Study Protocol

Ovarian Hormones, Reward Response, and Binge Eating in Bulimia Nervosa: An Experimental Design

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Ovarian Hormones, Reward Response, and Binge Eating in Bulimia Nervosa: An Experimental Design

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Funding Sponsor:	National Institute of Mental Health 6001 Executive Boulevard, Room 6200, MSC 9663 Bethesda, MD 20892-9663
Study Product:	**Estradiol** Active ingredient: micronized estradiol Chemical name: estra-1,3,5,(10)-triene-3, 17β-diol **Lupron Depot** Active ingredient: leuprolide acetate for depot suspension Chemical name: 5-oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-leucyl-L-leucyl-L-arginyl-N-ethyl-L-prolinamide **Progesterone** Active ingredient: micronized progesterone Chemical name: pregn-4-ene-3, 20-dione
IRB Protocol Number:	IRB# 19-2343
IND Number:	*Exempt*

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Protocol Updates:

November 11, 2021: Updated medication dispense protocol to require second pharmacist verification

October 20, 2021: Updated pharmacy medication dispense procedures

August 12, 2021: Updated exclusion criteria “pregnancy related excessive vomiting and high blood pressure.”

April 22, 2021: Approval to provide participants with mileage reimbursement for study visit travel

March 29, 2021: IRB approval to hire clinical trial recruitment company

November 13, 2021: Update BMI to <35

October 8, 2020: Additional COVID protocols

July 27, 2020: COVID protocols and procedures

List of Abbreviations

AE	Adverse Event
BAS	Behavioral approach system
BIS	Behavioral Inhibition system
BMI	Body Mass Index
BN	Bulimia Nervosa
CBT	Cognitive Behavior Therapy
CHD	Coronary Heart Disease
CMP	Comprehensive Metabolic Panel
CRF	Case Report Form
DA	Dopamine
DSMB	Data and Safety Monitoring Board
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, 5th Edition
DVT	Deep Vein Thrombosis
E2	Estradiol
EDs	Eating Disorders
EC	Ethics Committee

EPT	Combined Estrogen and Progestin Treatment (EPT)
ERT	Estrogen Replacement Therapy
FDA	Food and Drug Administration
FSH	Follicle Stimulating Hormone
GnRH	Gonadotropin-Releasing Hormone
HIPAA	Health Insurance Portability and Accountability Act
HCG	Human Chorionic Gonadotropin
IDAS	Inventory of Depression and Anxiety Symptoms
IDS	Investigational Drug Service
IM	Intramuscular
IRB	Institutional Review Board
IVF	In Vitro Fertilization
LH	Luteinizing Hormone
NIH	National Institutes of Health
P4	Progesterone
PHI	Protected Health Information

PMDD	Premenstrual Dysphoric Disorder
PMS	Premenstrual Syndrome
SAE	Serious Adverse Event
SCID	Structured Clinical Interview for DSM-5-TR Axis-I Disorders
UNC	University of North Carolina at Chapel Hill

Study Summary

Title	Ovarian Hormones, Reward Response, and Binge Eating in Bulimia Nervosa: An Experimental Design
Short Title	Neurobiology of Binge Eating
Protocol Number	IRB# 19-2343
Phase	II
Methodology	Double-blind, placebo-controlled, longitudinal comparison study
Study Duration	4 months
Study Center(s)	Single-center
Objectives	Proof-of-concept pilot study: examine the direct impact of ovarian hormones (i.e., E2 and P4) on binge eating and the behavioral reward response in women with BN
Number of Subjects	15
Diagnosis and Main Inclusion Criteria	A current DSM-5 diagnosis of BN, age 18-42, BMI < 35, and a regular menstrual cycle for at least three months; not pregnant, not lactating and in general good health, no medications or medical history contraindicated for use with study medications, no history of suicide attempts or bipolar/psychotic disorder, no current substance use disorder
Study Product, Dose, Route, Regimen	**Study Product:** **Estradiol** Active ingredient: micronized estradiol Chemical name: estra-1,3,5,(10)-triene-3, 17β-diol **Lupron Depot** Active ingredient: leuprolide acetate for depot suspension Chemical name: 5-oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-leucyl-L-leucyl-L-arginyl-N-ethyl-L-prolinamide **Progesterone** Active ingredient: micronized progesterone Chemical name: pregn-4-ene-3, 20-dione

Dose:

Drug	Dosage	Formulation
Estradiol	2 mg bid	Oral capsule
Lupron Depot	3.75 mg/month	Intramuscular injection
Progesterone	200 mg bid	Oral capsule

Route:

Drug	Route of Administration
Estradiol	Oral
Lupron Depot	Intramuscular injection
Progesterone	Oral

Regimen:

Induced Hypogonadism. After a baseline period, participants will receive their first injection of the gonadotropin-releasing hormone (GnRH) agonist leuprolide acetate (Lupron) 3.75 mg/month via intramuscular injection, which is administered to produce a stable hypogonadal condition (after the initial "flair"). Lupron is administered at monthly intervals thereafter for a total of 3 doses.

Addback. After 6-weeks of Lupron-alone treatment (i.e., hypogonadism), physiological plasma levels of E2 and P4 will be attained via micronized E2 and P4 tablets for two weeks (with continued Lupron administration), respectively. E2 and P4 will be administered in a double-blind, cross-over design with a 2-week washout period in-between E2 and P4 administration. E2 will be administered at a dose of 2 mg bid (i.e., a total of 4 mg per day). P4 will be administered at a dose of 200 mg bid (i.e., a total of 400 mg per day). The blood levels that we expect to achieve and

	sustain in each woman will be approximately 500 pg/ml of E2 and 30-40 ng/ml of P4.
Duration of administration	3 months
Reference therapy	Placebo
Statistical Methodology	A 2 x 2 double-blind crossover design, with 2-sequence, 2-period and 2-treatment will be implemented

1 Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

1.1 Background

EDs are serious mental health conditions affecting 15 million women in the United States[1] and have one of the highest mortality rates of any mental illness,[2] yet the underlying neurobiology remains poorly understood. EDs predominantly occur in women,[3] and the frequency of certain symptoms change in a predictable pattern over the menstrual cycle.[4] Specifically, symptom change appears to be triggered by ***normal*** fluctuations in the ovarian hormones E2 and P4;[5, 6] this is particularly evident for binge eating. In women with BN, high levels of E2 are associated with *reduced* binge-eating whereas high levels of P4 are associated with *increased* binge-eating[5] during a normal menstrual cycle. Thus, we have hypothesized BN represents a hormone sensitive phenotype: women with BN display differential sensitivity to normal changes in ovarian hormones.[4, 7] Unraveling the neurobiology of BN has the potential to open innovative avenues for treatment for this life-impairing illness.

Decades of preclinical work confirms E2 has a direct, protective effect on food intake, binge-type behaviors, and weight-related constructs.[8-10] No comparable experimental designs have been conducted in humans. Cumulatively, it appears that low E2 or P4 antagonized E2 (both E2 and P4 are *high*) are risky milieus for binge eating.[11] The exact hormone milieu that is "risky" may depend on the level of pathology present: subsetting a community sample into DSM-defined binge eaters vs. non- binge eaters showed a significant inverse main effect for E2.[12] This suggests that for those with higher levels of pathology, low E2 may be the catalyst for symptom exacerbation. In contrast, the follicular and ovulatory phases may be protective: across all studies, binge-eating frequency is lowest during these phases.[5, 6, 13-15] During the follicular phase, E2 is rising in preparation for ovulation and reaches peak levels at ovulation whereas P4 is low and relatively stable. Finally, two recent case reports show manipulation of E2/P4 directly effects symptomatology. The introduction of a P4-only hormonal contraceptive relapsed gains made in treatment for BN, which was reversed upon stopping the medication.[16] Impressively, complete recovery from subclinical binge eating disorder was also achieved following surgical ovarian suppression (i.e., bilateral salpingo-oophorectomy and hysterectomy) treatment for PMDD,[17] which results in the absence of E2/P4. This could suggest that, unlike animals, hypogonadism may result in symptom relief for humans: hormone sensitivity may reflect sensitivity to *changing* levels vs. the presence (or absence) of acute levels. This is corroborated by the fact menopause (i.e., hypogonadism) does not

result in increased ED symptoms, yet the menopause transition (i.e., chaotic changes in ovarian hormones) does.[18]

To date, the effects of ovarian hormones on eating behaviors have been inferred from animal studies and from changes in behavior occurring with presumed and measured levels of hormones during the menstrual cycle. Although animal studies established that E2 controls eating behaviors,[8, 9] studies have limitations: it is unclear if animal studies can translate to human behavior and observational human studies cannot conclude causality or tease apart the effects of E2 and P4 given the presence of both. Moreover, the neurobiological mechanisms underlying *why* ovarian hormones are responsible for symptom fluctuation remains unknown. E2 has pronounced effects on certain neuropathways[19-21] and neurotransmitters[22, 23] and in particular, may influence binge eating through its effect on reward processes that are altered in BN.[24-26] Women with BN show reduced brain activation in dopamine-related reward pathways,[27] increased reward sensitivity,[28, 29] and inhibitory control deficits[30] compared with healthy controls, which together, may result in increased reward-motivated behaviors (e.g., binge eating). Indeed, E2 replacement in ovariectomized rats decreases food reward-motivated behavior[31] and, in women, delay gratification is inversely associated with E2[32] whereas E2 suppression reduces reward responsivity.[33] Together, this suggests E2 neuromodulation of aspects of the reward response may be responsible for changes in binge eating observed across the menstrual cycle.

Indeed, women with BN tend to be more sensitive to reward,[28, 29, 34-36] display a preference for smaller rewards now vs. larger rewards later (i.e., delay discounting),[37-40] and have inhibitory control deficits associated with impulsivity.[30, 41-43] Certain aspects of reward processing and the reward response are powerfully modulated by E2,[22, 44] albeit the direction is inconsistent with some studies showing a beneficial effect[31, 45, 46] and others a worsening[47-49] effect. This directionality appears to, in part, differ by the aspect of reward addressed.[22, 50] Regardless, some work suggests that E2 replacement in ovariectomized rats decreases food-reward behavior[31] and in women, reward sensitivity is heightened during the late luteal phase,[51] which is marked by a steep decline in E2 and P4, and risk taking behavior and impulsivity are lowest when E2 levels are highest.[46, 52-54] Reward-motivated behavior related to choice (vs. action) has a more consistent inverse association with E2: delay discounting is greater in females than males in animals[50, 55, 56] yet E2 may attenuate delayed discounting and inhibition in humans and animals.[22, 32, 45, 57]

Individual characteristics may determine if E2 has a beneficial or worsening effect, which could also account for inconsistences observed in the direction of the effect of E2 on reward-motivated behaviors such as binge eating. Specifically, dopamine has an established effect on multiple aspects of reward[22, 23, 44, 58] and there is evidence that the effect of E2 may be dopamine activity dependent.[59-63] Preclinical studies established that E2 enhances dopamine activity,[64-66] but at its highest levels E2 may inhibit dopamine.[23] Also, dopamine appears to have an inverted U effect: an optimal amount of dopamine results in maximal function whereas insufficient or excessive levels lead to dysfunction,[58, 67, 68] suggesting that the effect of E2 on dopamine [reward] motivated behaviors depends on baseline dopamine.[23, 46, 60, 69] Specifically, E2 is beneficial for subgroups with *lower* baseline dopamine (enhancing activity to the optimal level) and worsening for subgroups with higher baseline dopamine (leading to an excessive amount of dopamine). Corroborating this, the beneficial effect of E2 on delay discounting is driven by *COMT* genotype, which is often used as a proxy for baseline dopamine activity: declines in delay discounting during high E2 were observed among *COMT* Val allele carriers,[32] the allele associated with less dopamine activity. This is highly relevant for BN:[70, 71] BN is associated with decreased dopamine activity,[72-74] reduced brain activation in dopamine-related reward pathways,[27, 75] and possibly a higher frequency of gene alleles associated with decreased dopamine;[71, 76] though this is inconsistent,[77] unconfirmed by genome-wide studies, and perhaps an indirect and/or interactive association.[78-80] Thus, because women with BN represent a subgroup of the population with low dopamine activity, low E2 may detrimentally affect the reward

response and further promote reward-motivated behavior via dopamine withdrawal, whereas high E2 is beneficial by enhancing dopamine activity to an optimal level, in turn decreasing the reward response and reward behaviors.

Together, we hypothesize women with BN are a hormone sensitive phenotype with precursory impaired dopamine activity, leading to increased reward response (e.g., reward-related inhibitory control deficits, heightened reward sensitivity, impaired delay discounting) and therefore, increased engagement in reward-motivated behavior, which is modulated by cycling E2 (Figure 1). Decreasing E2 triggers a cascade of neurobiological events leading to the onset of or increased engagement in reward-motivated behaviors (i.e., binge eating) in vulnerable women (i.e., low dopamine activity). While others made postulations regarding reward-related inhibitory control,[24, 34, 81] few considered the effect of E2[25, 82-84] Although some,[85] but not all,[5] studies suggest dysregulated hormone levels in women with BN, we do not hypothesize physiologically aberrant ovarian hormone levels are a precursor to BN, rather behavioral reactions to normal hormone changes are dysregulated in women with [or at risk for] BN due to an underlying sensitivity to this change. Currently, research in this area focuses on E2 and overlooks P4; assuming that the effect of P4 is indirect and not causal. However, we could be overlooking an important etiological construct given P4 also directly impacts dopamine levels,[86] which are at the core of our hypothesized model.

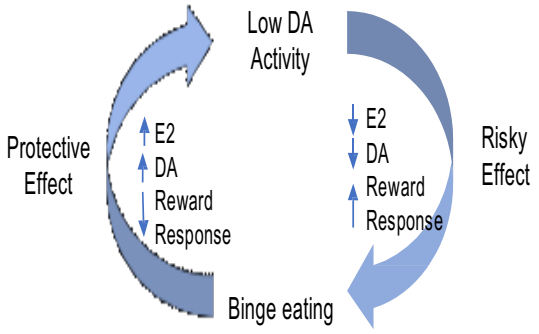


Figure 1. Hypothesized cycle of the effect of E2 on binge eating

The objective of this proposal is to examine the direct and mechanistic role of ovarian hormones on binge eating in women with BN (n = 15). Our overarching hypothesis is that BN represents a hormone sensitive phenotype and this sensitivity is modulated by E2's effects on aspects of the reward response such that reward-motivated behaviors increase in the context of low E2. For the first time in humans, we propose an experimental design that parallels animal models to directly manipulate ovarian hormones: temporarily stopping the menstrual cycle using a GnRH agonist and addback E2 and P4 independently in a double-blind crossover design. This utilizes an established design developed to determine the hormonal triggers of premenstrual dysphoric disorder PMDD and depression.[87-89] We propose this proof-of-concept study to obtain empirical evidence supporting our overarching hypothesis and the utility of this experimental design in a BN population. The experimental design proposed here is the only way to confidently explore the direct impact of ovarian hormones on binge eating in BN. Observational designs provide insight but cannot quantify the mechanism in which ovarian hormones impact binge eating. Further, the impact of E2 on neurocircuitry contributing to BN has been deprioritized by research to date.[25] Successful completion of the proposed aims will provide guidance in regard to if (and how) E2 and P4 directly affect binge eating in BN. This will not only provide the empirical direction needed for the investigators to complete larger, *hypothesis-driven* mechanistic trials, but will provide direction for future research addressing neuroendocrine, neurobiological, and brain activity and function in BN.

Rationale for experimental manipulation in BN population: the direct benefit to participants will be limited and *we are not conducting a clinical treatment trial* but a mechanistic clinical trial (as defined by the NIH). However, we do hypothesize a beneficial effect will be observed during some treatment arms, in particular during the E2 condition. Additionally, as described above, one case study showed complete recovery from subclinical binge eating disorder following surgical ovarian suppression via a bilateral salpingo-oophorectomy and hysterectomy for PMDD.[17] Compared with this surgery, ovarian suppression through an intervention such as Lupron is much less invasive. Thus, if our hypotheses are correct an obvious treatment implication could be the direct manipulation of ovarian hormones with the medications used here or the use of treatments that buffer the impact of these hormones on neurobiological function. Explicating the mechanism through which E2

inhibits (and/or P4 exacerbates) binge eating behavior could lead to the development of interventions based upon this mechanistic understanding. To date, there are no medications that have been developed specifically for the treatment of individuals with BN. Further, if a beneficial effect is observed for a specific treatment condition, follow-up clinical treatment trials could be conducted—for example, by beginning to explore the dosages at which a beneficial effect is observed and effectiveness when used in combination with other treatments (e.g., CBT). Additionally, one goal for this line of research is to further our mechanistic understanding of eating disorders/binge eating in order to develop more personalized approaches to treatment. Although we hypothesize that menstrual exacerbation of binge eating is due to an underlying sensitivity to normally fluctuating ovarian hormones, some women with an eating disorder may experience this sensitivity to a greater degree—or this sensitivity may not be present across all eating disorder subtypes. Thus, our larger studies (R01) and subsequent follow-up studies will aim to identify predictors of hormone sensitivity: women who binge eat who may benefit from the direct manipulation of ovarian hormones or treatments that buffer the impact of these hormones on neurobiological function. With this line of research, we hope to enable discovery of novel neural treatment targets and therapeutics to ultimately prevent illness expression.

The major benefit of this study is to aid in understanding the underlying neurobiological mechanisms in BN, leading to larger trials ultimately aimed at creating novel, individualized therapeutics. This line of research could lead to the development of medications that have less risk for side effects than the ones used here; for example, pharmacological interventions developed to target specific areas of the brain, brain receptors, or pathways identified to be involved in the mechanism underlying ovarian hormone change and binge eating. This pilot study will lay the groundwork for this line of research. For example, results from this pilot study and the larger mechanistic trial could lead to therapeutics that are developed to selectively target specific receptors in the brain or specific regions in the brain to alleviate binge eating (notably, in our future trials we plan to include an fMRI component), and other symptomatology, in BN. There is a major disconnect between the significant public health impact of BN and our knowledge about the neurobiology of the disorder. Further, patients with BN struggle with barriers to treatment and a fragmented health care system. Although psychotherapy is typically the first treatment of choice for BN,[90, 91] it is not readily accessible or affordable.[92,93] For those who receive evidence-based treatment, the effectiveness of psychotherapy varies across individuals[94] and over 60% of BN patients do not reach symptom abstinence from psychotherapy alone.[95] Moreover, there is only one FDA approved pharmacological treatment (Fluoxetine) for BN and innovative avenues for pharmacological treatment have been overlooked. Given that the cost per abstinent patient for Fluoxetine is nearly half that for psychotherapy ($12,146 vs. $20,317),[96] innovative avenues for pharmacological treatment must be pursued, which could improve treatment accessibility and outcome for BN. Implementing high risk research, yet that has the significant potential for high reward is the first step in opening doors for innovative new treatments to be developed. **Prior to study participation, all subjects will complete informed consent so that they can make a fully informed and educated decision regarding the possible risks associated with study participation.**

Women who participate in this study will received personalized feedback regarding their individual response to the experimental arms. Study participants will obtain personalized feedback at study completion of the study about any changes in BN symptoms observed during the experimental phases from the study PI. This *individual-level feedback* could provide an area of treatment for the participant to pursue and identify potential triggers for symptom exacerbation for that specific individual. We have also observed many altruistic benefits in our past eating disorder studies. Specifically, we frequently receive feedback from study participants that they are excited to participate in research studies that contribute to a better understanding of what causes an eating disorder, regardless of a direct benefit for them, in hopes that someone else in the future may not have to suffer.

1.2 Investigational Agent

Estradiol

Description

Estradiol Tablets USP for oral administration contain 2 mg of micronized estradiol per tablet. Estradiol (17β-estradiol) is a white, crystalline solid, chemically described as estra-1,3,5,(10)-triene-3, 17β-diol. The structural formula is:

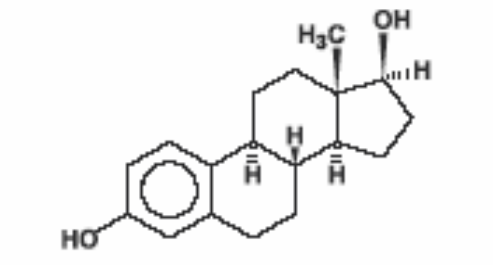


Figure 2. Structural formula for estra-1,3,5,(10)-triene-3, 17β-diol.

Inactive Ingredients: Colloidal silicon dioxide, corn starch, dibasic calcium phosphate, lactose monohydrate, magnesium stearate, and sodium starch glycolate. In addition, the 1 mg also contains FD&C blue no. 1 aluminum lake and D&C red no. 27 aluminum lake. The 2 mg also contains FD&C blue no. 1 aluminum lake and FD&C yellow no. 5 (tartrazine) aluminum lake.

Pharmacology

Endogenous estrogens are largely responsible for the development and maintenance of the female reproductive system and secondary sexual characteristics. Although circulating estrogens exist in a dynamic equilibrium of metabolic interconversions, estradiol is the principal intracellular human estrogen and is substantially more potent than its metabolites, estrone and estriol at the receptor level.

