DETAILED PROTOCOL

Title: The role of estrogen in the neurobiology of eating disorders: A study of cognitive flexibility, and reward in eating disorders

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I. BACKGROUND AND SIGNIFICANCE

Eating disorders (EDs) typically onset in adolescence at a time of gonadal hormonal changes and rapid brain development. EDs characterized by extreme dietary restriction and/or excessive exercise (ED-R/E) are associated with cognitive inflexibility,(1-5) reduced reward responsiveness,(6-10) and altered reward valuation,(11-14) which contribute to illness maintenance and poor outcomes.(15) Hypoestrogenemia is common in ED-R/E (~60%),(16, 17) and in other conditions has been linked to poor cognitive flexibility (18-22) and altered reward responsiveness and valuation.(23-29) ED-R/E are often chronic illnesses with high relapse rates; only ~40% recover fully following psychological treatment,(30) and to date, no specific pharmacological treatments are available for ED-R/E. Clarifying the link between estrogen status, cognitive flexibility, reward responsiveness and valuation, and ED pathology may facilitate identification of novel treatment targets and improve outcomes.

Low energy availability (as in ED-R/E) can suppress the hypothalamic-pituitary-gonadal axis, causing hypoestrogenemia,(16, 31) which may engender or exacerbate cognitive rigidity and reduced reward responsiveness/altered valuation, in turn, reinforcing the ED. Yet very limited data are available regarding the impact of hypoestrogenemia and its correction on Cognitive and Positive Valence Systems in young women with ED-R/E. Our preliminary data indicate that: (i) abnormalities in the National Institute of Mental Health Research Domain Criteria (RDoC) domains of Cognitive Systems (Cognitive Control [Updating, Representation and Maintenance i.e., Cognitive Flexibility]) and Positive Valence Systems (Reward Responsiveness [Initial Response to Reward] and Reward Valuation [Delay]) are evident in hypoestrogenic adolescents/young adults (independent of body weight) compared to normo-estrogenic controls, and (ii) hypoestrogenemia is associated with cognitive inflexibility, reduced Initial Response to Reward (neural response to palatable food images), altered Delay (increased preference for larger delayed over immediate smaller monetary rewards), and increased ED pathology.(9, 19, 32) Estrogen deficiency may thus play a key mechanistic role in maintenance of ED-R/E by acting on these RDoC domains. Importantly, hypogonadal adolescents/young women are commonly treated with estrogen replacement for other outcomes (e.g., optimizing bone health), and data from our team and others demonstrate that estrogen replacement improves Cognitive Flexibility and Initial Response to Reward and Delay.(18, 22, 29) Further, our preliminary data indicate that long-term estrogen replacement improves ED pathology and food intake.(33, 34) Published work in other hypogonadal states shows that even 8-12 weeks of estrogen/estrogen agonist administration can alter cognitive flexibility and reward processing.(22, 29, 35) Building on these compelling data, it is now critical to examine whether estrogen deficiency contributes to dysfunction across Cognitive and Positive Valence RDoC domains in ED-R/E, and whether correcting this deficiency improves ED pathology via its impact on these domains.

II. SPECIFIC AIMS

In a randomized, double-blind, placebo-controlled study of 120 hypoestrogenic females with ED-R/E (ages 14-35), we will use short-term (12 weeks) physiologic estrogen replacement as a mechanistic probe to study the impact of estrogen deficiency (and replacement) on Cognitive and Positive Valence Systems and ED pathology with the following aims:

Aim 1: Intermediate effects on Cognitive Flexibility, Initial Response to Reward, and Delay measured at 8 weeks. Hypothesis 1: Establishing physiologic estrogen levels in hypoestrogenemic ED-R/E compared to placebo will alter Cognitive Flexibility, Initial Response to Reward, and Delay at 8 weeks manifested by: (a) increased functional magnetic resonance imaging (fMRI) activation in the dorsolateral prefrontal cortex (DLPFC) and anterior cingulate cortex (ACC) during a task-switching paradigm and increased fMRI activation in the ventromedial PFC (VMPFC) and ventral striatum in response to reward outcome and during reward valuation (delay discounting); (b) improved inhibition-switching performance on the Delis-Kaplan Executive Function System Color-Word Interference Test (D-KEFS CWIT), increased Temporal Experience of Pleasure Scale (TEPS) Consummatory Pleasure scores, and an increased discounting rate in the Monetary Choice Questionnaire.

