used by clinical sites and by 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 research site.

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.

10.1.4 FUTURE USE OF STORED SPECIMENS AND DATA

Data collected for this study will be analyzed and stored at the research site. After the study is completed, the de-identified, archived data will be transmitted to and stored at the research site, for use by other researchers including those outside of the study. Permission to transmit data will be included in the informed consent.

With the participant's approval and as approved by local Institutional Review Boards (IRBs), de-identified biological samples will be stored at the research site with the same goal as the sharing of data with biospecimen repository. These samples could be used to research the factors that cause or contribute to the development of mood disorders in the menopause transition, its complications, and other conditions for which individuals in the menopause transition are at increased risk, and to improve treatment. The repository will also be provided with a code-link that will allow linking the biological specimens with the phenotypic data from each participant, maintaining the blinding of the identity of the participant.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to biosample storage may not be possible after the study is completed.

When the study is completed, access to study data and/or samples will be provided through the only personnel approved by the study investigator.

10.1.5 KEY ROLES AND STUDY GOVERNANCE

Principal Investigator	**Medical Monitor**
Susan Girdler, Ph.D.	*Maria Munoz, MD.*

UNC SOM - Psychiatry	*UNC OB/GYN*
101 Manning Dr. CH, 27514	*101 Manning Dr., CH, 27514*
(919) 966-2544	*(650) 815-9041*
Susan_girdler@med.unc.edu	*Cris_munoz@med.unc.edu*

10.1.6 SAFETY OVERSIGHT

The safety of the study will be monitored by a Data and Safety Monitoring Board (DSMB). This will ensure the independent oversight of issues related to safety and adverse events. The North Carolina Translational and Clinical Sciences (NC TraCS) Data and Safety Monitoring Board (NC TraCS DSMB) will be used for this purpose. The Board is composed of MDs from a variety of disciplines, an R.N. from the UNC School of Nursing, a Biostatistician, a Regulatory Expert, and for purposes of this study, a psychiatrist or clinical psychologist will serve as an ad hoc member.

The NC TraCS DSMB will monitor for AEs, including medical events (e.g., VTEs) and psychological events (e.g., significant deterioration in anxiety or anhedonia symptoms and emergent suicidal intent or behavior). The DSMB will specify the tables and data it wishes to have presented to it at all meetings, including but not limited to, all side effects and symptoms recorded at the study visits (see Adverse Events Form) and all interim phone calls initiated by research subjects to report AEs or concerns. DSMB review of this study will occur every six months (or more frequently if specified by the DSMB). At these meetings, the DSMB will also review data from subjects who have withdrawn or dropped out of the study and the reasons for drop out. The DSMB will evaluate issues of participant safety as well as enrollment information and the adequacy and integrity of accumulating data. The DSMB 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.

10.1.7 CLINICAL MONITORING

Not applicable.

10.1.8 QUALITY ASSURANCE AND QUALITY CONTROL

The research site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.1.9 DATA HANDLING AND RECORD KEEPING

10.1.9.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into Microsoft Access and Qualtrics, a 21 CFR Part 11-compliant data capture system provided by 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. Clinical data will be entered directly from the source documents.

10.1.9.2 STUDY RECORDS RETENTION

Study documents should be retained for 5 years after the last publication. At that point, all paper data will be shredded. Biological samples will be destroyed according to OSHA procedures. The linkage file will also be destroyed.

10.1.10 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations. All deviations must be addressed in study source documents and reported to NIH Program Official per NIH guidelines. Protocol deviations must be sent to the reviewing Institutional Review Board

(IRB) per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements.

10.1.11 PUBLICATION AND DATA SHARING POLICY

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.

Consistent with NOT-MH-14-015, we will submit data to the RDoC database. This database is built on the infrastructure of the National Database for Autism Research (NDAR). The project coordinator will work closely with the PIs and the data manager and be responsible for data preparation and submission to RDoCdb. We will use appropriate informed consent language to inform potential participants of the sharing of their data through RDoCdb. We commit to use RDoCdb template language during recruitment and to augment the informed consent process as necessary. To accomplish this objective, we will formulate an enrollment strategy that will obtain the information necessary to generate a GUID for each participant. We will build in procedures to be able to capture the personally identifiable information needed to create a valid GUID. We will utilize data structures already defined by RDoCdb as much as possible to ease the process of data submission and sharing. Where data structures have not yet been defined in RDoCdb, we will work with RDoCdb staff to define data structures for any data being collected as part of the study. Immediately upon award of this grant, we will complete and share the necessary RDoCdb Data Submission Documents so others can see what kind of research we are conducting. We commit to submit descriptive data two times per year and will provide supporting documentation as necessary for others to more fully understand the manner in which data were collected. We will submit cumulative data each submission cycle and will review the data to look for any personally identifiable information and ensure that data are loaded correctly. We commit to sharing data within 4 months after submission (both descriptive and experimental data) and will submit experimental data within 12 months after the study completion. We commit to create an RDoCdb Study for each publication related to the grant and to submit a link to the RDoCdb study along with any publications so readers of articles can link back to the data used in RDoCdb.

10.1.12 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 NIMH 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.

10.2 ADDITIONAL CONSIDERATIONS

Not applicable.

10.3 ABBREVIATIONS

AE	Adverse Event
AUC	Area Under the Curve
ACTH	Adrenocorticotropic Hormone
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
COC	Certificate of Confidentiality
CORT	Cortisol
CRF	Case Report Form
CVD	Cardiovascular Disease
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
E2	Estradiol
EEfRT	Effort Expenditure for Reward Task
ER	Estrogen Receptor
ERT	Estrogen Replacement Therapy
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GWAS	Genome-Wide Association Studies
HIPAA	Health Insurance Portability and Accountability Act
HPA-Axis	Hypothalamic-Pituitary-Adrenal Axis
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IND	Investigational New Drug Application
IRB	Institutional Review Board
ITT	Intention-To-Treat
LSMEANS	Least-squares Means
MDD	Major Depressive Disorder
MI	Myocardial Infarction
MT	Menopause Transition
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
OHRP	Office for Human Research Protections
P4	Progesterone
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
RCT	Randomized Controlled Trial
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SHAPS	Snaith-Hamilton Pleasure Scale
SOA	Schedule of Activities
SOP	Standard Operating Procedure
STAI	State-Trait Anxiety Inventory
STRAW+10	Staging of Reproductive Aging Workshop
UP	Unanticipated Problem
US	United States
WHI	Women's Health Initiative
VTE	Venous Thromboembolism

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