The primary source of estrogen in normally cycling adult women is the ovarian follicle, which secretes 70 to 500 mcg of estradiol daily, depending on the phase of the menstrual cycle. After menopause, most endogenous estrogen is produced by conversion of androstenedione, secreted by the adrenal cortex, to estrone by peripheral tissues. Thus, estrone and the sulfate conjugated form, estrone sulfate, are the most abundant circulating estrogens in postmenopausal women.

Estrogens act through binding to nuclear receptors in estrogen-responsive tissues. To date, two estrogen receptors have been identified. These vary in proportion from tissue to tissue. Circulating estrogens modulate the pituitary secretion of the gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH), through a negative feedback mechanism. Estrogens act to reduce the elevated levels of these hormones seen in postmenopausal women.

LUPRON DEPOT

Description

Leuprolide acetate is a synthetic nonapeptide analog of naturally occurring gonadotropin-releasing hormone (GnRH or LH-RH). The analog possesses greater potency than the natural hormone. The chemical name is 5-

oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-leucyl-L-leucyl-L-arginyl-N-ethyl-L-prolinamide acetate (salt) with the following structural formula:

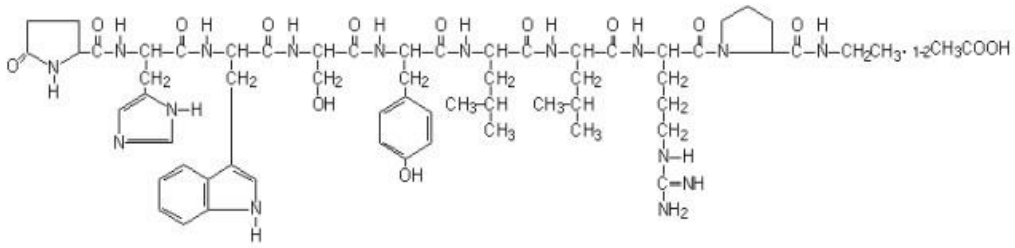


Figure 3. Structural formula for leuprolide acetate (5-oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-leucyl-L-leucyl-L-arginyl-N-ethyl-prolinamide acetate).

LUPRON DEPOT is available in a prefilled dual-chamber syringe containing sterile lyophilized microspheres which, when mixed with diluent, become a suspension intended as a monthly intramuscular injection.

The front chamber of LUPRON DEPOT 3.75 mg prefilled dual-chamber syringe contains leuprolide acetate (3.75 mg), purified gelatin (0.65 mg), DL-lactic and glycolic acids copolymer (33.1 mg), and D-mannitol (6.6 mg). The second chamber of diluent contains carboxymethylcellulose sodium (5 mg), D-mannitol (50 mg), polysorbate 80 (1 mg), water for injection, USP, and glacial acetic acid, USP to control pH. During the manufacture of LUPRON DEPOT 3.75 mg, acetic acid is lost, leaving the peptide.

Pharmacology

Leuprolide acetate is a long-acting GnRH analog. A single monthly injection of LUPRON DEPOT 3.75 mg results in an initial stimulation followed by a prolonged suppression of pituitary gonadotropins.

Repeated dosing at monthly intervals results in decreased secretion of gonadal steroids; consequently, tissues and functions that depend on gonadal steroids for their maintenance become quiescent. This effect is reversible on discontinuation of drug therapy. Leuprolide acetate is not active when given orally. Intramuscular injection of the depot formulation provides plasma concentrations of leuprolide over a period of one month.

PROGESTERONE

Description

PROMETRIUM (progesterone, USP) Capsules contain micronized progesterone for oral administration. Progesterone has a molecular weight of 314.47 and a molecular formula of C21H30O2. Progesterone (pregn-4-ene-3, 20-dione) is a white or creamy white, odorless, crystalline powder practically insoluble in water, soluble in alcohol, acetone and dioxane and sparingly soluble in vegetable oils, stable in air, melting between 126° and 131°C. The structural formula is:

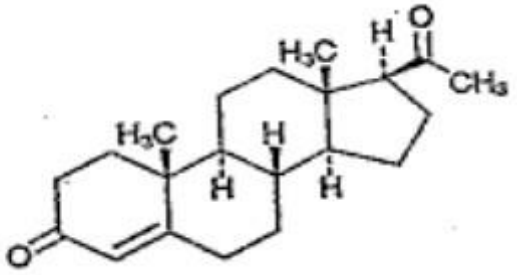


Figure 4. Structural formula for progesterone, USP (pregn-4-ene-3, 20-dione)

Progesterone is synthesized from a starting material from a plant source and is chemically identical to progesterone of human ovarian origin. PROMETRIUM Capsules are available in multiple strengths to afford dosage flexibility for optimum management. PROMETRIUM Capsules contain 200 mg micronized progesterone.

The inactive ingredients for PROMETRIUM Capsules 100 mg include: peanut oil NF, gelatin NF, glycerin USP, lecithin NF, titanium dioxide USP, D&C Yellow No. 10, and FD&C Red No. 40.
The inactive ingredients for PROMETRIUM Capsules 200 mg include: peanut oil NF, gelatin NF, glycerin USP, lecithin NF, titanium dioxide USP, D&C Yellow No. 10, and FD&C Yellow No. 6.

Pharmacology

PROMETRIUM Capsules are an oral dosage form of micronized progesterone which is chemically identical to progesterone of ovarian origin. The oral bioavailability of progesterone is increased through micronization.

Summary of Previous Human Experience

The proposed monthly 3.75-mg injection of leuprolide acetate (Lupron Depot) is FDA-approved for use to treat endometriosis and uterine fibroids. Lupron has also been widely researched to treat a variety of medical conditions in actively ill women, including ovarian epithelial tumor cells[97], insulin resistance[98], endometrial stromal sarcoma[99], and infertility[100] as well as in healthy women. Several studies have also investigated the use of Lupron and hormone addback (as is being completed here) to treat the symptoms of premenstrual syndrome and PMDD,[87, 101-103] which has resulted in combined Lupron and estrogen/progestin supplementation as a recommended long-term treatment for premenstrual syndrome.[104, 105] Notably, there is a significant association between PMDD and PMS and BN: women with PMDD are 7-times and with PMS 2-times more likely to have a BN diagnosis,[106] suggesting that an underlying pathophysiology may exist.

Infertile women undergoing IVF routinely receive luteal supplementation of 600 mg progesterone daily along with 6 mg oral micronized estradiol[107] in combination with GnRH agonists (e.g., Lupron). GnRH agonist treatment combined with hormone addback (i.e., high-dose estradiol and progesterone supplementation) has been previously studied in large randomized controlled trials for the purpose of IVF.[107-112] Of particular relevance to our study, researchers induced a hypogonadal state using 3.75 mg Decapeptyl, followed by high-dose (800 mg) oral progesterone treatment in 32 women without an adverse event.[111] Damario et al. (1999) also reported the use of combined Lupron treatment, 9 mg oral micronized estradiol, and 100 mg i.m. progesterone in 238 women presenting for IVF.

Dr. Schiller's research lab at UNC and research team's at the NIH have examined the combined administration of Lupron and estradiol and/or progesterone in premenopausal women in several studies without a significant adverse event.[113-117] Moreover, the same drug protocol proposed in the current study has been employed previously by Dr. Schiller's research lab at UNC and research teams at the NIH.[87, 117, 118] Notably, the hormone challenge we are using in this protocol has been truncated in length in order to decrease participant burden. Previous studies conducted at NIH indicate hypogonadism can be fully induced within 2 months and reliable symptom change occurs within 2 weeks of E2/P4 addback.[87, 118] As such, compared with the original protocol, the challenge has been shortened by 2 months yet still is able to capture maximal symptom change. Taken together, **a large number of women in the population have previously received combined Lupron with high-dose oral E2 and P4 supplementation either as part of medical care, a research study, or standard IVF treatment without serious side effects or adverse events.**

Status of Drug in Other Countries

To our knowledge, the proposed drugs have not been withdrawn from investigation or marketing in any other country.

1.3 Preclinical Data

Decades of preclinical work confirms E2 has a direct, protective effect on food intake, binge-type behaviors, and weight-related constructs whereas P4 may antagonize the effect of E2.[8-10]

1.4 Clinical Data to Date

Hormone Challenge Protocol. Dr. Schiller's research lab has employed similar hormone regimens to *elicit* symptoms of depression and is currently conducting an experimental study with a similar design to elicit irritability in healthy women. Team's at the NIH developed the protocol used here to address the hormonal mechanisms underlying PMS/PMDD. All participants will undergo the same hormonal challenge but in a double-blind cross-over design. Subjects will be randomly assigned to receive E2+P4 or P4+E2 based on the randomization table created by the study biostatistician.

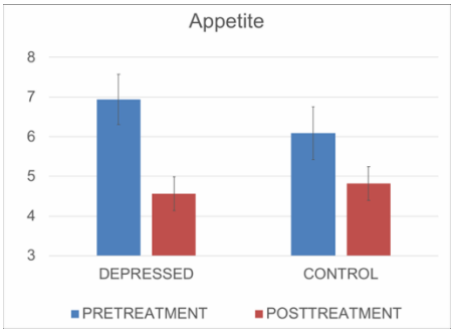
Recruitment and Retention. The investigative team has experience recruiting clinical populations for eating disorders research (i.e., Dr. Baker) and for reproductive hormone challenges (e.g., Dr. Schiller). In Dr. Schiller's most recent hormone challenge study, participants were recruited based on the following: targeted social media advertising (57%), university-wide mass emails (10%), Craigslist advertising (10%), ResearchMatch.org (7%), a UNC Center for Women's Mood Disorders research registry (7%), flyers (3%), Join the Conquest (3%), and referral from a friend in the study (3%).

Dr. Schiller's successful completion of a previous study (N=30) involving a similar hormone challenging plus a neuroimaging component demonstrates both the feasibility and ability to recruit and retain women to participate in hormone challenge studies. Importantly, the current protocol is less invasive. It is possible that recruiting women with BN willing to undergo the hormone experimentation will be more challenging, thus, we have built in additional retention plans: a) the hormone challenge has also been truncated in order to decrease participant burden given previous studies indicate hypogonadism can be fully induced with significant behavioral changes observed in 6-weeks and reliable symptom change occurs within 2 weeks of E2/P4 addback;[87, 118] b) we do not require participants to not be in treatment to participate in the study. However, we request that subjects make no changes to their treatment protocol for the duration of the study. While this does introduce a potential confound,

even in treatment, we would expect to see changes in binge eating during the hormone challenge if indeed ovarian hormones have a direct impact on binge eating—continuing treatment would not negate this effect; c) a unique aspect of our study, we will provide participants with individual feedback regarding any changes observed in binge eating during the hormone manipulation after study termination. Women can take advantage of this optional session with Dr. Baker to review their own data in relation to hormone changes and observed changes in binge eating. This represents real-time, personalized information about the participant's *individual response* to hormones. This may be helpful to women as they pursue treatment.

Use of the Hormone Challenge Protocol in BN and preliminary data. There is compelling evidence an experimental design is the next logical step in ovarian hormone research in BN. Preclinical work confirms E2 has a direct, protective effect on food intake, binge-type behaviors, and weight-related constructs. Additionally, observational studies implicate ovarian hormones in neurobiology such that E2 is inversely and P4 is positively associated with binge eating in women with BN.[5, 6] Cumulatively, for women with higher levels of binge eating pathology, findings indicate that E2 has a potential protective effect and P4 an exacerbating effect on binge eating. However, observational work in this area is limited: 1) both E2 and P4, albeit at differing levels, are present throughout the menstrual cycle: given the presence of both (and other hormones such as FSH and LH), the specific effects of each cannot be isolated, which is vital given that E2 and P4 may have opposite effects on binge eating; 2) there is a lack of ovarian suppression: in order to directly target and manipulate hormone sensitivity, the removal of both hormones must occur; 3) there is no randomization or manipulation: thus, causal, mechanistic conclusions cannot be made. Only with an experimental design can we begin to unravel the independent and mechanistic effects of ovarian hormones on BN symptoms—providing formative information about the nature of the hormone trigger on binge eating.

Co-I Schiller's early work on the effects of ovarian hormones on binge eating in women with BN provides the foundation for this study.[5] Increased binge eating was observed during the mid-luteal and premenstrual phases of the menstrual cycle compared with the follicular and ovulatory phases with mean Z-scores for binge-eating frequency as follows: ovulatory -.37, follicular -.30, premenstrual -.08, mid-luteal .61. Symptom fluctuation was attributable to change in E2 and P4: increases in binge eating were associated with decreases in E2 (-.13(0.05), $t(142.89) = -2.82$, $p<.01$) and increases in P4 (.15(0.04), $t(142.55) = 3.49$, $p<.001$), controlling for the effect of the other. Further, *women with BN did not have different E2 and P4 profiles compared with women without BN*[5] supporting our hypothesis: women with BN do not exhibit abnormal hormone levels, rather their behavioral reactions to normal ovarian hormone fluctuations are dysregulated.



Additionally, Dr. Schiller's pilot work of E2 treatment in midlife women suggests E2 has a direct effect on appetite: three weeks of E2 treatment significantly reduced appetite in those with major depression ($t=3.88$,$p<.001$) and those without depression ($t=2.70$, $p<.015$; **see Graph**) suggesting a Cohen's $d=2.3$. Finally, work at the NIH using this hormone challenge provides insight into changes in food intake during the challenge and proves its utility. In women with PMS, a significant increase in food cravings was reported from week 2 of the menstrual cycle (M=1.6,SD=.7) to week 4 (M=3.3,SD=1.5), which was entirely eliminated by the GnRH agonist (M=1.7,SD=.7 at week 4), but not placebo.[87] This suggests a strong effect (Cohen's $d=1.51$) of the GnRH agonist on food cravings. In a related protocol (GnRH agonist followed by combined E2+P4), women with PMDD had a significant increase in food cravings transitioning from hypogonadism to E2+P4.[118]

Pilot work by PI Baker provides further insight into the protective effect of E2 against dysregulated eating. In midlife women receiving estrogen replacement or placebo during the menopause transition, a moderate negative correlation was observed between loss of control eating and treatment group ($r= -.32$;unpublished data[119]) indicating a direct effect of E2 on loss of control eating such that *lower levels of loss of control eating were*

observed in women receiving E2 replacement. Dr. Baker's unpublished work further corroborates the association between ovarian hormones and ED as well as her experience in this area: a positive correlation was observed between binge eating and premenstrual symptoms (PMS; r=.24, p<.01) in college women (n=448) indicating that higher binge eating scores were associated with increased PMS, which by definition are ovarian hormone sensitive symptoms. Dr. Baker's published work shows pubertal development predicts ED symptoms[120] and some of the same genes are responsible for age of menarche and ED symptoms.[121]

1.5 Dose Rationale and Risks/Benefits

Dose Rationale

We use the same dose of each drug administered in previous published studies using this hormone challenge. We replicate the dosage used for these studies as they were able to reliable induce hypogonadism, physiological levels of E2 and P4, and measurable behavior change. Moreover, based on these published data[87, 118] we have shortened the protocol given that hypogonadism was reliably induced and measurable behavior change observed within 6-weeks of the first dose and maximal symptom change was observed with two weeks of E2 and P4 administration.[87, 118] Thus, the drug protocol, including the route of administration, dosage, dosage regimen, and dosage period, mirrors a truncated version of previous studies. Further, each medication is being used for their intended use (e.g., ovarian suppression).

This proposal is to support mechanistic work and *not* to support a new indication, dose, or route of administration, and not to support clinical use or treatment in a new population.

Risks/Benefits

We do not expect serious adverse side effects associated with the hormonal manipulations outlined in this protocol for the following reasons: First, the doses of Lupron, estradiol and progesterone, and the duration for which they will be administered in this protocol, will result in plasma hormone levels comparable to those commonly used for in vitro fertilization (IVF) protocols lasting 1-3 months. Therefore, based on current IVF procedures we do not anticipate any adverse incidents arising from the proposed doses of estradiol and progesterone.[122, 123] Second, no serious adverse reactions or events were encountered in past studies conducted with similar and more invasive protocols used in Dr. Schiller's research lab or labs at the NIH. Third, comparable extended, uninterrupted gonadal steroid treatment (such as oral contraceptives) for 6 to 12 weeks has been shown to be well-tolerated.[124] Fourth, we have truncated the length of the protocol compared with the initial studies using this hormone challenge.

Relevant studies in the EDs literature also indicate that an ED population would not be at increased risk for negative side effects or the decreased acceptability of such side effects. A relevant study in humans addressed the effectiveness of an oral contractive (synthetic E2/P4: 30 μg of ethinyl estradiol and 3 mg of drosperinone) for 3-months for the treatment of ED symptoms in women with BN.[125] The oral contraceptive reduced meal-related hunger and gastric distention and, for a subset of women only, it also reduced BN symptomatology. No serious adverse effects were reported.[125] Additionally, women and adolescents with low weight EDs, which are at heightened risk for severe medical complications, have been given oral contraceptives, physiological estrogen replacement, and progesterone, ranging from periods of time from 3-months to 18-months, without noted serious adverse events.[126-130] For example, in a study examining 18-months of physiological estrogen replacement in girls with anorexia nervosa compared with healthy controls, the most frequent (>25%) side effects were: bloating (32%), irritation at estrogen patch site (31%), breast tenderness (25%), and nausea/vomiting (25%).[127] Notably, for many of the reported side effects, girls receiving placebo reported side

effects at a similar rate to those girls receiving estradiol replacement. In a second, related study, of oligo-amenorrheic athletes who were randomized to an oral contraceptive, estradiol patch plus progesterone, or placebo for 12-months, the most common side effects noted from the patch (~4%) were bloating and headaches.[126] Finally, having an ED is not a recognized contraindicated population for use of these medications and the presence of an ED does not appear to have been an exclusionary criterion for the clinical trials of Lupron, specifically.[131] Given the high comorbidity with PMS/PMDD,[106] it is highly probable women with a current or past history of BN or binge eating have indeed been given these medications as part of routine treatment or in a research study. **Our study is not testing the effectiveness of these medications for the treatment of binge eating** but to further our mechanistic understanding of the disorder—which, in the future, could lead to new treatments or innovative targets to prevent illness expression.

Finally, any changes observed in ED symptoms that occur during E2 and P4 addback specifically would not be expected to surpass the subjects baseline levels of symptom expression. We do not expect symptom levels to significantly surpass baseline levels of severity (i.e., prior to hormone manipulation) given that the participants endogenous E2 and P4 levels are present and in flux during their natural menstrual cycle. For example, for women with PMS, a decrease in sadness was observed from baseline to hypogonadism. Although sadness increased with E2 and P4 addback (which is expected based on the hypothesized pathophysiology of PMS), the average-mean levels of sadness were *lower* at addback compared with mean scores observed during their natural menstrual cycle, prior to the manipulation.[87] Relatedly, oligo-amenorrheic athletes who were randomized to estrogen replacement showed a *stabilization* (i.e., no change) in ED symptoms compared with placebo, whereas girls on placebo showed an increase in symptoms over a one year period.[126] Second, physiologically relevant steroid hormones (E2 and P4) will be administered and hormone levels attained; thus, the E2 and P4 arms will mimic the presence of these hormones during a natural menstrual cycle—and thus, again, would not be expected to cause significant increases in symptomatology that surpass baseline levels. However, the E2 and P4 addback arms will allow us to directly manipulate hormone levels and remove additional confounds, while also attaining physiologic hormone levels. Third, we have truncated the experimental design to the minimal length of time necessary to elicit symptom change for each phase. Finally, as mentioned above, no adverse reactions or events were encountered in past studies conducted that were developed to induce symptoms of depression. Here, we are <u>not</u> selecting individuals with a past (or current) history of a mood disorder, which has been done in previous studies using this experimental design.[87] Although we are selecting individuals who have current BN and manipulating their symptoms, *their symptomatology is already present* at baseline; we are not directly causing their symptoms, and thus, symptoms are not expected to be more severe throughout the hormone challenge compared with the baseline levels that were already present at study enrollment.

As described below, the most serious potential adverse side effects associated with the pharmacological interventions used here would likely be considered: DVT/blood clots (<u>although a causal relationship has not been established</u>) and bone loss (<u>which is reversible upon stopping the medication</u>). Notably, there is no evidence to suggest women with BN are at increased risk for DVT/blood clots.[132] Further, whereas low weight EDs are at risk for bone loss, women with BN, who are of normal weight and above, may not be at this same risk.[133-135] The risk for bone loss in EDs is directly related to the loss of estrogen that can occur at a low weight status and with menstrual cycle irregularities (i.e., amenorrhea),[133l, 134] which are more common in anorexia nervosa. Notably, the current proposal <u>requires</u> participants to have a regular menstrual cycle and normal BMI for study inclusion.

We describe the adverse effects of Lupron, E2, P4, and combined Lupron+E2+P4 below.