Aim 2: Effects on ED pathology at 12 weeks. Hypothesis 2: Establishing physiologic estrogen levels (vs. placebo) will improve ED pathology at 12 weeks, demonstrated by reductions in: (a) Eating Disorder Inventory-3 (EDI-3) Drive for Thinness and Body Dissatisfaction scores; (b) Eating Disorder Examination (EDE) Dietary Restraint scale scores; and (c) increased caloric intake (4-day food record).

Aim 3: Whether changes in Cognitive Flexibility, Initial Response to Reward, and Delay at 8 weeks mediate estrogen effects on ED symptoms at 12 weeks. Hypothesis 3: In ED-R/E, changes in Cognitive Flexibility, Initial Response to Reward, and Delay at 8 weeks will mediate estrogen effects on ED pathology at 12 weeks.

III. SUBJECT SELECTION

Inclusion criteria:

- Female
- 14-35 years
- Bone age ≥ 13.5 years (applicable only for participants <16 years)
- Clinically significant ED characterized by restriction and/or excessive exercise and high drive for thinness (e.g. measured by Eating Disorder Inventory-3 [EDI-3; score >7]);(36-38) diagnoses to include DSM-5 anorexia nervosa (AN) restricting type, atypical AN, and other restricting type other specified feeding and eating disorder (OSFED) presentations assessed by Structured Clinical Interview for DSM-5 Research Version (SCID-5-RV) Feeding and Eating Disorder Module and ARFID questions
- Hypoestrogenemia: Oligo-amenorrhea defined as lack of menses for ≥3 months within a 6-month period of oligomenorrhea (cycle length ≥5 weeks) or absence of menses at >15 years if premenarchal or low estradiol levels on 2-3 weekly estradiol labs evaluated by study physician (39). For certain participants (e.g. for those an initial estradiol level < 25 pg/mL, or for those where the estradiol level can be timed to a last menstrual period), we will accept only one low estradiol level at the discretion of the study physician.

Exclusion criteria:

- Suicidal ideation where outpatient treatment is determined unsafe by study clinician
- Other causes of oligo-amenorrhea, e.g., polycystic ovary syndrome, thyroid dysfunction (unless euthyroid on medications for ≥3 months), primary ovarian insufficiency (elevated follicle-stimulating hormone [FSH]), or hyperprolactinemia. A participant with PCOS, thyroid dysfunction or mild hyperprolactinemia may be included if a study clinician determines that missed menstrual periods are more likely a consequence of restrictive eating.
- Medications that contain estrogen ± progesterone (e.g., oral contraceptive pills) within the past 2 months

- Levonorgestrel-releasing intrauterine device if subject is unable to provide two to three weekly blood samples for estradiol or if estradiol levels are determined to be too high by study doctor.
- Neurological or psychiatric disorders that may impact neural circuitry of interest, such as schizophrenia, schizoaffective disorder, delusional disorder, psychotic disorders NOS, patients with mood-congruent or mood-incongruent psychotic features, active substance use disorder (substance use disorders in remission are permissible), assessed using the MINI International Neuropsychiatric Interview (M.I.N.I.) (SCID-5-RV for ADD/ADHD only)
- Lifetime history of seizure disorder or electroconvulsive therapy
- Pregnancy/breastfeeding
- Gastrointestinal tract surgery
- Contraindications to estrogen use: Family history of thromboembolism, myocardial infarction, or stroke at <50 years; history of thromboembolism, complex migraine, hypertension, undiagnosed abnormal genital bleeding, known or suspected history of breast or genital cancer or estrogen-dependent neoplasia, liver dysfunction, or abnormal LFTs (>2 times the upper limit of normal); current history of smoking >10 cigarettes a day; history of an allergic reaction to estrogen/progesterone preparations. In case of concerns of a hypercoagulation disorder, a hypercoagulation profile will be performed. Subjects who have no evidence of such a disorder will be permitted to enroll in the study.
- Any other significant illness or condition that the investigator determines could interfere with study participation or safety or put the subject at any unnecessary risk