Lupron: The most frequent adverse effect of Lupron is hot flushes (flashes) reportedly occurring in 4-89% of patients receiving the drug. Lupron-induced hot flushes have ranged in severity from occasional mild flushing

to frequent sweating. Episodes of flushing appear to decrease with continued therapy in most patients receiving Lupron; however, in at least one study, the incidence of hot flushes did not appear to decrease with continued therapy. In a recently completed study of 400 women of reproductive age with either uterine fibroids or endometriosis who each received 3.75mg depot Lupron every month for a period of six months, a timeframe longer than the current protocol, the most common side effects were as follows: 1) hot flashes of mild to moderate intensity (89%); 2) headache (22%); 3) nervousness or irritability (11%); and 4) insomnia (10%). Local irritation at the injection site was complained of in less than 10% of the patients in this sample, and there was a mean decrease in bone density, as measured by bone densitometry, of 3.4 to 4.0%, which totally reversed after the medication had been discontinued for six months. Approximately 10 patients of the original sample of 400 found the side effects to be severe enough to discontinue therapy (2%). In the majority of women regular menstrual cycle function returned within two months following the last injection of depot Lupron (Tapp Pharmaceuticals, personal communication). Complete reversibility of fertility suppression has been observed for administration of Lupron for periods of up to 24 weeks[131]—much longer then the use of Lupron proposed in this study.

Blurred vision, myalgias, lethargy, memory disorder, and numbness have been reported in less than 3% of patients receiving the drug. Thrombophlebitis, pulmonary embolus, and congestive heart failure have occurred rarely in patients receiving Lupron, but a causal relationship to the drug has not been established. Adverse GI effects occurring in 2% or more of patients receiving Lupron include nausea and/or vomiting, constipation, and loss of appetite (non-eating disorder related food intake). Diarrhea and a sour or unusual taste in the mouth have been reported less frequently. Other adverse effects of Lupron occurring in less than 3% of patients include decreased hematocrit and hemoglobin concentration, fatigue, fever, facial swelling, rash, hives, hair loss, and itching. In 20 PMS patients and 20 controls, Lupron was well tolerated (no dropouts) with the most common side effects being hot flushes and a decrease in libido. Limited information is available on the acute toxicity of Lupron. Following subcutaneous administration of Lupron in rats at dosages 250-500 times the usual human dosage, dyspnea, decreased activity, and local irritation at the injection site wereobserved; however, there is no evidence to date that overdosage in humans produces similar adverse effects. Lupron dosages up to 20mg daily for up to two years have not produced unusual adverse effects in humans. There has been one report of an anaphylactic reaction in a patient following administration of a GnRH agonist. Recent longitudinal follow-up studies of girls and boys receiving GnRH agonists as a treatment for precocious puberty report the development of normal reproductive function, skeletal growth, and fertility.[136,137]

Estradiol: Nausea is the most common side effect of estrogen administration. At conventional replacement doses, higher than those employed in this protocol, this complaint seldom interferes with food intake (i.e., non-eating disordered), and no weight loss has been reported. Breast engorgement, endometrial hyperplasia, and bleeding are also common side effects of estrogen administration. Pre-existing fibroid tumors of the uterus may enlarge under the effects of estrogen; however, at the dosage and for the duration of estrogen administration in this protocol (2-weeks) this risk is quite small.

The relationship between estrogen, both endogenous and exogenous, and the development of endometrial carcinoma has been suggested by several different lines of investigation.[138] Numerous retrospective case control studies published since 1975 have indicated that post-menopausal exposure to unopposed estrogens for more than one-year results in a two to 12-fold increased relative risk for endometrial cancer. A relationship between the dose and duration of estrogen use and the risk for endometrial cancer has also been shown, the risk being increased after one to four years of estrogen use and rising also with the dosage employed. However, the addition of progesterone to estrogen replacement therapy (ERT) appears to decrease the risk of endometrial hyperplasia and endometrial cancer to equal or below that of women receiving no hormonal treatment. Recent studies suggest that the optimal regimen to prevent hyperplasia during long term ERT and thus, inferentially, the risk of carcinoma, consists of 12 to 13 days of progesterone treatment each month when estrogens are

administered.[139] There is an increase in thromboembolism in women receiving non-contraceptive estrogen therapy.[140-142] Additionally, some but not all studies report an increase in risk of stroke[143, 144] in older women taking estrogen therapy. However, these complications are unlikely at the dose and duration of estrogen replacement employed in this protocol (2-weeks), and in the younger age group (18-42) of women who participate in this study. One study[122] reported no effect of the estrogen patch on the four clotting indices previously shown to be altered by oral contraceptive use.[145, 146] Blood pressure, on average, appears to be unaffected by estrogen therapy, although both increases and decreases have been reported. In observational studies, post-menopausal estrogen therapy has been observed to lower the relative risk of cardiovascular disease in some but not all studies.[143, 147] In contrast, randomized controlled trials in older postmenopausal women (e.g., Women's Health Initiative [WHI]) report an increased risk of cardiovascular disease.[148]

Emerging data suggest that these disparities in findings may be related to the timing of initiation of estrogen therapy in relation to the proximity of menopause. Subgroup analyses of the combined estrogen and progestin (EPT) arm of the WHI demonstrated a significant interaction between coronary heart disease (CHD) risk and time since initiation of EPT, with an increased risk in the early years following initiation and a decreased risk in later years. Additionally, the increased risk of CHD was observed in older but not younger perimenopausal women.[149-152] High doses of oral estrogens have been reported to elevate hepatocellular enzyme levels and, less commonly, cause cholestatic jaundice. The risk for gall stones and hepatocellular adenomas has been reported to be increased in association with oral contraceptive use, and although uncommon these complications may also occur with the use of replacement doses of estrogen.[153, 154] Estrogen therapy also may increase the risk of urinary incontinence in older postmenopausal women.[155] Further, most studies have suggested an increased relative risk of breast cancer after four or five years' use,[156-160] similar to the risk expected if the onset of menopause was delayed for a comparable length of time.

Women and adolescents with low weight EDs, which are at heightened risk for severe medical complications, have been given physiological estrogen replacement for periods ranging from 3-months to 18-months, without any noted serious adverse events.[126-130] In a study examining 18-months of physiological estrogen replacement in girls with anorexia nervosa compared with healthy controls, the most frequent (>25%) side effects were: bloating (32%), irritation at estrogen patch site (31%), breast tenderness (25%), and nausea/vomiting (25%).[127] For a majority of the reported side effects, girls receiving placebo reported side effects at a similar rate to those girls receiving estradiol replacement. Notably, the experimental arm of E2 addback is time-limited, lasting only 2-weeks. Thus, any side effects that may occur are expected to be minimal and transient in nature.

Progesterone: Progesterone and the synthetic progestins are widely prescribed to women in the population, with indications including dysfunctional uterine bleeding, endometriosis, mastodynia, galactorrhea, and precocious puberty.[162] Progestin contraceptives are also widely used. Side effects reported in women taking progestins may include breakthrough bleeding, edema, change in weight (increase or decrease), cholestatic jaundice, rash (with or without pruritus), depression, easy fatigue and sedation, lack of initiative, and chloasma. Since progestins are often used in women with antecedent menstrual irregularity, it is not clear whether the breakthrough bleeding represents an effect of the medication or refractoriness to treatment. In the large majority of patients, menstruation occurs predictably following withdrawal of progestins and is usually more regular than in spontaneous cycles. In a recent study, an average dose of 1750mg of oral micronized P4 was given to 59 women with PMS for a period of three months and was well tolerated by this sample. The side effects reported on progesterone were lightheadedness, fatigue, forgetfulness, and headaches. These were very mild and caused no dropouts.

Women and adolescents with low weight eating disorders have been given physiological estrogen replacement with cyclic progesterone for up to 18-months, without any noted serious adverse events.[126-130]

Given the short timeframe of P4 addback in this study (2-weeks), we expect the risk for any side effects to be minimal and any side-effects that are experienced will be brief.

Lupron, Estradiol, and Progesterone Combined Administration: The proposed monthly 3.75-mg injection of leuprolide acetate (Lupron Depot) is FDA-approved for use in premenopausal women to treat endometriosis and uterine fibroids.[163] Lupron has also been widely researched and used to treat a variety of medical conditions, including ovarian epithelial tumor cells,[97] insulin resistance,[98] endometrial stromal sarcoma,[99] infertility,[100] and premenstrual syndrome and premenstrual dysphoric disorder.[101-103] Combined Lupron and estrogen/progestin supplementation is a recommended long-term treatment for premenstrual syndrome.[104, 105] Women undergoing IVF to treat infertility routinely receive luteal supplementation of 600 mg progesterone daily along with 6 mg oral micronized estradiol[107] in combination with GnRH agonists (e.g., Lupron).

GnRH agonist treatment combined with hormone addback (i.e., high-dose estradiol and progesterone supplementation) has been previously studied in large randomized controlled trials for the purpose of IVF to treat infertility.[107, 109-111] Several studies have examined the use of combined Lupron treatment (1 mg daily or 3.75 mg monthly), oral micronized estradiol (max doses ranged from 4 mg to 9 mg daily), and either oral (900 mg daily), vaginal (90-600 mg daily), or i.m. (50-200 mg daily) progesterone administration in >1800 women.[108, 109, 111, 164-172]

Dr. Schiller's research lab at UNC and labs at NIH have examined the combined administration of Lupron and estradiol and/or progesterone in premenopausal women in several studies without a serious adverse event.[113-117] Thus, a large number of women have previously received combined Lupron with high-dose oral estradiol and progesterone supplementation either as treatment for a medical condition, a research study, or routine IVF treatment without serious adverse events. It is possible that women with BN or who binge eat may have taken part, as assessing for an eating disorder is not necessarily common practice during medical evaluations.

Below we describe the adverse events reported in a recent protocol completed by Dr. Schiller that entailed a more invasive study design and higher doses of E2/P4. **This protocol was classified as IND exempt by the FDA.**

Table 2. Medication Regimens in a previous IND Exempt Protocol and the Proposed Protocol

	Previous IND Exempt Protocol				Proposed Protocol		
Phase	1: Hypogonadism	2: Low Dose Addback	3: High Dose Addback	4: High Dose Withdrawal	1: Hypogonadism	2: Addback	3: Addback
Duration	4 weeks	2 weeks	6 weeks	4 weeks	6 weeks	2 weeks	2 weeks
Lupron	3.75 mg IM dose #1	3.75 mg IM dose #2	3.75 mg IM dose #3	3.75 mg IM dose #4	3.75 mg IM dose #1,2	3.75 mg IM dose #3	
Estrace	Placebo	2 mg bid	5 mg bid	Placebo	Placebo	2 mg bid	
Prometrium	Placebo	200 mg bid	400 mg bid	Placebo	Placebo		200 mg bid

Below (Table 3) is a list of the adverse events that occurred in the previous, IND exempt protocol conducted at UNC by Dr. Schiller described above. All of the symptoms summarized in Table 3 were either transient and remitted without intervention or were addressed either by consolidating the dose to nighttime or by decreasing the dose of estradiol by 2 mg or progesterone by 200 mg, depending on the symptom. The adverse events reported in purple most replicate the experimental arms proposed here. Dizziness was the most common reported side effect and side effects were more common during the addback conditions compared with the Lupron-only condition. In the current protocol, the addback conditions are only 4-weeks total (2-weeks each) whereas in the previous protocol they were 8-weeks total. Few adverse events were reported as "severe." Importantly, there were no serious adverse events in this study and no subjects were discontinued due to an adverse event.

Table 3. Adverse Events in a Previous IND Exempt Protocol

	1: Hypogonadism	***2: Low Dose Addback***
Cognitive:		
Drowsiness	1	1
Sedation		3
Dizziness	1	5
Lightheadedness		1
Memory impairment		
Psychological:		
Depression		2
Anxiety		1
Irritability		2
Mood Swings		1
Trouble concentrating	1	
Night Terrors		1
Gastrointestinal:		
Nausea		1

Diarrhea	1	
Upset Stomach		
Constipation	1	
Heart Burn	1	1
Cardiac:		
Transient Heart Palpitations*	1	
Chest Pain*		1
Arrhythmia*	1	
Bradycardia*	1	
Menstrual:		
Spotting	1	
Prolonged menstrual bleeding		
Heavy menstrual bleeding		
Breast Tenderness	2	2
Vaginal itching		
Cramps		
Physical/Somatic:		
Weight Gain		
Hot Flashes	1	
Headache	3	1
Hair Loss		
Cracked Nipple		1
Rash on legs	1	
Tingling		1
Dry Mouth		1
Frequent Urination		1
Hip pain	1	
TOTAL	**18**	**27**

Note: Bolded individual symptoms were rated as severe. Headaches were rated as severe two times and all other bolded symptoms were rated as severe only once. All others symptoms were rated minimal, mild, or moderate.

**Symptoms were determined by an independent* physician to be a result of participant's excessive physical training for Iron Man combined with poor *self-care (i.e., working 3rd shift, sleeping 4 hours/night, and poor diet) rather than the study. All symptoms resolved when participant reduced training schedule and increased time available for sleep.*

2 Study Objectives

The objective of this study is to examine whether BN is an ovarian hormone sensitive phenotype and whether this hormone sensitivity is modulated by reward processing by directly manipulating ovarian hormone levels in women with BN (n = 15), using an established experiential design. For the first time in humans, we propose an experimental design that parallels animal models to directly manipulate ovarian hormones: temporarily stopping the menstrual cycle (i.e., hypogonadism) and then adding back E2 and P4 independently in a double-blind cross-over design in order to examine the direct effect of E2 and P4 on the BN symptom of binge eating. Participants will complete behavioral tasks and self-report questionnaires of reward processing and response during each phase of the experimental design. Our overarching hypothesis for the following aims is that BN represents a hormone sensitive phenotype and this sensitivity is displayed as an impaired reward response within the context of low E2 such that E2 addback will be beneficial for all outcomes of interest. We plan to accomplish the objectives of this application by pursuing the following specific aims:

Aim 1: Quantify the direct effect of E2 and P4 on binge eating in women with BN.

Aim 2: Determine the effect of E2 on reward response and related correlates (e.g., behavioral inhibition) in women with BN during behavioral tasks and through self-report questionnaire.

Aim 3: Examine the association between reward response (defined in Aim 2) and binge eating before and after E2 addback.

3 Study Design

3.1 General Design

This single-site study will include 8 study visits in total and a reproductive hormone challenge. Participants will undergo screening and consent (T0), a baseline assessment (T1), testing at the end of each experimental phase (T2-T4), and a follow-up assessment 8-weeks post-intervention (T5). Brief testing to examine side effects will also occur in addition to the end-of-phase primary study visits. During the hormone challenge, subjects will have an equal number of brief check-in visits (n=3) and end-of-phase study visits (n=3) The study timeline is depicted in **Figure 5**, and the specific procedures that will take place are outlined in **Table 4** and detailed below in Section 6.

3.2 Outcome Variables

The **primary outcomes** for the Specific Aims are: 1) binge eating, and 2) reward response.

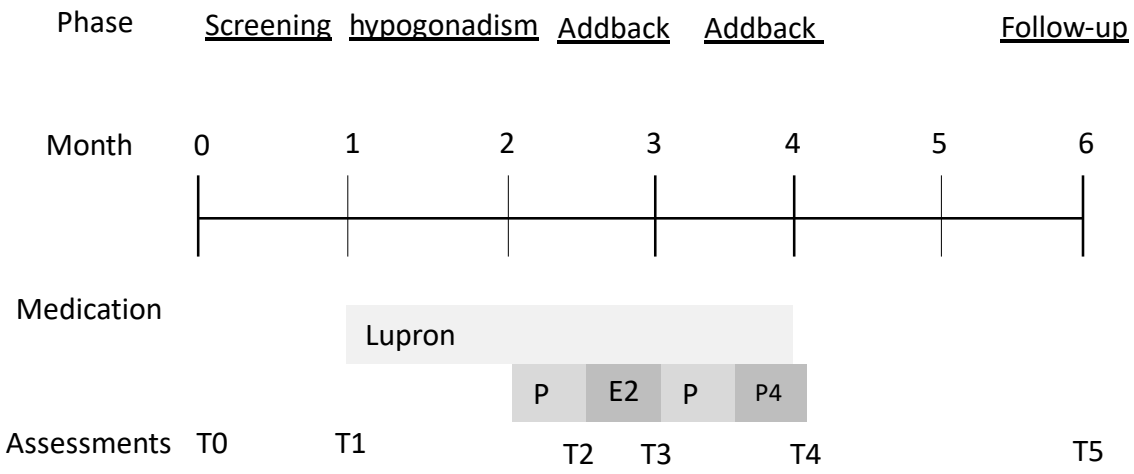
Binge eating will be defined as: 1) the EPSI subscale binge eating score obtained at each study visit (T1-T4), which represents symptoms over the previous week; 2) a weekly average based on daily frequency obtained from the DSRP. For both the subscale score and weekly average, the primary outcome is the last week of each phase of the hormone challenge to capture the period of time with maximal symptom change.

Reward response and related correlates will be defined by the Monetary Incentive Delay (MID),[174] the Delayed Discounting (DD) and Go No/Go behavioral tasks, and the BIS/BAS and SPSRQ self-report questionnaires. MID operationalizes reward response as *motivated behavior*: the average speed of responses to reward vs. non-

reward trials during a reward task. DD operationalizes reward response as "delay gratification": the tendency to choose small, immediate rewards over larger, delayed rewards. The task determines the rate of devaluation over time for the larger, delayed reward, which is defined as the k parameter. We use behavioral tasks of monetary reward vs. food because the value of food is dependent on hunger state[202, 203] and it reduces the confounding effect of the task provoking symptoms.[34] The Go/No Go Task is a behavioral measure of inhibitory control. Inhibitory control is defined by the response accuracy of the go no/go trials with fewer errors ("go" response on a "no/go" trial) indicating better inhibitory control.

The BIS/BAS and SPSRQ will be included as self-report measures relevant to the reward response. The BIS/BIAS will be used to assess behavioral inhibition and activation. The SPSRQ will be used to assess sensitivity to reward. As described in the statistical analysis plan below, reward response will be examined within the E2 arm of the study.

Figure 5. Within-Subject Hormone Challenge (study visits occur every 2 weeks)



4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

Participants will be women aged 18-42 with a current BN. Only participants capable of giving informed consent and understanding the risks associated with the study will be enrolled. Participants will be compensated upon completion of the study.

Inclusion Criteria.

1) Current BN
2) Aged 18-42
3) A regular menstrual cycle for at least three months;
4) BMI < 35
5) Free of medication that impacts ovarian hormones or is contraindicated for use with study interventions

4.2 Exclusion Criteria

Patients will not be permitted to enter this protocol if they have important abnormalities including any of the following:

- peanut allergy
- endometriosis (an illness related to abnormal tissue growth around the uterus)
- enlargement of the ovaries
- liver disease
- breast cancer (self or family history)
- a personal or family history of blood clots
- undiagnosed/abnormal vaginal bleeding
- porphyria (a rare genetic blood disorder)
- diabetes mellitus
- osteoporosis or osteopenia
- malignant melanoma (a type of skin cancer)
- gallbladder or pancreatic disease
- heart or kidney disease
- cerebrovascular disease (stroke)
- currently smoking >10 cigarettes daily
- epilepsy or history of seizures
- a history of suicide attempts or bipolar disorder/psychotic episodes
- current substance misuse
- frequently use diuretics or laxatives
- recurrent migraine headaches with aura
- history of pregnancy-related deep vein thrombosis
- irregular menstrual cycle
- body mass index (BMI) greater than 35
- currently pregnant, planning to become pregnant, or lactating
- taking any medication or have any other medical history that is contraindicated for the medications used in this study
- unwilling to use barrier contraceptive during the study

· Any condition or symptoms considered by the study team to detrimentally impact subject safety.
· First degree relative (immediate family) with breast cancer that occurred before menopause, or breast cancer presenting in both breasts, or if you have multiple family members (greater than three relatives) with breast cancer.

4.3 Subject Recruitment and Screening

Methods of recruiting for this study include:

We will utilize recruitment methods that have been highly successful in the past including:

1) **Recruiting directly from the UNC Center of Excellence for Eating Disorders (CEED) outpatient treatment and research program**: CEED evaluates between 200-220 new patients per year and has an excellent track record of successful recruitment of women with EDs for research studies.[175] Specifically, we will recruit women from the CEED via flyers, brochures, and direct recruitment by speaking with women who expressed interest in research on their referral form. In a recent clinical treatment trial for BN,[175] 214 women contacted study staff and completed the phone screen and 80% were randomized.

2) **Established research registries at CEED and within the Department of Psychiatry at UNC:** CEED houses a research registry, which any member of the community (including current CEED patients) can join and consent to be contacted about future studies. There are currently 535 individuals signed up for the registry, 81 of whom self-report a BN diagnosis. CEED also has a registry of previous research study participants who consented to be re-contacted for future studies. Additionally, Dr. Schiller has an established registry of individuals (3,000+) who previously completed online screening questionnaires for her hormone manipulation studies who agreed to be contacted for future studies.

3) **Targeted social media and website advertisements** (e.g., Facebook; ResearchMatch.org; Craigslist): In past studies, we have found social media to be an extremely effective strategy for recruitment at CEED.[176, 177]

4) **Large-scale research registries:** UNC houses two large-scale research registries available to researchers: The Carolina Data Warehouse (CDW), a central data repository containing clinical, research, and administrative data sourced from the UNC Health Care System, and the website Join the Conquest. Within CDW currently, 557 female patients aged 18-35 have a diagnosis of BN within the medical record, with 151 of these diagnoses being made since 2017.

5) **Flyers, brochures, and mass emails**: these will be disbursed in UNC Hospitals, across the university, across the larger community, and with established community partners who allow us to advertise and recruit for active studies (e.g., local medical offices).

6) **NC TraCS Research Recruitment Service**: We will capitalize on the TraCS Research Recruitment Service's expertise in enrolling members of communities historically under-represented in research.

7) **TrialFacts Clinical Trial Recruitment**: As of March 2021, we will also work with the clinical trial recruitment/marketing company TrialFacts. <u>During recruitment and initial screening process, direct interaction occurs between the individual and TrialFacts, not study staff</u>. The TrialFacts website specifically informs individuals that they are a distinct company from the trial site and a distinct organization from the site conducting the research study. Individuals consent to provide their data to TrialFacts, receive communication from TrialFacts, and for TrialFacts to provide us with their contact information. TrialFacts are the owners of the

data they collect and it does not become part of the research study data. TrialFacts primarily recruits via social media avenues (facebook, youtube, instagram etc). Individuals are self-identified by responding to the advertisements. Once an individual self-identified by TrialFacts is determined as potentially eligible for our study, contact information is provided to us by Trialfacts - only at this point does our study team begin/initiate any contact (i.e., phone call) with this individual.

Eligibility screening will include:

- An initial phone or online screening that includes questions about past medical and mental health history to assess potential participants eligibility based on the criteria listed in sections 4.1 and 4.2.
- Participants will undergo a Clinical and Health Screening process to determine whether they are healthy enough to participate in this study.
- All participants will receive a pregnancy test. No pregnant women will be entered into the study, because the study drugs (Lupron, estrogen, and progesterone) may be associated with birth defects.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

- Participants with significant clinical/laboratory abnormalities will not be enrolled/discontinued from the study prior to GnRH agonist administration.
- Participants deemed at elevated suicide risk at enrollment will not be enrolled in the study.
- If a subject is deemed at elevated suicide risk at any point after enrollment, they will be discontinued from the study protocol.
- Any subject experiencing clinically significant side effects that cannot be relieved will be discontinued.
- If menopausal-like symptoms occurring secondary to GnRH agonist treatment are intolerable, drug treatment will be discontinued.
- Any subject experiencing > 50% increase in BN symptom severity, as indicated by the frequency of binge eating and/or purging behaviors, during the medication protocol will be assessed for discontinuation. Continuing/discontinuing will, in part, depend on subject safety, whether the increase in severity is likely a direct result of the study protocol, and if the subject is in current, study approved treatment, for their eating disorder.

Participant safety will be managed in several ways. First, risk is managed through study eligibility criteria. Although we do not expect the hormone manipulation to elevate suicide risk based on previous studies,[87, 88, 117, 118] as an added measure of safety, we exclude subjects who have a history of elevated suicide risk. Specifically, exclusionary criteria include a history of a suicide attempt. Second, risk is also managed by the truncated hormone regimen. The protocol has been shortened to only the amount of time necessary for each condition to elicit symptom change.

Third, to ensure safety of the participant's enrolled in the study we will monitor risk *daily*, throughout the study protocol. Once enrolled, subjects will complete a daily questionnaire that assesses the presence and intensity of behavioral (e.g., binge eating frequency) and mood symptoms. This will include items related to suicide risk. The questionnaire will be completed online by subjects, and ratings will be transmitted to the study team in real-time. Members of the study team will receive immediate notification if a subject endorses active risk on the study form. Subjects will be contacted directly by Dr. Baker and a safety assessment will be conducted. Any subject that is deemed at elevated suicide risk will be followed-up for at least three days with The Hamilton Rating Scale for Depression and the Beck Scale for Suicidal Ideation (SSI). Anyone scoring > 20 on the

Hamilton (indicating severe depressive symptoms[178]) for three days will be considered to have severe mood symptoms and be discontinued from the protocol. The SSI does not have a defined cutoff (> 6 is suggested as high risk for psychiatric patients with a suicide attempt history[179]); thus, total scores and item-level responses will be examined for severity and change. If a subject does not complete the daily form, a member of the study staff will contact them and conduct a safety assessment over the phone.

Fourth, to further ensure the safety of enrolled subjects, participants will complete bi-weekly assessments of change in physical symptoms, mood, suicide risk, and eating disorder symptoms throughout the hormone manipulation—this is in addition to the daily assessment. Mood symptoms and suicide risk will be monitored by administering the Inventory of Depression and Anxiety Symptoms (IDAS). The IDAS will be scored immediately and if IDAS suicidality risk scores > 8 are observed (mean score observed in non-psychiatric community sample; we use this mean to conservatively evaluate risk[180]), then the Hamilton Rating Scale for Depression and SSI will be administered as described above. BN symptoms (e.g., binge eating, purging) will be monitored at bi-weekly appointments with the Eating Disorder Examination Questionnaire (EDEQ). The EDEQ will be scored during bi-weekly visits and scores will be compared with baseline levels and previous study visits. Dr. Baker will follow-up with any participant with a >50% increase in symptoms as indicated by the EDEQ questions regarding frequency of binge eating and purging behaviors.

We are not conducting a clinical treatment study and thus are not providing care or management of the subject's BN symptoms. We will not provide treatment or pay for treatment/medical costs for subjects who are discontinued from the study or in need of medical care during the course of the study. However, we will ensure subjects have appropriate treatment contacts before terminating contact with the subject.

Notably, no serious adverse reactions or events were encountered in past studies conducted with this protocol. No subjects to date have been discontinued from a study protocol due to heightened suicide risk. Indeed, this is true of similar experimental designs that have been longer in nature and selected individuals with a history of a mood disorder. It is possible that women with current BN may be at increased risk; however, this risk is decreased by excluding women with a significant suicide history. Additionally, previous studies implementing this hormone regimen have shown that E2 and P4 addback are the conditions which negatively impact mood[87, 118] (i.e., depressive symptoms worsen). In the current study these conditions are brief, lasting only 2-weeks. Thus, any impact on mood that may occur is expected to be transient.

Any patient experiencing clinically significant side effects such as nausea, vomiting, or extreme fluid retention from the medication will have the dose titrated to achieve relief of the symptoms. If adequate relief cannot be achieved in this manner, drug treatment will be discontinued. Similarly, if menopausal-like symptoms occurring secondary to GnRH agonist treatment are intolerable, drug treatment will be discontinued.

Should an adverse event occur, we will comply with the NIMH reporting requirements for adverse events. As Principal Investigator, Dr. Baker will be responsible for the documentation, investigation, and follow-up of all study-related adverse events. All adverse events will be reported according to the NIMH expectations and reporting timeframes and provided to the study Program Officer in writing. Dr. Baker will also be responsible to report any individual occurrence of an adverse event to the Chair of the DSMB according to the guidelines established at the initial DSMB meeting. All moderate or severe adverse events will be reported to the UNC IRB within 7 calendar days. The NIMH Program Officer will be notified of any study modifications or suspension imposed by the DSMB or local IRB in response to an adverse event.

The determination of when and how to withdrawal subjects will be overseen by the study's Data and Safety Monitoring Board and will be reported to the UNC Biomedical IRB.

5 Study Drug

5.1 Description

Drug	Dosage	Formulation
Estradiol	2 mg bid (total of 4 mg/day)	Oral capsule
Lupron Depot	3.75 mg/month	Intramuscular injection
Progesterone	200 mg bid (total of 400 mg/day)	Oral capsule

5.2 Treatment Regimen

Regimen

Induced Hypogonadism. After a screening period, participants will receive their first injection of the gonadotropin-releasing hormone (GnRH) agonist leuprolide acetate (Lupron) 3.75 mg/month via i.m. injection, which is administered to produce a stable hypogonadal condition (after the initial "flair"). The first Lupron injection will be administered at approximately day six of the participants' first menstrual cycle. After 4-weeks of Lupron alone, participants will begin taking two capsules daily of either placebo, estradiol, or progesterone for 8-weeks. They will be told at some points during this final 8-week period of the study, the pills will be active medication and at some points they will be placebo. Subjects will be blinded to when medication switches to active medication; all participants will first receive 2-weeks of placebo. After 2-weeks of placebo, the end-of-phase Lupron assessment (T2) will occur and placebo capsules will be switched to active medication in a double blind, cross over design.

Addback. After 6-weeks of Lupron-alone treatment (i.e., hypogonadism), physiological levels of E2 or P4 will be attained via oral micronized E2 (2mg b.i.d) or P4 (200mg b.i.d), respectively (along with continued Lurpon administration), with a 2-week washout period in-between. Placebo pills continue during the washout. The blood levels that we expect to achieve and sustain in each woman will be approximately 500 pg/ml of estradiol and 30-40 ng/ml of progesterone.

5.3 Method for Assigning Subjects to Treatment Groups

All subjects will receive the same drug protocol in a double-blind, cross over fashion. The study biostatistician will create a randomization table. Subjects will be randomized into two arms by permutated block of small size 2 with 1:1 ratio and maximum imbalance of 1. We will also restrict E2-P4 group to have the n = 8 and P4-E2 to have n=7 because the response from E2-P4 group is the primary phase of interest. The pre-generated randomization sequence will be uploaded to REDCap, with assignment group information coded. REDCap will randomly assign a possible number from table when a subject is entered. Study personnel will only receive that randomization number and will not receive information on the coded assignment group, so not only will they not know what group a subject is in, but they won't know which subjects have been similarly assigned. We will

not store any information on what assignment groups codes actually correspond to in REDCap, further ensuring that randomization remains concealed.

5.4 Preparation and Administration of Study Drug

All study drugs will be stored, prepared, and dispensed from the UNC Investigational Drug Service (IDS).

Assigned Study Pharmacist:
Investigational Drug
Service Department of
Pharmacy UNC Hospitals
CB 7600, Room 3001
101 Manning Drive
Chapel Hill, NC 27514

5.5 Subject Compliance Monitoring

We will monitor participants' compliance with the drug regimen through self-report. Compliance will also be monitored by having participant's return any missed pills during the study visits.

5.6 Prior and Concomitant Therapy

Women are required to be free of any medications that influence ovarian hormones or are contraindicated for use with the study medications; however, prior medication usage will not preclude participation in the study.

5.7 Packaging

The UNC Investigational Drug Service will receive the active drug from their Pharmacy storeroom and will provide the capsules for blinding.

5.8 Blinding of Study Drug (if applicable)

Placebo and active medication will be in like-colored capsules with identical labeling. Subjects will take two capsules daily during each phase once oral medication administration begins.

5.9 Receiving, Storage, Dispensing and Return

The UNC Investigational Drug Service will receive the study drugs from the UNC Pharmacy Storeroom and will dispense the drug to the PI or study coordinator to deliver to participants. Any unused drug will be returned to the UNC Investigational Drug Service.

5.9.1 Receipt of Drug Supplies

To dispense medications, IDS sets up a standing medication order in the electronic medical record for each study. When a medication is needed, the project coordinator will pull up the order, enter the participant's subject ID number and confirm they provided informed consent. Within the order there is also a comment box to provide additional information to the pharmacy. When oral medications are dispensed, the project coordinator will note in this box if the medication dispensed is to be active hormone or placebo. The order is then forwarded to Co-Investigator Young for review and signature. Only an MD has the ability to sign off on an order. Orders are sent electronically to IDS who then dispenses the study medication according to the study protocol.

According to IDS protocol, prior to the first oral medication release, IDS is provided with the participant's randomization number. The research coordinator will be primarily responsible for picking up the prescription from IDS. Upon receipt, the coordinator will check the medication to ensure the prescription is for the correct participant. The research coordinator will be blinded to the sequence of the medication (E2 and P4).

5.9.2 Storage

Lupron, Estradiol, and Progesterone will be stored at 20° to 25° C in a temperature-controlled facility.

5.9.3 Dispensing of Study Drug

Drugs will be dispensed in tight, light-resistant containers and defined in the USP, with a child-resistant closure. Regular study drug reconciliation will be performed to document drug assigned, drug consumed, and drug remaining. This reconciliation will be logged on the drug accountability form and signed and dated by the study team.

5.9.4 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

6 Study Procedures

Overview. This single-site study will include 8 study visits and the reproductive hormone challenge (**Figure 5**). The experimental protocol will last approximately 3-months. Participants will undergo screening and consent, a baseline assessment, testing at the end of each experimental phase, brief testing to examine side effects and protocol adherence, and a follow-up assessment 8-weeks post-intervention.

The study timeline is depicted in **Figure 5** in Section 3, and the specific procedures that will take place are outlined in **Table 4** and detailed below.

Participants. Women between the ages of 18-42 with a current DSM-5 diagnosis of BN. Our primary research question does not require a control group and requires a within-subjects design. All participants will undergo the same hormonal challenge but in a double-blind cross-over design. Subjects will be randomly assigned to receive E2+P4 or P4+E2 based on the randomization table created by the study biostatistician.

Table 4. Study Visit Procedures

Procedure	T0	T1	T2	T3	T4	T5	Check-in
Eligibility and Enrollment							
Informed Consent	x						
Demographics	x						
SCID-5	x						
GYN Exam/Medical History	x						
Venipuncture	x						
Saliva Samples		x	x	x	x		x
Side Effects & Adherence			x	x	x		x
Self-report Questionnaires							
IDAS*	x	x	x	x	x	x	x
EDEQ*	x	x	x	x	x	x	x
EPSI	x	x	x	x	x	x	x
BIS/BAS		x	x	x	x	x	x
SPSRQ		x	x	x	x	x	x
Behavioral Tasks							
MIDT		x	x	x	x		
DD		x	x	x	x		
Go/No Go		x	x	x	x		
PANAS		x	x	x	x		
24-hour Food Intake		x	x	x	x		
*used to monitor subject safety only							

Hormone Administration. The hormone administration protocol replicates a design used by co-investigators,[87, 117] with slight modifications made based on previous study findings in order to reduce the burden of the protocol and the risk for side effects. The hormone challenge consists of three study phases: 1) hypogonadism; 2) E2 addback; 3) P4 addback. Lupron administration begins at T1 and continues throughout the duration of the challenge. Medication administration (placebo or addback) begins after 4-weeks of Lupron-alone. This is done in order to add an additional blind to participants as to whether they are on active medication vs. placebo. Because the end-of-phase assessments are longer in duration than the check-in visits, it is possible a subject could detect when active mediation begins based on the timing of the change from check-in visits to end-of-phase assessments if medication administration begins with the first dose of Lupron. This also standardizes each study phase to be 2-weeks in length as the first month of Lupron is not a study phase of interest, yet a 'waiting period' for ovarian suppression to occur.

Induced Hypogonadism. At T1, participants will receive their first injection of the gonadotropin-releasing hormone (GnRH) agonist leuprolide acetate (Lupron) 3.75 mg/month via i.m. injection, which is administered to produce a stable hypogonadal condition (after the initial "flair"). The first Lupron injection will be administered at approximately day six of the participants' first menstrual cycle and monthly thereafter. Subjects will be on Lupron alone for 6-weeks. Previous studies using this experimental design show significant change in behavioral measures of interest after 6-weeks.[87]

During the first month of GnRH agonist administration, participants will not receive any other medication. After 4-weeks of Lupron alone, all participants will receive 2-weeks of placebo capsules (blinded to the participant) and continue daily capsules throughout the duration of the study. Subjects will be told at some points the medication will be active and at other points it will be placebo. Subjects will not know when medication is active vs. placebo. An end-of-phase (T2) assessment will occur before addback, 2-weeks after medication administration begins.

Addback. After 6-weeks of Lupron-alone treatment (i.e., hypogonadism), physiological levels of E2 or P4 will be attained via oral micronized E2 (2mg b.i.d) or P4 (200mg b.i.d), respectively (along with continued Lurpon administration), with a 2-week washout period in-between. Placebo pills continue during the washout. Each addback phase will be 2-weeks (compared with 4-weeks) as previous studies using this experimental design have shown significant symptom change can be obtained within 2-weeks.[87] Subjects will be randomized to receive E2 followed by P4 or P4 followed by E2. The study biostatistician will create a randomization table and study investigators will be blinded to the randomization order. End-of-phase assessments will occur at the end of E2 and P4 addback (T3/T4).

Once medication administration begins, subjects will be instructed to take pills twice daily, at approximately the same time each day. To improve medication compliance, we will recommend subjects schedule a daily reminder on their phone to take the study medication each day. The daily assessments will also ask participant's whether they took the study medication each day.

Clinical Assessments. Measures used to obtain primary outcomes and monitor subject safety are shown in **Table 4**. Study visits will occur every 2-weeks. Participants will attend end-of-phase assessments at the end of each hormone phase as well as check-in visits to monitor symptoms. There will be an equal number of end-of-phase assessments and check-in visits (3 each) during the hormone challenge. All study measures are empirically valid and reliable.

Clinical Interviews

a) SCID-5: Structured Clinical Interview for DSM-5[181] will be administered by trained study staff at T0, and supervised by Dr. Baker, to confirm eligibility and BN diagnosis. Based on DSM-5 criteria, the interview guides trained interviewers in determining whether a psychiatric diagnosis is present or absent.

Self-report Questionnaires

Self-report questionnaires will be completed through secure, encrypted, online survey (i.e., Qualtrics), and scored by standard conventions. During the reproductive hormone challenge, (i.e., T1-T4) self-report questionnaires will be modified to represent symptomatology over the previous week only, in order to capture the period of time with maximal or minimal pathology during each hormone manipulation phase.

Primary outcome measures are bolded. Non-bolded measures are included to replicate the projected protocol for the larger mechanistic trial we will conduct based off of these pilot data but will not be examined as outcomes in the current study.

a) Demographics: at baseline, subjects will be asked to self-report relevant demographic information in order to address eligibility and to characterize the sample: age, race, ethnicity, marital status, education level, and participation in any current treatment for BN.

b) Adverse Life Events Checklist: will be completed at baseline only to obtain information on the number of adverse life events experienced by the participant as previous studies have suggested that ovarian hormone sensitivity is greater in women with a history of adverse life events.[182] Respondents indicate varying levels of exposure to each type of potentially traumatic event. A count score will be obtained which indicates the number of negative adverse life events experienced. It will be completed at baseline only and we plan to include it in our follow-up clinical trial.

a) UPPS-P: The UPPS-P Impulsive Behavior Scale[183] is a 59-item self-report questionnaire that assesses distinct dimensions of impulsivity. Here we include the negative urgency, lack of premeditation, and sensation seeking subscales. It will be completed at baseline only and we plan to include it in our follow-up clinical trial.

b) Inventory of Depression and Anxiety Symptoms (IDAS):[184] is a 64-item questionnaire that comprehensively assesses anxiety and depression symptoms, including ill temper, dysphoria, appetite change, lassitude, well-being, and suicidality. The IDAS is only used to monitor subject safety.

c) EDEQ: the Eating Disorder Examination Questionnaire,[185] will be used to monitor subject safety. Items include frequency of specific behaviors (e.g., binge eating, purging) as well as a subscale score.

d) **EPSI:** the Eating Pathology Inventory[186] is a 45-item self-report questionnaire assessing various aspects of eating disorder symptomatology and will be included in order to obtain a more detailed examination of symptoms compared with frequency counts only. The EPSI includes eight subscales; however, we only include the following subscales in the current project: binge eating, body dissatisfaction, cognitive restraint, purging, excessive exercise, and restricting. A majority of the previous studies to date examining symptom change over the menstrual cycle have focused on symptom-scores, including pilot data from our own group using the EPSI.[177]

e) **BIS/BAS Scales:** the Behavioral Inhibition/Behavioral Activation Scales[187] is a 24-item measure designed to measure behavioral inhibition and behavioral activation. It is comprised of four subscales: BIS, Reward Responsiveness, Drive, and Fun Seeking, and 20 items total that assess behavioral inhibition and behavioral activation. Primary outcomes of interest are reward responsiveness, behavioral inhibition, and behavioral activation. The BIS/BAS scales are associated with neural markers of psychopathology.

f) **SPSRQ**: The Sensitivity to Punishment/Sensitivity to Reward Questionnaire[188] is a 48-item self-report questionnaire used to assess sensitivity to reward and sensitivity to punishment.

g) The Food Cravings Questionnaire (FCQ): the Food Cravings Questionnaire,[189, 190] includes an assessment of both trait-cravings and state-cravings. Only the craving as a physiological and preoccupation with food subscales will be given. The FCQ will be included in our larger, follow-up clinical trial.

h) Three-Factor Eating Questionnaire-Hunger Subscale: The TFEQ-Hunger subscale[191] will be used to assess changes in self-reported hunger across the experimental design. Hunger will be included in the larger mechanistic trial given previous studies show in change in appetite, including our own pilot work, with E2 administration. The TFEQ will be included in our larger, follow-up clinical trial.

i) EEI: The Eating Expectancies Inventory (EEI) will be given at T2-T5. The EEI measures learned expectations about eating and contains five subscales. We include the eating helps manage negative affect, eating is useful as a reward, and eating leads to feeling out of control subscales. It will be included in our follow-up, larger clinical trial.

Scoring for Primary Outcome Self-Report Questionnaires			
Scale	**Likert Scale**	**Minimum Score**	**Maximum Score**
EPSI	5 point		
Binge eating		0	32
BIS/BAS	4 point		
Behavioral inhibition		7	28
Fun seeking		4	16
Reward responsiveness		5	20
SPSRQ	2 point(T/F)		
Sensitivity to reward		0	24

Reward Response Behavioral Tasks

a) Monetary Incentive Delay Task (MIDT)[174]: Two event-related MID runs consist of 6-second trials during which women will be presented with a cue shape, a fixation crosshair (for variable duration), the target, and performance (win/loss/neutral) feedback. Cues indicate whether it is an incentive (gain, loss) or non-incentive trial. In incentive trials, women can gain or lose money by pressing a button during target presentation; difficulty is based on individual reaction times. MID defines reward response as the average speed of responses to reward vs. non-reward trials during a reward task.

b) Delayed Discounting (DD) Task: The DD task[192] is used to understand how subjects make a choice between an immediate, smaller reward and a larger reward given after a time delay. Discounting is assessed across 4 time delays: 2, 30, 180, and 365 days later. Delays are presented in a mixed fashion. Questions are posed to participants asking whether they would prefer to receive a given amount of money immediately or a larger amount after a delay. The amount of money available immediately is adjusted with each trial to calculate an indifference point for each delay periods. Discounting rates of hypothetical and real reward tasks are comparable.[193-195] DD defines reward response as the tendency to choose small, immediate rewards over larger, delayed rewards. The task determines the rate of devaluation over time for the larger, delayed reward, which is defined as the indifference point.[192] The k parameter will be obtained and used as the primary outcome.

c) Go No/Go Task: A behavioral Go No/Go Task will be used as a measure of inhibitory control. Each trial consists of one stimulus indicating either "go" (response) or "no/go" (do not respond). The response accuracy of each no/go trial is used as the indicator for inhibitory control such that fewer errors ("go" response on a "no/go" trial) signifies better inhibitor control.

d) Positive Affect Negative Affect Schedule: The PANAS[180] is a 10-item questionnaire that measures current positive and negative affect. The measure will be given to subjects prior to the behavioral tasks to include as a potential covariate in statistical analyses. Items are answered according to a 1 to 5 Likert scale and then summed to create the positive and negative affect subscales with scores ranging from 10 to 50.

e) 24-hour Food Intake: We will ask women to self-report their food intake for the 24-hours prior to study appointments. Detailed information will be obtained so that estimated caloric intakes can be determined based on the provided information as well as the number of meals and snacks eaten.

Daily Symptom Assessment

a) Daily Rating Form:[36] 20-item questionnaire that assesses the presence and intensity of physical and mood symptoms that accompany ovarian hormone changes, will be used to assess daily symptoms and safety. This will be completed by subjects online (i.e., Qualtrics) each morning starting at T0 and ending at T4. Thus, there will be 17 weekly scores for each participant. The form will be slightly modified to include BN symptom ratings (e.g., binge eating frequency). **Ratings are transmitted to the study team in real-time, which is essential for monitoring changes throughout the study and ensuring participant safety.**

Assessment Schedule.

Eligibility. Interested participants will initially complete an online screening survey to assess potential eligibility. No more information than required to determine an accurate representation of eligibility will be collected. This can also be completed by telephone interview if needed. Women who screen eligible on the survey and are interested in participating in the study will be contacted by a member of the study staff to schedule a review consent and schedule a screening assessment.

Screening. At this initial screening visit (T0), consent forms will be reviewed, eligibility confirmed, and a Clinical and Health Screening will be completed. Women will be required to have a GYN exam within the past year. Women who have had this exam completed already will be asked to provide medical records for review. Women who have not had this exam within the past year can go to their own provider to complete the exam—providing the records for review—or can be scheduled to see the study GYN (Dr. Schiff). This screening will include a laboratory panel (CMP, CBC, pregnancy test). After enrollment (if eligible), participants will complete the Daily Rating Form for one natural menstrual cycle. This form will be completed each morning, throughout the study duration. We expect the completion of the Daily Rating Form to take <5 minutes. The Daily Rating Form will also be used to monitor subject safety regarding changes in mood and eating disorder symptoms throughout the protocol.

Baseline (T1) and end-of-phase assessments (T2-T4). Prior to beginning the reproductive hormone challenge, participants will complete a baseline assessment (T1) including self-report questionnaires and behavior tasks. Most procedures will be repeated at the end of each experimental phase: after 6-weeks of Lupron (T2); after 2-weeks of E2/P4 (T3/T4). After the baseline assessment, subjects will complete three end-of-phase assessments.

Check-in Visits. In additional to the Daily Rating Form, subjects will have study visits (either end-of-phase or check-in visits) every two weeks. Check-in visits will be completed in order to assess side effects and protocol adherence. Two check-in visits will occur during Lupron only before medication administration begins and one will occur during medication administration (after the washout period). The number of check-in visits and end-of-phase assessments are standardized across the study protocol. The same adherence, monitoring, and side-effect checks will occur at every study visit once medication administration begins. Along with monitoring side effects and adherence, this information will be used for protocol development in our future studies. From these visits, we can pinpoint when ovarian suppression has occurred, and thus, the protocol could be modified for future studies based on this information.

Medication Administration and Study Visits. The first dose of Lupron will be given at T1 and monthly thereafter. Medication administration (placebo, E2, or P4) will begin after 4-weeks of Lupron alone. Subjects will be blinded as to whether capsules contain placebo, E2, or P4. Investigators will be blinded to the order of E2 and P4. Subjects will be told that at some points the medication will be active and at other points the medication will be inactive. Once medication administration begins, participants will have four remaining study visits; three end-of-phase assessments and one check-in visit. The behavioral tasks that are part of the end-of-phase assessments are the primary difference between the end-of-phase visits and the check-in visit (we believe participants would be unlikely to notice small differences in the type and number of self-report questionnaires given). Participants will be told that, over the final 8-weeks of the study, they will attend three long study visits and one short study visit, and the addition of the behavioral tasks to the study visit will be randomly scheduled. They will be informed of the next study visit type at their current study visit. Although the assignment of the behavioral tasks is not random, this is done in order to decrease the likelihood that the participant would detect

whether they are on inactive or active medication by the timing and duration of the study visits. Given the significant length of time the addition of the behavioral tasks adds to the study visits (~ 60 minutes), it would add significant and unreasonable participant burden to add the behavioral tasks to the post-washout period visit, with no empirical/scientific justification to do so given we are not addressing symptom change on the behavioral tasks during the washout period.

Follow-up. Eight weeks after study completion a brief follow-up assessment, via online self-report questionnaire, will be completed by participants.

7 Statistical Plan

Mr. Russ Dean is the database manager and Kai Xia, PhD, is the biostatistician who will complete the statistical analyses.

7.1 Sample Size Determination

This is a pilot study to obtain pilot data for larger studies. Previous observational studies in humans suggest at least a moderate effect of ovarian hormones on binge eating with an effect size approximating $d = 1.5$,[5, 196, 19] including Dr. Schiller's pilot work described above. Additionally, Dr. Schiller's pilot work of E2 treatment in midlife women indicates $d = 2.3$ for the effect of E2 replacement decreasing appetite in women with and without major depression whereas other published work showed $d = 1.51$ for the effect of Lupron on decreasing food cravings in women with PMS.[87] Because we are directly manipulating ovarian hormones with an experimental design, which removes other potential confounds found in observational studies, we expect that the effect sizes from observational studies are an underestimate.

According to a power estimation for Aims 1 and 2, with a projected sample of $n = 15$, an effect size of .77 is required to detect a treatment difference at a two-sided .05 significance level at 80% power (calculated based off of projected sample size and desired power). Additionally, based on $n = 15$ and estimated effect size of 1.5 for E2 on binge eating (the average effect size observed in previous studies),[5, 196, 197] a power estimation for Aim 1 (our primary aim) estimates our power at significance of .05 is > 90%. Thus, despite the smaller sample size of this pilot study, power analyses indicate we should have sufficient power based on hypothesized effect sizes (which are empirically based) for E2 on binge eating.

7.2 Statistical Methods

Statistical analyses are completed using the most recent version of R. Primary outcome measures, as described below, are binge eating and aspects of the reward response. Methods are modeled after similar, successfully implemented studies.[87, 117] All measures included are empirically valid, and well-established, thus, measures will be scored according to standard conventions and scoring procedures. Descriptive statistics and graphics will be used to screen for errors, outliers, and potential influential observations and to check distributional assumptions. Where appropriate statistical estimates will be tabulated. We first complete analysis without covariates. As appropriate, relevant covariates may also be included in data analysis (e.g., negative affect, self-reported food intake).

PRIMARY OUTCOMES

Binge eating will be defined as: 1) the EPSI subscale binge eating score obtained at each study visit (T1-T4), which represents a continuous score of symptoms over the previous week (higher scores indicate more symptoms); 2) a weekly average based on daily frequency obtained from the DSRP. For both the subscale score and weekly average, the primary outcome is the last week of each phase of the hormone challenge to capture the period of time with maximal symptom change.

Reward response will be defined by the Monetary Incentive Delay (MID),[174] the Delayed Discounting (DD), and Go No/Go behavioral tasks and the BIS/BAS and SPSRQ self-report questionnaires. MID operationalizes reward response as *motivated behavior*: the average speed of responses to reward vs. non-reward trials during a reward task.

DD operationalizes reward response as "delay gratification": the tendency to choose small, immediate rewards over larger, delayed rewards. The task determines the rate of devaluation over time for the larger, delayed reward, which is defined as the k parameter. We use behavioral tasks of monetary reward vs. food because the value of food is dependent on hunger state[202, 203] and it reduces the confounding effect of the task provoking symptoms.[34]

The Go/No Go Task is a behavioral measure of inhibitory control. Inhibitory control is defined by the response accuracy of the go no/go trials with fewer errors ("go" response on a "no/go" trial) indicating better inhibitory control.

The BIS/BAS and SPSRQ will be included as a self-report measures of reward response. Specifically, we will include the reward responsiveness, behavioral inhibition, and fun seeking subscales of the BIS/BAS as self-report correlates of the reward response. The SPSRQ will be used to assess sensitivity to reward.

Hypothesis. Our overarching hypothesis for the following aims is that BN represents a hormone sensitive phenotype and this sensitivity is displayed as an impaired reward response within the context of low E2 such that E2 addback will be beneficial for all outcomes of interest (i.e., binge eating, aspects of the reward response). As such, our null hypothesis is that there is no effect of E2 on binge eating or the reward response. For all hypothesis tests, we will use the Benjamini-Hochberg Procedure to correct for multiple comparisons.

Aim 1: Quantify the direct effect of E2 and P4 on binge eating in women with BN.

Dependent variables: EPSI binge eating subscale score; DSRP binge eating weekly average.
Independent variables: Treatment sequence, treatment condition, and selected covariates

The two longitudinal measures of binge eating (i.e., EPSI subscale score and DSRP weekly average) will be fit to linear mixed effect models with subject-specific random intercept assuming an unstructured covariance among different time points. Such a covariance allows different variance and covariance parameters to be estimated for each time point allowing for potential increasing or decreasing variability in outcomes during each of the follow-up time points for each outcome and will include fixed effect predictors such as binary treatment sequence (E2-P4 or P4-E2) and treatment condition of categorical variable with three levels: Lupron-alone, E2, or P4, where effect size and standard error of fixed effect parameters will be estimated in the described model followed by *t*-test. Study hypotheses comparing outcomes between group outcomes of E2 vs. Lupron-alone, E2 vs. P4, and P4 vs. Lupron-alone, will be tested using contrasts test through *t*-test of least squares means estimate in the context of the main effects model.

Along with the primary contrasts of interest described above, additional contrasts between each end point of treatment condition (Lupron-alone, E2, or P2) and baseline (T1), which occurs prior to medication administration, will be tested using the statistical model just described above.

Aim 2: Determine the effect of E2 on reward response and related correlates (e.g., behavioral inhibition) in women with BN.

Dependent variables: Behavioral measures of reward response, self-report measures of reward response.
Independent variables: Treatment sequence, treatment condition, and selected covariates

Behavioral measures of reward response (MID, DD) and related correlates (Go No/Go, BIS/BAS, SPSRQ) will be fit to linear mixed effect models as described above with binary treatment sequence (E2-P4 or P4-E2), treatment condition, and covariates as independent variables and reward response as dependent variables. The contrasts between E2 and baseline (T1) or Lupron-alone (T2) will be tested in the same model.

For the behavioral tasks, beneficial impacts of E2 are defined as an accelerated speed of response to rewards and improvement in reward learning during the MID, higher indifference points during the DD task, and fewer errors on the Go No/Go Task. For self-report questionnaires, a decrease in scores represents a beneficial response as higher scores indicate more "pathological" responses.

Aim 3: Examine the association between reward response (defined in Aim 2) and binge eating before and after E2 addback.

Null hypothesis: There is no association between reward response and binge eating before and after E2 addback
Alternative hypothesis: There is an association between reward response and binge eating before and after E2 addback.

We will explore Pearson correlations between change in self-reported reward responses and change in binge eating between baseline and E2 addback. Binge eating will be defined by the EPSI subscale score. Reward response will include the BIS/BAS reward responsiveness subscale, and SPSRQ reward sensitivity subscale. Specifically, a change score will be created that signifies the amount and direction of change that occurred in binge eating and reward responses between baseline and E2 addback for each subject. A Pearson correlation will then be examined between binge eating and reward response change scores.

If Aims 1 and 2 alternative hypotheses are supported, we will conduct a tertiary exploration to address whether changes in reward behavior mediate the link between E2 addback and changes of binge eating. A four-step mediation analysis (Baron & Kenny 1986) will be applied to preliminarily investigate the mediation effect of reward-motivated behavior on changes of binge eating. An approximated t-test will be used to test the significance of the mediation effect.

Missingness. We will use multiple imputation to correct for data determined to be missing at random. Data for any dropouts will be compared with data for those who complete the study to determine the extent to which excluding their data will introduce selection bias, and selection bias will be identified as a weakness in any resulting publications or presentations.

Sensitivity Analysis. In addition to the statistical tests described above, we will use sensitivity analyses to evaluate the robustness of the main results of the study to reasonable perturbations of the statistical methods and assumptions used and to help ensure reproducibility of the main results. Sensitivity analysis will address the inclusion/exclusion of questionable data values (i.e., outliers), the use of alternative methods of coping with missing values and dropouts, and modeling assumptions. We will additionally examine whether associations differ based on the severity (e.g., DSM-5 mild, moderate, severe, extreme) of baseline binge eating and purging frequency.

7.3 Subject Population(s) for Analysis

Most studies addressing the impact of ovarian hormones on binge eating have included heterogenous samples. While this increases generalizability, findings may be less robust or inconsistent because associations between variables of interest may differ by sub-groups within these populations. Specifically, we hypothesize these individuals would vary in their baseline dopamine activity which, in turn, would affect whether E2 has a beneficial or worsening effect on symptoms: the behavioral effect of E2 would not be consistent unless individual differences in dopamine are accounted for. This may account for why when samples are separated by high and low pathology (with higher pathology predicted to represent low dopamine), low E2 is consistently the catalyst for increased symptomatology.[5, 12] Thus, the sample for this proof of concept study is focused on a clinical population of BN, a sub-group proxy for low baseline dopamine.

8 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of EC/IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. See Attachment for a copy of the Subject Informed Consent Form. This consent form will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a subject, using the EC/IRB- approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

9 Study Finances

9.1 Funding Source

This study is financed through a R21 grant from the National Institutes of Health.

9.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) will have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All UNC investigators will follow the University conflict of interest policy.

9.3 Subject Stipends or Payments

Participants will be compensated $900 upon completion of the study according to the schedule below. For study visits that occur at UNC, parking vouchers will be provided as well as mileage reimbursement for study. Payment is processed through the UNC Department of Psychiatry.

Long Study Visits	
Clinical Health Screening Visit	$20
Long Visit 1 (before medication administration)	$50
Long Visit 2 (during medication administration)	$75
Long Visit 3 (during medication administration)	$75
Long Visit 4 (during medication administration	$75
Other Study Activities	
GYN exam	$30
Screening phase (1 menstrual cycle)	$30
Short Clinic Visits (3 visits, $20/each)	$60
Lupron Injection (3 injections, $5/each)	$15
Completion of daily survey and medication adherence during hormone challenge ($5.00/day for 91 days)	$455
Follow-up Survey	$15

10 References

1. American Psychiatric Association, *Diagnostic and Statistical Manual of Mental Disorders. Fifth Edition*2013, Washington, DC: American Psychiatric Press.
2. Chesney, E, Goodwin, GM, and Fazel, S. Risks of all-cuse and suicide mortality in mental disorders: a meta-review. World Psychiatry. 2014;13:153-160.
3. Hudson, JI, Hiripi, E, Pope, HG, Jr., and Kessler, RC. The prevalence and correlates of eating disorders in the National Comorbidity Survey Replication. Biol Psychiatry. 2007;61:348-358.
4. Baker, JH, Girdler, SS, and Bulik, CM. The role of reproductive hormones in the development and maintenance of eating disorders. Expert Rev Obstet Gynecol. 2012;7(6):573-583.
5. Edler, C, Lipson, SF, and Keel, PK. Ovarian hormones and binge eating in bulimia nervosa. Psychol Med. 2007;37(1):131-141.
6. Klump, KL, Keel, PK, Culbert, KM, and Edler, C. Ovarian hormones and binge eating: exploring associations in community samples. Psychol Med. 2008;38(12):1749-1757.
7. Baker, JH and Runfola, CD. Eating disorders in midlife women: A perimenopausal eating disorder? Maturitas. 2016;85:112-116.
8. Asarian, L and Geary, N. Modulation of appetite by gonadal steroid hormones. Philos Trans R Soc Lond B Biol Sci. 2006;361(1471):1251-1263.
9. Eckel, LA. The ovarian hormone estradiol plays a crucial role in the control of food intake in females. Physiol Behav. 2011;104(4):517-524.
10. Yu, Z, Geary, N, and Corwin, RL. Ovarian hormones inhibit fat intake under binge-type conditions in ovariectomized rats. Physiol Behav. 2008;95(3):501-507.
11. Klump, KL, Culbert, KM, and Sisk, CL. Sex Differences in Binge Eating: Gonadal Hormone Effects Across Development. Annual review of clinical psychology. 2017;13:183-207.
12. Klump, KL, Racine, SE, Hildebrandt, B, Burt, SA, Neale, M, Sisk, CL, Boker, S, and Keel, PK. Ovarian Hormone Influences on Dysregulated Eating: A Comparison of Associations in Women with versus without Binge Episodes. Clin Psychol Sci. 2014;2(4):545-559.
13. Lester, NA, Keel, PK, and Lipson, SF. Symptom fluctuation in bulimia nervosa: relation to menstrual-cycle phase and cortisol levels. Psychol Med. 2003;33(1):51-60.
14. Price, WA, Torem, MS, and DiMarzio, LR. Premenstrual exacerbation of bulimia. Psychosomatics. 1987;28(7):378-379.
15. Gladis, MM and Walsh, BT. Premenstrual exacerbation of binge eating in bulimia. Am J Psychiatry. 1987;144(12):1592-1595.
16. Mac Neil, BA, Hudson, CC, Dempsey, K, and Nadkarni, P. A case of symptom relapse post placement of intrauterine device (IUD) in a patient with bulimia nervosa: consequence or coincidence. Eat Weight Disord. 2017;22(2):369-372.
17. Dahlgren, CL and Qvigstad, E. Eating disorders in premenstrual dysphoric disorder: a neuroendocrinological pathway to the pathogenesis and treatment of binge eating. J Eat Disord. 2018;6:35.
18. Mangweth-Matzek, B, Hoek, HW, Rupp, CI, Kemmler, G, Pope, HG, Jr., and Kinzl, J. The menopausal transition--a possible window of vulnerability for eating pathology. Int J Eat Disord. 2013;46(6):609-616.
19. Barth, C, Villringer, A, and Sacher, J. Sex hormones affect neurotransmitters and shape the adult female brain during hormonal transition periods. Front Neurosci. 2015;9:37.
20. Maki, PM and Dumas, J. Mechanisms of action of estrogen in the brain: insights from human neuroimaging and psychopharmacologic studies. Semin Reprod Med. 2009;27(3):250-259.
21. Comasco, E, Frokjaer, VG, and Sundstrom-Poromaa, I. Functional and molecular neuroimaging of menopause and hormone replacement therapy. Front Neurosci. 2014;8:388.

22. Yoest, KE, Cummings, JA, and Becker, JB. Estradiol, dopamine and motivation. Cent Nerv Syst Agents Med Chem. 2014;14(2):83-89.
23. Yoest, KE, Quigley, JA, and Becker, JB. Rapid effects of ovarian hormones in dorsal striatum and nucleus accumbens. Horm Behav. 2018.
24. Berner, LA and Marsh, R. Frontostriatal circuits and the development of bulimia nervosa. Front Behav Neurosci. 2014;8:395.
25. Berner, LA, Brown, TA, Lavender, JM, Lopez, E, Wierenga, CE, and Kaye, WH. Neuroendocrinology of reward in anorexia nervosa and bulimia nervosa: Beyond leptin and ghrelin. Mol Cell Endocrinol. 2018.
26. McAdams, CJ and Smith, W. Neural correlates of eating disorders: translational potential. Neurosci Neuroecon. 2015;4:35-49.
27. Frank, GK. Advances from neuroimaging studies in eating disorders. CNS spectrums. 2015;20(4):391-400.
28. Wu, M, Brockmeyer, T, Hartmann, M, Skunde, M, Herzog, W, and Friederich, HC. Reward-related decision making in eating and weight disorders: A systematic review and meta-analysis of the evidence from neuropsychological studies. Neurosci Biobehav Rev. 2016;61:177-196.
29. Eneva, KT, Murray, S, O'Garro-Moore, J, Yiu, A, Alloy, LB, Avena, NM, and Chen, EY. Reward and punishment sensitivity and disordered eating behaviors in men and women. J Eat Disord. 2017;5:6.
30. Wu, M, Hartmann, M, Skunde, M, Herzog, W, and Friederich, HC. Inhibitory control in bulimic-type eating disorders: a systematic review and meta-analysis. PLoS One. 2013;8(12):e83412.
31. Richard, JE, Lopez-Ferreras, L, Anderberg, RH, Olandersson, K, and Skibicka, KP. Estradiol is a critical regulator of food-reward behavior. Psychoneuroendocrinology. 2017;78:193-202.
32. Smith, CT, Sierra, Y, Oppler, SH, and Boettiger, CA. Ovarian cycle effects on immediate reward selection bias in humans: a role for estradiol. J Neurosci. 2014;34(16):5468-5476.
33. Macoveanu, J, Henningsson, S, Pinborg, A, Jensen, P, Knudsen, GM, Frokjaer, VG, and Siebner, HR. Sex-Steroid Hormone Manipulation Reduces Brain Response to Reward. Neuropsychopharmacology. 2016;41(4):1057-1065.
34. Wierenga, CE, Ely, A, Bischoff-Grethe, A, Bailer, UF, Simmons, AN, and Kaye, WH. Are Extremes of Consumption in Eating Disorders Related to an Altered Balance between Reward and Inhibition? Front Behav Neurosci. 2014;8:410.
35. Monteleone, AM, Monteleone, P, Esposito, F, Prinster, A, Volpe, U, Cantone, E, Pellegrino, F, Canna, A, Milano, W, Aiello, M, Di Salle, F, and Maj, M. Altered processing of rewarding and aversive basic taste stimuli in symptomatic women with anorexia nervosa and bulimia nervosa: An fMRI study. Journal of psychiatric research. 2017;90:94-101.
36. Harrison, A, O'Brien, N, Lopez, C, and Treasure, J. Sensitivity to reward and punishment in eating disorders. Psychiatry research. 2010;177(1-2):1-11.
37. Kekic, M, Bartholdy, S, Cheng, J, McClelland, J, Boysen, E, Musiat, P, O'Daly, OG, Campbell, IC, and Schmidt, U. Increased temporal discounting in bulimia nervosa. Int J Eat Disord. 2016;49(12):1077-1081.
38. Bartholdy, S, Rennalls, S, Danby, H, Jacques, C, Campbell, IC, Schmidt, U, and O'Daly, OG. Temporal Discounting and the Tendency to Delay Gratification across the Eating Disorder Spectrum. Eur Eat Disord Rev. 2017;25(5):344-350.
39. McClelland, J, Dalton, B, Kekic, M, Bartholdy, S, Campbell, IC, and Schmidt, U. A systematic review of temporal discounting in eating disorders and obesity: Behavioural and neuroimaging findings. Neurosci Biobehav Rev. 2016;71:506-528.
40. Steward, T, Mestre-Bach, G, Vintro-Alcaraz, C, Aguera, Z, Jimenez-Murcia, S, Granero, R, and Fernandez-Aranda, F. Delay Discounting of Reward and Impulsivity in Eating Disorders: From Anorexia Nervosa to Binge Eating Disorder. Eur Eat Disord Rev. 2017;25(6):601-606.

41. Smith, KE, Mason, TB, Johnson, JS, Lavender, JM, and Wonderlich, SA. A systematic review of reviews of neurocognitive functioning in eating disorders: The state-of-the-literature and future directions. Int J Eat Disord. 2018;51(8):798-821.
42. Merlotti, E, Mucci, A, Volpe, U, Montefusco, V, Monteleone, P, Bucci, P, and Galderisi, S. Impulsiveness in patients with bulimia nervosa: electrophysiological evidence of reduced inhibitory control. Neuropsychobiology. 2013;68(2):116-123.
43. Rosval, L, Steiger, H, Bruce, K, Israel, M, Richardson, J, and Aubut, M. Impulsivity in women with eating disorders: problem of response inhibition, planning, or attention? Int J Eat Disord. 2006;39(7):590-593.
44. Diekhof, EK. Estradiol and the Reward System in Humans. Current Opinion in Behavioral Sciences. 2018;23:58-64.
45. Colzato, LS, Pratt, J, and Hommel, B. Estrogen modulates inhibition of return in healthy human females. Neuropsychologia. 2012;50(1):98-103.
46. Diekhof, EK. Be quick about it. Endogenous estradiol level, menstrual cycle phase and trait impulsiveness predict impulsive choice in the context of reward acquisition. Horm Behav. 2015;74:186-193.
47. Colzato, LS, Hertsig, G, van den Wildenberg, WP, and Hommel, B. Estrogen modulates inhibitory control in healthy human females: evidence from the stop-signal paradigm. Neuroscience. 2010;167(3):709-715.
48. Flores, RJ, Pipkin, JA, Uribe, KP, Perez, A, and O'Dell, LE. Estradiol promotes the rewarding effects of nicotine in female rats. Behav Brain Res. 2016;307:258-263.
49. Galankin, T, Shekunova, E, and Zvartau, E. Estradiol lowers intracranial self-stimulation thresholds and enhances cocaine facilitation of intracranial self-stimulation in rats. Horm Behav. 2010;58(5):827-834.
50. Weafer, J and de Wit, H. Sex differences in impulsive action and impulsive choice. Addictive behaviors. 2014;39(11):1573-1579.
51. Banis, S and Lorist, MM. The combined effects of menstrual cycle phase and acute stress on reward-related processing. Biological psychology. 2017;125:130-145.
52. Kaighobadi, F and Stevens, JR. Does fertility status influence impulsivity and risk taking in human females? Adaptive influences on intertemporal choice and risky decision making. Evol Psychol. 2013;11(3):700-717.
53. Saunders, KE and Hawton, K. Suicidal behaviour and the menstrual cycle. Psychol Med. 2006;36(7):901-912.
54. Broder, A and Hohmann, N. Variations in risk taking behavior over the menstrual cycle: An improved replication. Evolution and Human Behavior. 2003;24(6):391-398.
55. Beck, RC and Triplett, MF. Test-retest reliability of a group-administered paper-pencil measure of delay discounting. Exp Clin Psychopharmacol. 2009;17(5):345-355.
56. Van Haaren, F, Van Hest, A, and Van De Poll, NE. Self-control in male and female rats. J Exp Anal Behav. 1988;49(2):201-211.
57. Uban, KA, Rummel, J, Floresco, SB, and Galea, LA. Estradiol modulates effort-based decision making in female rats. Neuropsychopharmacology. 2012;37(2):390-401.
58. Dalley, JW and Roiser, JP. Dopamine, serotonin and impulsivity. Neuroscience. 2012;215:42-58.
59. Shansky, RM, Glavis-Bloom, C, Lerman, D, McRae, P, Benson, C, Miller, K, Cosand, L, Horvath, TL, and Arnsten, AF. Estrogen mediates sex differences in stress-induced prefrontal cortex dysfunction. Mol Psychiatry. 2004;9(5):531-538.
60. Jacobs, E and D'Esposito, M. Estrogen shapes dopamine-dependent cognitive processes: implications for women's health. J Neurosci. 2011;31(14):5286-5293.
61. Evans, SM, Haney, M, and Foltin, RW. The effects of smoked cocaine during the follicular and luteal phases of the menstrual cycle in women. Psychopharmacology (Berl). 2002;159(4):397-406.

62. Justice, AJ and de Wit, H. Acute effects of d-amphetamine during the follicular and luteal phases of the menstrual cycle in women. Psychopharmacology (Berl). 1999;145(1):67-75.
63. Jakob, K, Ehrentreich, H, Holtfrerich, SKC, Reimers, L, and Diekhof, EK. DAT1-Genotype and Menstrual Cycle, but Not Hormonal Contraception, Modulate Reinforcement Learning: Preliminary Evidence. Front Endocrinol (Lausanne). 2018;9:60.
64. Bazzett, TJ and Becker, JB. Sex differences in the rapid and acute effects of estrogen on striatal D2 dopamine receptor binding. Brain Res. 1994;637(1-2):163-172.
65. Di Paolo, T. Modulation of brain dopamine transmission by sex steroids. Rev Neurosci. 1994;5(1):27-41.
66. Xiao, L and Becker, JB. Quantitative microdialysis determination of extracellular striatal dopamine concentration in male and female rats: effects of estrous cycle and gonadectomy. Neurosci Lett. 1994;180(2):155-158.
67. Egan, MF, Goldberg, TE, Kolachana, BS, Callicott, JH, Mazzanti, CM, Straub, RE, Goldman, D, and Weinberger, DR. Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. Proc Natl Acad Sci U S A. 2001;98(12):6917-6922.
68. Mattay, VS, Goldberg, TE, Fera, F, Hariri, AR, Tessitore, A, Egan, MF, Kolachana, B, Callicott, JH, and Weinberger, DR. Catechol O-methyltransferase val158-met genotype and individual variation in the brain response to amphetamine. Proc Natl Acad Sci U S A. 2003;100(10):6186-6191.
69. Colzato, LS and Hommel, B. Effects of estrogen on higher-order cognitive functions in unstressed human females may depend on individual variation in dopamine baseline levels. Front Neurosci. 2014;8:65.
70. Frank, GK. The Perfect Storm - A Bio-Psycho-Social Risk Model for Developing and Maintaining Eating Disorders. Front Behav Neurosci. 2016;10:44.
71. Bello, NT and Hajnal, A. Dopamine and binge eating behaviors. Pharmacology, biochemistry, and behavior. 2010;97(1):25-33.
72. Jimerson, DC, Lesem, MD, Kaye, WH, and Brewerton, TD. Low serotonin and dopamine metabolite concentrations in cerebrospinal fluid from bulimic patients with frequent binge episodes. Arch Gen Psychiatry. 1992;49(2):132-138.
73. Broft, A, Shingleton, R, Kaufman, J, Liu, F, Kumar, D, Slifstein, M, Abi-Dargham, A, Schebendach, J, Van Heertum, R, Attia, E, Martinez, D, and Walsh, BT. Striatal dopamine in bulimia nervosa: a PET imaging study. Int J Eat Disord. 2012;45(5):648-656.
74. Kaye, WH, Wierenga, CE, Bailer, UF, Simmons, AN, Wagner, A, and Bischoff-Grethe, A. Does a shared neurobiology for foods and drugs of abuse contribute to extremes of food ingestion in anorexia and bulimia nervosa? Biol Psychiatry. 2013;73(9):836-842.
75. Frank, GK, Reynolds, JR, Shott, ME, and O'Reilly, RC. Altered temporal difference learning in bulimia nervosa. Biol Psychiatry. 2011;70(8):728-735.
76. Mikolajczyk, E, Grzywacz, A, and Samochowiec, J. The association of catechol-O-methyltransferase genotype with the phenotype of women with eating disorders. Brain Res. 2010;1307:142-148.
77. Collantoni, E, Solmi, M, Gallicchio, D, Santonastaso, P, Meneguzzo, P, Carvalho, AF, Stubbs, B, Clementi, M, Pinato, C, Forzan, M, Cassina, M, Fontana, F, Piva, I, Siani, R, Salvo, P, Tenconi, E, Veronese, N, Correll, CU, and Favaro, A. Catechol-O-Methyltransferase (COMT) Val158Met Polymorphism and Eating Disorders: Data From a New Biobank and Meta-Analysis of Previously Published Studies. Eur Eat Disord Rev. 2017;25(6):524-532.
78. Gervasini, G, Gonzalez, LM, Mota-Zamorano, S, Gamero-Villarroel, C, Carrillo, JA, Flores, I, and Garcia-Herraiz, A. Association of COMT Val158Met Polymorphism with Psychopathological Symptoms in Patients with Eating Disorders. Curr Mol Med. 2018;18(1):65-70.
79. Donofry, SD, Roecklein, KA, Wildes, JE, Miller, MA, Flory, JD, and Manuck, SB. COMT met allele differentially predicts risk versus severity of aberrant eating in a large community sample. Psychiatry research. 2014;220(1-2):513-518.

80. Yilmaz, Z, Kaplan, AS, Zai, CC, Levitan, RD, and Kennedy, JL. COMT Val158Met variant and functional haplotypes associated with childhood ADHD history in women with bulimia nervosa. Progress in neuro-psychopharmacology & biological psychiatry. 2011;35(4):948-952.
81. Appelhans, BM, Woolf, K, Pagoto, SL, Schneider, KL, Whited, MC, and Liebman, R. Inhibiting food reward: delay discounting, food reward sensitivity, and palatable food intake in overweight and obese women. Obesity (Silver Spring, Md. 2011;19(11):2175-2182.
82. Monteleone, AM, Castellini, G, Volpe, U, Ricca, V, Lelli, L, Monteleone, P, and Maj, M. Neuroendocrinology and brain imaging of reward in eating disorders: A possible key to the treatment of anorexia nervosa and bulimia nervosa. Progress in neuro-psychopharmacology & biological psychiatry. 2018;80(Pt B):132-142.
83. Sinclair, EB, Hildebrandt, BA, Culbert, KM, Klump, KL, and Sisk, CL. Preliminary evidence of sex differences in behavioral and neural responses to palatable food reward in rats. Physiol Behav. 2017;176:165-173.
84. Klump, KL, Fowler, N, Mayhall, L, Sisk, CL, Culbert, KM, and Burt, SA. Estrogen moderates genetic influences on binge eating during puberty: Disruption of normative processes? J Abnorm Psychol. 2018;127(5):458-470.
85. Monteleone, P, Luisi, M, Colurcio, B, Casarosa, E, Monteleone, P, Ioime, R, Genazzani, AR, and Maj, M. Plasma levels of neuroactive steroids are increased in untreated women with anorexia nervosa or bulimia nervosa. Psychosom Med. 2001;63(1):62-68.
86. Russo, SJ, Festa, ED, Fabian, SJ, Gazi, FM, Kraish, M, Jenab, S, and Quinones-Jenab, V. Gonadal hormones differentially modulate cocaine-induced conditioned place preference in male and female rats. Neuroscience. 2003;120(2):523-533.
87. Schmidt, PJ, Nieman, LK, Danaceau, MA, Adams, LF, and Rubinow, DR. Differential behavioral effects of gonadal steroids in women with and in those without premenstrual syndrome. The New England journal of medicine. 1998;338(4):209-216.
88. Bloch, M, Schmidt, PJ, Danaceau, M, Murphy, J, Nieman, L, and Rubinow, DR. Effects of gonadal steroids in women with a history of postpartum depression. Am J Psychiatry. 2000;157(6):924-930.
89. Ben Dor, R, Harsh, VL, Fortinsky, P, Koziol, DE, Rubinow, DR, and Schmidt, PJ. Effects of pharmacologically induced hypogonadism on mood and behavior in healthy young women. Am J Psychiatry. 2013;170(4):426-433.
90. Linardon, J, Wade, TD, de la Piedad Garcia, X, and Brennan, L. The efficacy of cognitive-behavioral therapy for eating disorders: A systematic review and meta-analysis. Journal of consulting and clinical psychology. 2017;85(11):1080-1094.
91. Agras, WS, Walsh, T, Fairburn, CG, Wilson, GT, and Kraemer, HC. A multicenter comparison of cognitive-behavioral therapy and interpersonal psychotherapy for bulimia nervosa. Arch Gen Psychiatry. 2000;57(5):459-466.
92. Mussell, MP, Crosby, RD, Crow, SJ, Knopke, AJ, Peterson, CB, Wonderlich, SA, and Mitchell, JE. Utilization of empirically supported psychotherapy treatments for individuals with eating disorders: A survey of psychologists. Int J Eat Disord. 2000;27(2):230-237.
93. Crow, S. The economics of eating disorder treatment. Curr Psychiatry Rep. 2014;16(7):454.
94. Halmi, KA, Agras, WS, Mitchell, J, Wilson, GT, Crow, S, Bryson, SW, and Kraemer, H. Relapse predictors of patients with bulimia nervosa who achieved abstinence through cognitive behavioral therapy. Arch Gen Psychiatry. 2002;59(12):1105-1109.
95. Linardon, J and Wade, TD. How many individuals achieve symptom abstinence following psychological treatments for bulimia nervosa? A meta-analytic review. Int J Eat Disord. 2018;51(4):287-294.
96. Crow, SJ, Agras, WS, Halmi, KA, Fairburn, CG, Mitchell, JE, and Nyman, JA. A cost effectiveness analysis of stepped care treatment for bulimia nervosa. Int J Eat Disord. 2013;46(4):302-307.
97. Thompson, MA, Adelson, MD, and Kaufman, LM. Lupron retards proliferation of ovarian epithelial tumor cells cultured in serum-free medium. J Clin Endocrinol Metab. 1991;72(5):1036-1041.

98. Elkind-Hirsch, KE, Valdes, CT, and Malinak, LR. Insulin resistance improves in hyperandrogenic women treated with Lupron. Fertility and sterility. 1993;60(4):634-641.
99. Scribner, DR, Jr. and Walker, JL. Low-grade endometrial stromal sarcoma preoperative treatment with Depo-Lupron and Megace. Gynecol Oncol. 1998;71(3):458-460.
100. Padilla, SL, Smith, RD, and Garcia, JE. The Lupron screening test: tailoring the use of leuprolide acetate in ovarian stimulation for in vitro fertilization. Fertility and sterility. 1991;56(1):79-83.
101. Lee, EE, Nieman, LK, Martinez, PE, Harsh, VL, Rubinow, DR, and Schmidt, PJ. ACTH and cortisol response to Dex/CRH testing in women with and without premenstrual dysphoria during GnRH agonist-induced hypogonadism and ovarian steroid replacement. J Clin Endocrinol Metab. 2012;97(6):1887-1896.
102. Mitwally, MF, Gotlieb, L, and Casper, RF. Prevention of bone loss and hypoestrogenic symptoms by estrogen and interrupted progestogen add-back in long-term GnRH-agonist down-regulated patients with endometriosis and premenstrual syndrome. Menopause (New York, N.Y. 2002;9(4):236-241.
103. Wyatt, KM, Dimmock, PW, Ismail, KM, Jones, PW, and O'Brien, PM. The effectiveness of GnRHa with and without 'add-back' therapy in treating premenstrual syndrome: a meta analysis. BJOG. 2004;111(6):585-593.
104. Kaur, G, Gonsalves, L, and Thacker, HL. Premenstrual dysphoric disorder: a review for the treating practitioner. Cleve Clin J Med. 2004;71(4):303-305, 312-303, 317-308 passim.
105. Mezrow, G, Shoupe, D, Spicer, D, Lobo, R, Leung, B, and Pike, M. Depot leuprolide acetate with estrogen and progestin add-back for long-term treatment of premenstrual syndrome. Fertility and sterility. 1994;62(5):932-937.
106. Nobles, CJ, Thomas, JJ, Valentine, SE, Gerber, MW, Vaewsorn, AS, and Marques, L. Association of premenstrual syndrome and premenstrual dysphoric disorder with bulimia nervosa and binge-eating disorder in a nationally representative epidemiological sample. Int J Eat Disord. 2016;49(7):641-650.
107. Lukaszuk, K, Liss, J, Lukaszuk, M, and Maj, B. Optimization of estradiol supplementation during the luteal phase improves the pregnancy rate in women undergoing in vitro fertilization-embryo transfer cycles. Fertility and sterility. 2005;83(5):1372-1376.
108. Bourgain, C, Devroey, P, Van Waesberghe, L, Smitz, J, and Van Steirteghem, AC. Effects of natural progesterone on the morphology of the endometrium in patients with primary ovarian failure. Human reproduction (Oxford, England). 1990;5(5):537-543.
109. Damario, MA, Hammitt, DG, Galanits, TM, Session, DR, and Dumesic, DA. Pronuclear stage cryopreservation after intracytoplasmic sperm injection and conventional IVF: implications for timing of the freeze. Fertility and sterility. 1999;72(6):1049-1054.
110. Fatemi, HM, Kolibianakis, EM, Camus, M, Tournaye, H, Donoso, P, Papanikolaou, E, and Devroey, P. Addition of estradiol to progesterone for luteal supplementation in patients stimulated with GnRH antagonist/rFSH for IVF: a randomized controlled trial. Human reproduction (Oxford, England). 2006;21(10):2628-2632.
111. Friedler, S, Raziel, A, Schachter, M, Strassburger, D, Bukovsky, I, and Ron-El, R. Luteal support with micronized progesterone following in-vitro fertilization using a down-regulation protocol with gonadotrophin-releasing hormone agonist: a comparative study between vaginal and oral administration. Human reproduction (Oxford, England). 1999;14(8):1944-1948.
112. Humaidan, P, Bredkjaer, HE, Bungum, L, Bungum, M, Grondahl, ML, Westergaard, L, and Andersen, CY. GnRH agonist (buserelin) or hCG for ovulation induction in GnRH antagonist IVF/ICSI cycles: a prospective randomized study. Human reproduction (Oxford, England). 2005;20(5):1213-1220.
113. Berman, KF, Schmidt, PJ, Rubinow, DR, Danaceau, MA, Van Horn, JD, Esposito, G, Ostrem, JL, and Weinberger, DR. Modulation of cognition-specific cortical activity by gonadal steroids: a positron-emission tomography study in women. Proc Natl Acad Sci U S A. 1997;94(16):8836-8841.

114. Bloch, M, Rubinow, DR, Schmidt, PJ, Lotsikas, A, Chrousos, GP, and Cizza, G. Cortisol response to ovine corticotropin-releasing hormone in a model of pregnancy and parturition in euthymic women with and without a history of postpartum depression. J Clin Endocrinol Metab. 2005;90(2):695-699.
115. Daly, RC, Schmidt, PJ, Davis, CL, Danaceau, MA, and Rubinow, DR. Effects of gonadal steroids on peripheral benzodiazepine receptor density in women with PMS and controls. Psychoneuroendocrinology. 2001;26(6):539-549.
116. Roca, CA, Schmidt, PJ, Deuster, PA, Danaceau, MA, Altemus, M, Putnam, K, Chrousos, GP, Nieman, LK, and Rubinow, DR. Sex-related differences in stimulated hypothalamic-pituitary-adrenal axis during induced gonadal suppression. J Clin Endocrinol Metab. 2005;90(7):4224-4231.
117. Schmidt, PJ, Steinberg, EM, Negro, PP, Haq, N, Gibson, C, and Rubinow, DR. Pharmacologically induced hypogonadism and sexual function in healthy young women and men. Neuropsychopharmacology. 2009;34(3):565-576.
118. Schmidt, PJ, Martinez, PE, Nieman, LK, Koziol, DE, Thompson, KD, Schenkel, L, Wakim, PG, and Rubinow, DR. Premenstrual Dysphoric Disorder Symptoms Following Ovarian Suppression: Triggered by Change in Ovarian Steroid Levels But Not Continuous Stable Levels. Am J Psychiatry. 2017;174(10):980-989.
119. Gordon, JL, Rubinow, DR, Eisenlohr-Moul, TA, Xia, K, Schmidt, PJ, and Girdler, SS. Efficacy of Transdermal Estradiol and Micronized Progesterone in the Prevention of Depressive Symptoms in the Menopause Transition: A Randomized Clinical Trial. JAMA Psychiatry. 2018;75(2):149-157.
120. Baker, JH, Thornton, LM, Lichtenstein, P, and Bulik, CM. Pubertal development predicts eating behaviors in adolescence. The International journal of eating disorders. 2012;45(7):819-826.
121. Baker, JH, Thornton, LM, Bulik, CM, Kendler, KS, and Lichtenstein, P. Shared genetic effects between age at menarche and disordered eating. The Journal of adolescent health : official publication of the Society for Adolescent Medicine. 2012;51(5):491-496.
122. Chetkowski, RJ, Meldrum, DR, Steingold, KA, Randle, D, Lu, JK, Eggena, P, Hershman, JM, Alkjaersig, NK, Fletcher, AP, and Judd, HL. Biologic effects of transdermal estradiol. The New England journal of medicine. 1986;314(25):1615-1620.
123. Rice, VC, Richard-Davis, G, Saleh, AA, Ginsburg, KA, Mammen, EF, Moghissi, K, and Leach, R. Fibrinolytic parameters in women undergoing ovulation induction. American journal of obstetrics and gynecology. 1993;169(6):1549-1553.
124. Sulak, PJ, Cressman, BE, Waldrop, E, Holleman, S, and Kuehl, TJ. Extending the duration of active oral contraceptive pills to manage hormone withdrawal symptoms. Obstet Gynecol. 1997;89(2):179-183.
125. Naessén, S, Carlstrom, K, Bystrom, B, Pierre, Y, and Hirschberg, AL. Effects of an antiandrogenic oral contraceptive on appetite and eating behavior in bulimic women. Psychoneuroendocrinology. 2007;32(5):548-554.
126. Plessow, F, Singhal, V, Toth, AT, Micali, N, Eddy, KT, and Misra, M. Estrogen administration improves the trajectory of eating disorder pathology in oligo-amenorrheic athletes: A randomized controlled trial. Psychoneuroendocrinology. 2019;102:273-280.
127. Misra, M, Katzman, D, Miller, KK, Mendes, N, Snelgrove, D, Russell, M, Goldstein, MA, Ebrahimi, S, Clauss, L, Weigel, T, Mickley, D, Schoenfeld, DA, Herzog, DB, and Klibanski, A. Physiologic estrogen replacement increases bone density in adolescent girls with anorexia nervosa. J Bone Miner Res. 2011;26(10):2430-2438.
128. Misra, M, Katzman, DK, Estella, NM, Eddy, KT, Weigel, T, Goldstein, MA, Miller, KK, and Klibanski, A. Impact of physiologic estrogen replacement on anxiety symptoms, body shape perception, and eating attitudes in adolescent girls with anorexia nervosa: data from a randomized controlled trial. The Journal of clinical psychiatry. 2013;74(8):e765-771.
129. DiVasta, AD, Feldman, HA, Beck, TJ, LeBoff, MS, and Gordon, CM. Does hormone replacement normalize bone geometry in adolescents with anorexia nervosa? J Bone Miner Res. 2014;29(1):151-157.

130. Strokosch, GR, Friedman, AJ, Wu, SC, and Kamin, M. Effects of an oral contraceptive (norgestimate/ethinyl estradiol) on bone mineral density in adolescent females with anorexia nervosa: a double-blind, placebo-controlled study. J Adolesc Health. 2006;39(6):819-827.
131. Abbvie. *Lupron Depot Monograph*. 2019 July 6, 2019]; Available from: https://www.rxabbvie.com/pdf/lupron3_75mg.pdf.
132. Lippi, G, Franchini, M, and Favaloro, EJ. Holiday thrombosis. Semin Thromb Hemost. 2011;37(8):869-874.
133. Solmi, M, Veronese, N, Correll, CU, Favaro, A, Santonastaso, P, Caregaro, L, Vancampfort, D, Luchini, C, De Hert, M, and Stubbs, B. Bone mineral density, osteoporosis, and fractures among people with eating disorders: a systematic review and meta-analysis. Acta Psychiatr Scand. 2016;133(5):341-351.
134. Newton, JR, Freeman, CP, Hannan, WJ, and Cowen, S. Osteoporosis and normal weight bulimia nervosa--which patients are at risk? J Psychosom Res. 1993;37(3):239-247.
135. Zipfel, S, Seibel, MJ, Lowe, B, Beumont, PJ, Kasperk, C, and Herzog, W. Osteoporosis in eating disorders: a follow-up study of patients with anorexia and bulimia nervosa. J Clin Endocrinol Metab. 2001;86(11):5227-5233.
136. Feuillan, PP, Jones, JV, Barnes, KM, Oerter-Klein, K, and Cutler, GB, Jr. Boys with precocious puberty due to hypothalamic hamartoma: reproductive axis after discontinuation of gonadotropin-releasing hormone analog therapy. J Clin Endocrinol Metab. 2000;85(11):4036-4038.
137. Feuillan, PP, Jones, JV, Barnes, K, Oerter-Klein, K, and Cutler, GB, Jr. Reproductive axis after discontinuation of gonadotropin-releasing hormone analog treatment of girls with precocious puberty: long term follow-up comparing girls with hypothalamic hamartoma to those with idiopathic precocious puberty. J Clin Endocrinol Metab. 1999;84(1):44-49.
138. Gambrell, RD, Jr. The role of hormones in the etiology and prevention of endometrial cancer. Clin Obstet Gynaecol. 1986;13(4):695-723.
139. Nieman, LK and Loriaus, DL. Estrogens and progestins. Textbook of Pharmacology. 1992:695-716.
140. Jick, H, Derby, LE, Myers, MW, Vasilakis, C, and Newton, KM. Risk of hospital admission for idiopathic venous thromboembolism among users of postmenopausal oestrogens. Lancet. 1996;348(9033):981-983.
141. Cushman, M, Kuller, LH, Prentice, R, Rodabough, RJ, Psaty, BM, Stafford, RS, Sidney, S, Rosendaal, FR, and Women's Health Initiative, I. Estrogen plus progestin and risk of venous thrombosis. JAMA. 2004;292(13):1573-1580.
142. Daly, E, Vessey, MP, Hawkins, MM, Carson, JL, Gough, P, and Marsh, S. Risk of venous thromboembolism in users of hormone replacement therapy. Lancet. 1996;348(9033):977-980.
143. Stampfer, MJ, Colditz, GA, Willett, WC, Manson, JE, Rosner, B, Speizer, FE, and Hennekens, CH. Postmenopausal estrogen therapy and cardiovascular disease. Ten-year follow-up from the nurses' health study. The New England journal of medicine. 1991;325(11):756-762.
144. Wassertheil-Smoller, S, Hendrix, SL, Limacher, M, Heiss, G, Kooperberg, C, Baird, A, Kotchen, T, Curb, JD, Black, H, Rossouw, JE, Aragaki, A, Safford, M, Stein, E, Laowattana, S, Mysiw, WJ, and Investigators, WHI. Effect of estrogen plus progestin on stroke in postmenopausal women: the Women's Health Initiative: a randomized trial. JAMA. 2003;289(20):2673-2684.
145. Mandel, FP, Geola, FL, Lu, JK, Eggena, P, Sambhi, MP, Hershman, JM, and Judd, HL. Biologic effects of various doses of ethinyl estradiol in postmenopausal women. Obstet Gynecol. 1982;59(6):673-679.
146. Melis, GB, Fruzzetti, F, Paoletti, AM, Carmassi, F, and Fioretti, P. Fibrinopeptide A plasma levels during low-estrogen oral contraceptive treatment. Contraception. 1984;30(6):575-583.
147. Barrett-Connor, E and Bush, TL. Estrogen and coronary heart disease in women. JAMA. 1991;265(14):1861-1867.
148. Manson, JE, Hsia, J, Johnson, KC, Rossouw, JE, Assaf, AR, Lasser, NL, Trevisan, M, Black, HR, Heckbert, SR, Detrano, R, Strickland, OL, Wong, ND, Crouse, JR, Stein, E, Cushman, M, and Women's

Health Initiative, I. Estrogen plus progestin and the risk of coronary heart disease. The New England journal of medicine. 2003;349(6):523-534.
149. Prentice, RL, Langer, RD, Stefanick, ML, Howard, BV, Pettinger, M, Anderson, GL, Barad, D, Curb, JD, Kotchen, J, Kuller, L, Limacher, M, Wactawski-Wende, J, and Women's Health Initiative, I. Combined analysis of Women's Health Initiative observational and clinical trial data on postmenopausal hormone treatment and cardiovascular disease. Am J Epidemiol. 2006;163(7):589-599.
150. Grodstein, F, Manson, JE, and Stampfer, MJ. Hormone therapy and coronary heart disease: the role of time since menopause and age at hormone initiation. J Womens Health (Larchmt). 2006;15(1):35-44.
151. Prentice, RL, Langer, R, Stefanick, ML, Howard, BV, Pettinger, M, Anderson, G, Barad, D, Curb, JD, Kotchen, J, Kuller, L, Limacher, M, Wactawski-Wende, J, and Women's Health Initiative, I. Combined postmenopausal hormone therapy and cardiovascular disease: toward resolving the discrepancy between observational studies and the Women's Health Initiative clinical trial. Am J Epidemiol. 2005;162(5):404-414.
152. Lobo, RA. Evaluation of cardiovascular event rates with hormone therapy in healthy, early postmenopausal women: results from 2 large clinical trials. Archives of internal medicine. 2004;164(5):482-484.
153. Cirillo, DJ, Wallace, RB, Rodabough, RJ, Greenland, P, LaCroix, AZ, Limacher, MC, and Larson, JC. Effect of estrogen therapy on gallbladder disease. JAMA. 2005;293(3):330-339.
154. Petitti, DB, Sidney, S, and Perlman, JA. Increased risk of cholecystectomy in users of supplemental estrogen. Gastroenterology. 1988;94(1):91-95.
155. Steinauer, JE, Waetjen, LE, Vittinghoff, E, Subak, LL, Hulley, SB, Grady, D, Lin, F, and Brown, JS. Postmenopausal hormone therapy: does it cause incontinence? Obstet Gynecol. 2005;106(5 Pt 1):940-945.
156. Wingo, PA, Layde, PM, Lee, NC, Rubin, G, and Ory, HW. The risk of breast cancer in postmenopausal women who have used estrogen replacement therapy. JAMA. 1987;257(2):209-215.
157. Chen, WY, Manson, JE, Hankinson, SE, Rosner, B, Holmes, MD, Willett, WC, and Colditz, GA. Unopposed estrogen therapy and the risk of invasive breast cancer. Archives of internal medicine. 2006;166(9):1027-1032.
158. Chlebowski, RT, Hendrix, SL, Langer, RD, Stefanick, ML, Gass, M, Lane, D, Rodabough, RJ, Gilligan, MA, Cyr, MG, Thomson, CA, Khandekar, J, Petrovitch, H, McTiernan, A, and Investigators, WHI. Influence of estrogen plus progestin on breast cancer and mammography in healthy postmenopausal women: the Women's Health Initiative Randomized Trial. JAMA. 2003;289(24):3243-3253.
159. Gann, PH and Morrow, M. Combined hormone therapy and breast cancer: a single-edged sword. JAMA. 2003;289(24):3304-3306.
160. Rossouw, JE, Anderson, GL, Prentice, RL, LaCroix, AZ, Kooperberg, C, Stefanick, ML, Jackson, RD, Beresford, SA, Howard, BV, Johnson, KC, Kotchen, JM, Ockene, J, and Writing Group for the Women's Health Initiative, I. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA. 2002;288(3):321-333.
161. Anderson, GL, Chlebowski, RT, Aragaki, AK, Kuller, LH, Manson, JE, Gass, M, Bluhm, E, Connelly, S, Hubbell, FA, Lane, D, Martin, L, Ockene, J, Rohan, T, Schenken, R, and Wactawski-Wende, J. Conjugated equine oestrogen and breast cancer incidence and mortality in postmenopausal women with hysterectomy: extended follow-up of the Women's Health Initiative randomised placebo-controlled trial. Lancet Oncol. 2012;13(5):476-486.
162. Henzel, MR. Contraceptive hormones and their clinical use. Reproductive Endocrinology: Physiology, Pathophysiology, and Clinical Management. 1986:643-682.
163. FDA. *CENTER FOR DRUG EVALUATION AND RESEARCH* 2012; Available from: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/203696Orig1s000SumR.pdf.

164. Bustillo, M, Krysa, LW, and Coulam, CB. Uterine receptivity in an oocyte donation programme. Human reproduction (Oxford, England). 1995;10(2):442-445.
165. Engmann, L, DiLuigi, A, Schmidt, D, Benadiva, C, Maier, D, and Nulsen, J. The effect of luteal phase vaginal estradiol supplementation on the success of in vitro fertilization treatment: a prospective randomized study. Fertility and sterility. 2008;89(3):554-561.
166. Legro, RS, Ary, BA, Paulson, RJ, Stanczyk, FZ, and Sauer, MV. Premature luteinization as detected by elevated serum progesterone is associated with a higher pregnancy rate in donor oocyte in-vitro fertilization. Human reproduction (Oxford, England). 1993;8(9):1506-1511.
167. Mirkin, S, Gimeno, TG, Bovea, C, Stadtmauer, L, Gibbons, WE, and Oehninger, S. Factors associated with an optimal pregnancy outcome in an oocyte donation program. J Assist Reprod Genet. 2003;20(10):400-408.
168. Nagy, ZP, Chang, CC, Shapiro, DB, Bernal, DP, Elsner, CW, Mitchell-Leef, D, Toledo, AA, and Kort, HI. Clinical evaluation of the efficiency of an oocyte donation program using egg cryo-banking. Fertility and sterility. 2009;92(2):520-526.
169. Noyes, N, Hampton, BS, Berkeley, A, Licciardi, F, Grifo, J, and Krey, L. Factors useful in predicting the success of oocyte donation: a 3-year retrospective analysis. Fertility and sterility. 2001;76(1):92-97.
170. Peress, MR and Phillips, HM. Quintuplet gestation following transfer of five cryopreserved embryos: conditions stimulating thawed-embryo implantation. J Assist Reprod Genet. 1998;15(8):521-523.
171. Sauer, MV, Paulson, RJ, and Lobo, RA. Reversing the natural decline in human fertility. An extended clinical trial of oocyte donation to women of advanced reproductive age. JAMA. 1992;268(10):1275-1279.
172. Tonguc, E, Var, T, Ozyer, S, Citil, A, and Dogan, M. Estradiol supplementation during the luteal phase of in vitro fertilization cycles: a prospective randomised study. Eur J Obstet Gynecol Reprod Biol. 2011;154(2):172-176.
173. Frost, R and McNaughton, N. The neural basis of delay discounting: A review and preliminary model. Neurosci Biobehav Rev. 2017;79:48-65.
174. Knutson, B and Greer, SM. Anticipatory affect: neural correlates and consequences for choice. Philos Trans R Soc Lond B Biol Sci. 2008;363(1511):3771-3786.
175. Zerwas, SC, Watson, HJ, Hofmeier, SM, Levine, MD, Hamer, RM, Crosby, RD, Runfola, CD, Peat, CM, Shapiro, JR, Zimmer, B, Moessner, M, Kordy, H, Marcus, MD, and Bulik, CM. CBT4BN: A Randomized Controlled Trial of Online Chat and Face-to-Face Group Therapy for Bulimia Nervosa. Psychotherapy and psychosomatics. 2017;86(1):47-53.
176. Thornton, LM, Munn-Chernoff, MA, Baker, JH, Jureus, A, Parker, R, Henders, AK, Larsen, JT, Petersen, L, Watson, HJ, Yilmaz, Z, Kirk, KM, Gordon, S, Leppa, VM, Martin, FC, Whiteman, DC, Olsen, CM, Werge, TM, Pedersen, NL, Kaye, W, Bergen, AW, Halmi, KA, Strober, M, Kaplan, AS, Woodside, DB, Mitchell, J, Johnson, CL, Brandt, H, Crawford, S, Horwood, LJ, Boden, JM, Pearson, JF, Duncan, LE, Grove, J, Mattheisen, M, Jordan, J, Kennedy, MA, Birgegard, A, Lichtenstein, P, Norring, C, Wade, TD, Montgomery, GW, Martin, NG, Landen, M, Mortensen, PB, Sullivan, PF, and Bulik, CM. The Anorexia Nervosa Genetics Initiative (ANGI): Overview and methods. Contemp Clin Trials. 2018;74:61-69.
177. Baker, JH, Eisenlohr-moul, TA, Bulik, CM, Schiller, CE, and Girdler, SS, *Impact of Estrogen and Progesterone on Changes in Eating Disorder Symptoms During the Menopause Transition*, in *International Conference on Eating Disorders*2018: Chicago, IL.
178. Hamilton, M. A rating scale for measuring depression. Journal of Neurological and Neurosurgical Psychiatry. 1960;23:56-62.
179. de Beurs, DP, Fokkema, M, and O'Connor, RC. Optimizing the assessment of suicidal behavior: The application of curtailment techniques. J Affect Disord. 2016;196:218-224.
180. Watson, DC, LA. Tellegen, A. Development and validation of brief measures of positive and negative affect: the PANAS scales. Journal of Personality & Social Psychology. 1988;54(6):1063-1070.

181. First, MB, Williams, JBW, Karg, RS, and Spitzer, RL, *Structured Clinical Interview for DSM-5: Research Version (SCID-5 for DSM-5, Research Version; SCID-5-RV)*2015, Arlington, VA: American Psychiatric Association.
182. Gordon, JL, Rubinow, DR, Eisenlohr-Moul, TA, Leserman, J, and Girdler, SS. Estradiol variability, stressful life events, and the emergence of depressive symptomatology during the menopausal transition. Menopause (New York, N.Y. 2016;23(3):257-266.
183. Whiteside, SP and Lynam, D. The Five Factor Model and impulsivity: using a structural model of personality to understand impulsivity. Personality and Individual Differences. 2001;30(4):669-689.
184. Watson, D, O'Hara, MW, Simms, LJ, Kotov, R, Chmielewski, M, McDade-Montez, EA, Gamez, W, and Stuart, S. Development and validation of the Inventory of Depression and Anxiety Symptoms (IDAS). Psychol Assess. 2007;19(3):253-268.
185. Fairburn, C and Cooper, Z, *The Eating Disorders Examination (12th Edition)*, in *Binge-Eating: Nature, Assessment and Treatment*, C. Fairburn and G. Wilson, Editors. 1993, Guilford Press: New York. p. 317-360.
186. Forbush, KT, Wildes, JE, Pollack, LO, Dunbar, D, Luo, J, Patterson, K, Petruzzi, L, Pollpeter, M, Miller, H, Stone, A, Bright, A, and Watson, D. Development and validation of the Eating Pathology Symptoms Inventory (EPSI). Psychol Assess. 2013;25(3):859-878.
187. Carver, CS and White, TL. Behavioral inhibition, behavioral activation, and affective responses to impending reward and punishment: The BIS/BAS scales. Journal of Personality and Social Psychology. 1994;67:319-333.
188. Torrubia, R, Avila, C, Molto, J, and Caseras, X. The Sensitivity to Punishment and Sensitivity to Reward Questionnaire (SPSRQ) as a measure of Gray's anxiety and impulsivity dimensions. Personality and Individual Differences. 2001;31(6):837-862.
189. Rodriguez-Martin, BC and Meule, A. Food craving: new contributions on its assessment, moderators, and consequences. Front Psychol. 2015;6:21.
190. Nijs, IM, Franken, IH, and Muris, P. The modified Trait and State Food-Cravings Questionnaires: development and validation of a general index of food craving. Appetite. 2007;49(1):38-46.
191. Stunkard, A and Messick, S. Three-factor eating questionnaire to measure dietary restraint, disinhibition, and hunger. J Psychosom Res. 1985;29:71-83.
192. Richards, JB, Zhang, L, Mitchell, SH, and de Wit, H. Delay or probability discounting in a model of impulsive behavior: effect of alcohol. J Exp Anal Behav. 1999;71(2):121-143.
193. Madden, GJ, Begotka, AM, Raiff, BR, and Kastern, LL. Delay discounting of real and hypothetical rewards. Exp Clin Psychopharmacol. 2003;11(2):139-145.
194. Locey, ML, Jones, BA, and Rachlin, H. Real and hypothetical rewards. Judgm Decis Mak. 2011;6(6):552-564.
195. Scheres, A, Sumiya, M, and Thoeny, AL. Studying the relation between temporal reward discounting tasks used in populations with ADHD: a factor analysis. Int J Methods Psychiatr Res. 2010;19(3):167-176.
196. Klump, KL, Suisman, JL, Culbert, KM, Kashy, DA, Keel, PK, and Sisk, CL. The effects of ovariectomy on binge eating proneness in adult female rats. Horm Behav. 2011;59(4):585-593.
197. Yu, Z, Geary, N, and Corwin, RL. Individual effects of estradiol and progesterone on food intake and body weight in ovariectomized binge rats. Physiol Behav. 2011;104(5):687-693.
198. Halbreich, U, Backstrom, T, Eriksson, E, O'Brien, S, Calil, H, Ceskova, E, Dennerstein, L, Douki, S, Freeman, E, Genazzani, A, Heuser, I, Kadri, N, Rapkin, A, Steiner, M, Wittchen, HU, and Yonkers, K. Clinical diagnostic criteria for premenstrual syndrome and guidelines for their quantification for research studies. Gynecol Endocrinol. 2007;23(3):123-130.
199. Eisenlohr-Moul, TA, Girdler, SS, Schmalenberger, KM, Dawson, DN, Surana, P, Johnson, JL, and Rubinow, DR. Toward the Reliable Diagnosis of DSM-5 Premenstrual Dysphoric Disorder: The Carolina Premenstrual Assessment Scoring System (C-PASS). Am J Psychiatry. 2017;174(1):51-59.

200. SAS Institute Inc, *SAS/STAT® Software: Version 9*2004, Cary, NC: SAS Institute, Inc.
201. Myerson, J, Green, L, and Warusawitharana, M. Area under the curve as a measure of discounting. J Exp Anal Behav. 2001;76(2):235-243.
202. Stockburger, J, Schmalzle, R, Flaisch, T, Bublatzky, F, and Schupp, HT. The impact of hunger on food cue processing: an event-related brain potential study. Neuroimage. 2009;47(4):1819-1829.
203. Charbonnier, L, van Meer, F, Johnstone, AM, Crabtree, D, Buosi, W, Manios, Y, Androutsos, O, Giannopoulou, A, Viergever, MA, Smeets, PAM, and Full4Health, c. Effects of hunger state on the brain responses to food cues across the life span. Neuroimage. 2018;171:246-255.
204. Grodstein, F, Stampfer, MJ, Goldhaber, SZ, Manson, JE, Colditz, GA, Speizer, FE, Willett, WC, and Hennekens, CH. Prospective study of exogenous hormones and risk of pulmonary embolism in women. Lancet. 1996;348(9033):983-987.
205. Mandel, FP, Geola, FL, Meldrum, DR, Lu, JH, Eggena, P, Sambhi, MP, Hershman, JM, and Judd, HL. Biological effects of various doses of vaginally administered conjugated equine estrogens in postmenopausal women. J Clin Endocrinol Metab. 1983;57(1):133-139.
206. Anderson, GL, Limacher, M, Assaf, AR, Bassford, T, Beresford, SA, Black, H, Bonds, D, Brunner, R, Brzyski, R, Caan, B, Chlebowski, R, Curb, D, Gass, M, Hays, J, Heiss, G, Hendrix, S, Howard, BV, Hsia, J, Hubbell, A, Jackson, R, Johnson, KC, Judd, H, Kotchen, JM, Kuller, L, LaCroix, AZ, Lane, D, Langer, RD, Lasser, N, Lewis, CE, Manson, J, Margolis, K, Ockene, J, O'Sullivan, MJ, Phillips, L, Prentice, RL, Ritenbaugh, C, Robbins, J, Rossouw, JE, Sarto, G, Stefanick, ML, Van Horn, L, Wactawski-Wende, J, Wallace, R, Wassertheil-Smoller, S, and Women's Health Initiative Steering, C. Effects of conjugated equine estrogen in postmenopausal women with hysterectomy: the Women's Health Initiative randomized controlled trial. JAMA. 2004;291(14):1701-1712.
207. Stefanick, ML, Anderson, GL, Margolis, KL, Hendrix, SL, Rodabough, RJ, Paskett, ED, Lane, DS, Hubbell, FA, Assaf, AR, Sarto, GE, Schenken, RS, Yasmeen, S, Lessin, L, Chlebowski, RT, and Investigators, WHI. Effects of conjugated equine estrogens on breast cancer and mammography screening in postmenopausal women with hysterectomy. JAMA. 2006;295(14):1647-165

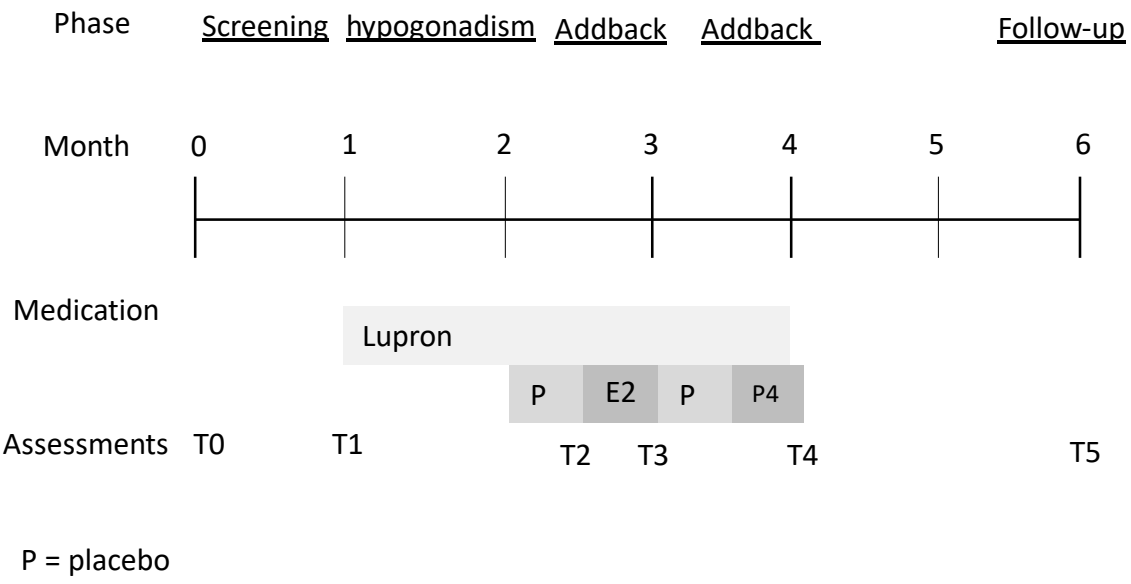
15 Appendices

Appendix A. Flow Chart of Study Procedures

Appendix B. COVID Procedures

Appendix A. Study Procedures Flow Chart

Figure 5. Within-Subject Hormone Challenge (study visits occur every 2 weeks)



Appendix B. COVID Response

Due to the COVID-19 pandemic, this study will be modified as described below. These procedures are only in response to the COVID-19 pandemic. If we are able to begin recruitment, enrollment, and study visits 'as usual,' study procedures will resume as described in the original protocol.

All remote portions of study visits will be conducted via WebEx calls. All scheduled WebEx visits will be password protected and only those with the password can enter the call. WebEx has been approved by the UNC ITS as secure programs for PHI and sensitive information and the School of Medicine specifically recommends the use of WebEx for research study visits. WebEx is HIPPA complaint, ISO/IEC 27001:2013 certified, and approved for the transmission of PHI. Visits will be password protected and only those with the password can enter the study visit. Although Internet access is not a specific inclusion/exclusion criteria, because COVID19 requires converting many aspects of this study to virtual visits, we acknowledge participants will need to have access to the Internet.

Overview:
Recruitment will be conducted as described, we will make no changes to our recruitment procedures or methods. We previously created a modification for participants who appear eligible on a study screener to be added to a research study waiting list and will be re-contacted once the study is able to resume in-person visits. Once our revised study protocol is IRB approved, we will no longer create this waiting list and study enrollment will begin as described in this revised, COVID19 protocol addendum.

This current addendum includes our plans to begin enrolling participants and the updated measures to prevent against the spread of SARS-CoV-2. We have altered study activities to make many of them remote. When contact is essential for certain activities, we have included procedures to protect participants and our study team. We are limiting the number of study personnel that conduct in-person visits to two individuals— the Clinical Translational Research Center nurse and coordinator. Both will be dressed in personal protective equipment (PPE) and will follow procedures to prevent the opportunity of transmission.

PPE. During all interactions with participants, participants will be given a surgical mask and the study personnel will wear scrubs, gloves, and a surgical mask. Eye protection will be worn during Lupron injection and during blood draws. All items will be immediately removed as soon as possible after the study visit and washed or thrown away.
COVID screening. A COVID health check will be completed with all participants 24 hours prior to study visit. If this is not completed, the study visit will not occur. In addition, a departmental Qualtrics survey will be completed by research personnel prior to any study visits or interactions with human subjects, regardless of the location.
Preventive procedures. Study personnel, additionally, will follow preventive procedures. All equipment that will be shared between a participant and research staff (except paper) will be cleaned immediately prior to the study visit, immediately after use, and upon returning to CEED.
Social distancing. Distance will be maintained between people any time closer contact is not absolutely required (e.g., blood draws). As allowable, study visits will be scheduled as in-home. If a subject prefers to come to the hospital for a study visit, this request will be considered. As much of a study visit that can be completed without being in person will occur remotely in order to decrease interaction with subjects.

Study Activities

Recruitment & Eligibility

Distance. Due to the need to complete as many study visits offsite as possible, we have expanded our radius for in-home study visits to a radius of approximately 45 miles or 1-hour of UNC Hospitals (as described in the consent form). Although this change is in response to COVID19, for fairness, this will remain a permanent change to the study protocol.

Eligibility Screening: We will screen individuals remotely via Qualtrics and telephone as described in the original protocol. This includes screening individuals via phone or online Qualtrics screener. As previously approved, we were screening participants and adding them to a waitlist to be contacted once in-person visits resumed. Now that we are planning in-person visits, the individuals that were on the waitlist will be contacted to confirm study eligibility. A portion of the phone/clinic eligibility screener will be used to confirm eligibility. These participants will only be asked the menstrual cycle status and eating behaviors sections of the screener. In the event study activities need to be suspended again due to COVID19, we will resume use of the waitlist. This waitlist is a password-protected document that contains the potential subjects' name, temporary ID, and contact information.

Enrollment: If participants are deemed eligible based on their screening, we will follow-up with a remote Informed Consent (T0a) and a remote Clinical Health Screening and SCID Interview (T0b) visit. T0b will be scheduled after informed consent is obtained and the signed consent forms are received by study staff. No study procedures will be conducted until the signed consent forms are received by study staff. We also added 3 questions to the T0 questionnaires survey that assess the impacts of COVID-19 on eating behaviors.

Informed consent. The informed consent documents have been modified to reflect the changes to the study protocol outlined below. Dr. Jessica Baker or her trained study coordinator will obtain informed consent from those individuals who pass the initial screening and are interested in participating. Notably, the informed consent process during COVID (virtual/remote) will mirror the procedures for an in-person visit, the major difference being the visit will occur remotely via WebEx and the consent forms will be mailed, reviewed, signed, and mailed back. Participants will not review the consent documents on their own, but will be walked through the forms, verbatim, as would be completed during an in-person visit.

Two copies of the consent forms will be mailed to the person in unmarked envelopes. For any participant that does not receive this most recent consent form, they will be given the COVID-19 Consent Addendum. This document includes text from the updated study consent form that discusses COVID-19 exposure risks and the measures that may be taken to prevent exposure.

Consent forms will be reviewed remotely during WebEx (or telephone if necessary) and will be returned to study staff signed in a pre-paid envelope. The participant will be given multiple opportunities to ask questions. Prior to signing the consent form, participants will be asked the consent quiz to ensure understanding of the consent form. We will obtain contact information and social security number verbally from the participant after the consent process. Contact information and social security number are only required if the participant consents to participant in the study. Future studies consent will also only be obtained verbally.

Regarding WedEx, it is HIPPA complaint, ISO/IEC 27001:2013 certified, and approved for the transmission of PHI. Visits will be password protected and only those with the password can enter the study visit. UNC SOM specifically recommends the use of WebEx for virtual research study visits.

Study Procedures and COVID19 Study Visit Modifications:

Screening Period Prior to Medication Administration. Prior to medication administration, participants complete a screening period which examines their eating disorder symptoms daily, during 1 menstrual cycle. No changes have been made to this aspect of the protocol as the original protocol included completing this screening virtually/remotely through daily Qualtrics surveys.

Study Questionnaires. A majority of study questionnaires were already completed through Qualtrics so this is easily converted to remote completion. Two surveys, IDAS and EDE-Q, have moved from paper and pencil versions to Qualtrics during COVID-19. These are used to monitor safety throughout the duration of the study and will be scored automatically for study staff to review within the survey flow. When the study returns standard procedures, we will keep the option of completing these in Qualtrics or completing them in their paper/pencil versions. The Delay Discounting questionnaire has now been added to T1-T5 Qualtrics surveys. This would have previously been completed via a paper and pencil survey, but now will be completed electronically via Qualtrics. This will remain a permanent change. The COVID Stress Scale (CSS) has been added to the electronic questionnaires for each in person visit that includes in-person behavioral tasks (T1, T2, T3, T4) to assess distress in relation to COVID-19 during tasks.

Side Effect Checks and Interviews. The side effects and adherence interview will be conducted via interview through WebEx. Participants responses to the daily surveys will continue to be monitored daily and participants endorsing any concerning symptoms will be followed up with by the PI (as outlined in the original protocol).

Behavioral Tasks. Behavioral tasks must be completed in person due to the software used (i.e., E-prime). Although a remote option for E-prime is available, it is not ideal for tasks that are based on response time this could vastly differ across computers and operating systems. Using our computer standardizes this. The remote version also saves the data directly to the participants computer and requires it to be transferred to the study staff. This would also require participants have access to an appropriate computer.

Before using the study laptop, the research coordinator will thoroughly wipe down the entire laptop, especially the parts that are frequently touched. When setting up the tasks for the participant, the coordinator will wear gloves. Directions will be given to the participant at a distance, and the participant and coordinator will remain 6 feet apart whenever possible during this task. The Pre-Behavioral Task Food Intake Questionnaire will be completed with the participant by the study coordinator. In the event we must stop completing behavioral tasks in person, we will use the software application Millisecond as a back-up. Notably, our initial IRB approval proposed to use Millisecond; however, we converted to E-prime given we intend to include fMRI imaging in our future studies and UNC imaging center uses E-prime to run imaging tasks.

Millisecond allows for psychological tasks to be administered online, on any device able to download the application, and provides centralized and secure storage for data. Their servers are protected by high-end firewall systems, and scans are performed regularly to ensure that any vulnerabilities are quickly found and patched. All services have quick failover points and redundant hardware, with complete backups performed nightly. Data are stored redundantly across data centers for resiliency and availability during disasters. Millisecond provides each customer a unique username and strong

password that must be entered each time a customer logs on. The user remains authenticated only for the duration of the session and is automatically logged off after 30 minutes of inactivity. This system ensures that customer data can only be accessed by authenticated and authorized users. Customer data are processed and stored in world-class data center facilities in Oregon, USA. Data are not moved around to other locations. The data centers are housed in nondescript facilities. Physical access is strictly controlled both at the perimeter and at building ingress points by professional security staff utilizing video surveillance, intrusion detection systems, and other electronic means. Authorized staff must pass two-factor authentication a minimum of two times to access data center floors. All visitors and contractors are required to present identification and are signed in and continually escorted by authorized staff. The servers reside behind high-availability firewalls and are monitored using state of the art systems for detection and prevention of various threats including denial of service, man in the middle, IP spoofing, port scanning, and packet sniffing. Automated network security audits using the industry standard SSAE-16 method are conducted to the standards and requirements of the SANS/FBI security test, the U.S. Department of Homeland Security's published recommendations and the Payment Card Industry Data Security Standard. Millisecond encrypts all data in transit by enforcing Transport Layer Security (TLS) encryption (also known as HTTPS). Millisecond encrypts all data at rest using the industry standard AES-256 cypher. Millisecond deploys the general requirements set forth by many Federal Acts, including the FISMA Act of 2002. They meet or exceed the minimum requirements as outlined in FIPS Publication 200. HITECH (Health Information Technology for Economic and Clinical Health Act) updated HIPAA rules to ensure that data are properly protected and best security practices followed. Millisecond safeguards all customer data, and uses secure data centers to ensure the highest protection as per HITECH requirements.

Medication administration. Medication will be administered similar to our original protocol as in person contact is required (i.e., Lupron injection). However, for any study visits where giving the study participant their next set of oral medications is the *only in-person activity* needed, the study coordinator will pass off the pills directly to the participant during a drive by the participant's house. Alternatively, the participant can drive by the hospital and the research assistant will give them the pills when they pull up to the hospital. If the visit is already requiring in-person contact for another reason (i.e. Lupron injection or behavioral tasks), the personnel that is involved in the visit will give the participant the study pills.

Blood draws. Due to significant safety concerns regarding in-person contact and biological specimen processing and handling, all study blood draws will be removed from the protocol except those that are for labs in order to determine if participant is healthy and can participate in the study (T0). If the participant attends the research study gynecological exam (which must be conducted in person and cannot be modified), this will be collected during the exam. If they instead provide their own gynecological records, the blood draw for labs will be performed by the coordinator at the participant's home or at the hospital.

Saliva samples. Saliva samples will now be collected for E2 and P4 assay. This will be collected by the participant in their home. All saliva sample materials will be given to participants with instructions during the first in-person contact during (T1) to be completed remotely. We have created collection documents and a log for participants to complete in. At visit T4, all tubes will be collected from the participant and taken back to the UNC Core laboratory for storage. Consent forms have been modified to reflect this change.

End of Study Feedback. We have expanded the information participants will be provided at the end of study participation. All participants who completed the study have the option to learn how their eating behaviors changed in response to the study medications. However, given the current pandemic we have expanded this to include a more comprehensive report vs overview as this detailed information could be immediately beneficial. This report will include how eating behaviors changed but now also include a discussion of (1) how this may translate to natural menstrual cycles (e.g., high

or low risk periods for symptoms); (2) strategies that can be used to manage symptoms; (3) triggers that may suggest a high risk period of the menstrual cycle is forthcoming (e.g., ovulation, mensuration); (4) additional resources and recommendations that may be useful based on the participants individual response. This will remain a permanent change to the study protocol. We have added additional text regarding this for interested participants on the study website and as part of the Qualtrics and phone/clinic screen.

COVID Risk. If a participant has direct or secondary contact with any suspected or confirmed cases of COVID-19 or experience any known symptoms of COVID-19, the study team will reschedule any scheduled in person study visits until the case is confirmed negative or until the subject has quarantined for at least 14 days from the first symptom.