We anticipate screening up to 200 participants to enroll 120 who will qualify for the study based on study inclusion and exclusion criteria. We expect at least 102 participants to complete the 12-week study. Participants will be recruited through: (i) the MGH Eating Disorders Clinical and Research Program (EDCRP), which principal investigator Dr. Eddy and co-investigator Dr. Thomas co-direct and which evaluates ≥200 patients per year, 33% of whom are females with ED-R/E; (ii) principal investigator Dr. Misra and co-investigator Dr. Lawson at MGH see many patients with ED-R/E and oligo-amenorrhea in their respective adolescent and adult endocrine clinics, which will be other sources of recruitment; (iii) electronic and hard-copy mailings/online posts to potential participants, pediatricians, nutritionists, therapists, sports medicine specialists, high schools, and colleges in New England and beyond, such as Pennsylvania State University; (iv) other local ED treatment centers, such as the Cambridge Eating Disorder Center and the Klarman Eating Disorders Center at McLean Hospital.

Recruitment efforts will be enhanced by utilization of the pediatric, adolescent medicine clinic (at Massachusetts General Hospital), and Klarman Eating Disorders Center (at McLean Hospital) databases. Providers of eligible subjects identified through the database may be contacted about sending letters to these subjects informing them of the study and study contacts. Providers and/or patients may be contacted by study staff or via the Research Patient Data Registry at Massachusetts General Hospital, which allows targeted searches of individuals within the Partners system by age, sex, diagnosis with the ability to filter out patients by study-specific exclusion criteria. This results in a highly targeted recruitment list that allows investigators to contact potential subjects at the approval of their treating doctor and/or through direct contact if patients have consented to this service. Using the targeted recruitment list from RPDR, in conjunction, we will also recruit subjects through Patient Gateway. Medical records of potential subjects may be accessed for the sole purpose of confirming eligibility. We may also use the Research Opportunities Direct to You (RODY) program and research invitations sent through Patient Gateway or regular mail.

For patients of study investigators, the following procedures will be followed to avoid situations where patients may feel obligated to participate: Participants will be presented with written study recruitment materials and asked whether they would be interested in talking to a study nurse practitioner to learn more about the study. If interested, the study nurse practitioner will explain the study details and provide potential participants with the consent form to take home and discuss with friends, family, or other treating clinicians. All participants must be in treatment with a physician (e.g., pediatrician, primary care physician) monitoring the participant during and after the study. For severe low weight or other concerns, the research team will discuss fitness for the study with the participant and her treatment team before enrollment. Moreover, information about existing comorbidities and major life events may be accessed with permission from subjects, guardians, and/or treatment team in order to determine whether it is appropriate or necessary to administer certain portions of the screening interviews.

IV. SUBJECT ENROLLMENT

Informed consent and assent

Written informed consent will be obtained from participants ≥18 years, and written consent from parents and assent from participants <18 years old by a physician investigator or nurse practitioner. A licensed physician investigator will always be available to speak with the subjects during the consent process. All adult participants and parents of minors will receive the Informed Consent form as well as the Human Subject's Bill of Rights. These documents will be read by adult participants and parents of those <18 years old and reviewed by a physician investigator or nurse practitioner with these individuals prior to study participation. The physician investigator or nurse practitioner obtaining Informed Consent will thoroughly review the consent form with the adult participant or the child and her parents, including study procedures and potential risks and benefits of study participation. The adult participant or child and her parents will be encouraged to ask questions throughout the process. It will be emphasized that research is voluntary and that the subject can opt out of this project at any time without jeopardizing her treatment in any way. If a nurse practitioner is obtaining consent/assent, subjects will be explicitly offered the opportunity to also speak to a physician investigator. Any questions, concerns, or ambiguities will be clarified by the physician investigator and/or nurse practitioner prior to signing of consent.

In cases where the subjects and/or guardians live out of state and/or find it difficult or inconvenient to come to MGH, informed consent may be obtained remotely via paper or eConsent. We will explain the study to subjects and/or guardians over the phone/other secure platform (i.e. Zoom), and consent and/or assent forms can be mailed/faxed/e-mailed to the subjects and/or guardians to sign and send back to us. If the subject is currently residing in a different location, separate from their guardians (i.e. residential treatment setting) we will conduct the consent process by telephone/other secure platform and consent forms can be mailed/faxed/emailed to the guardians to sign and send back to us. We will accept a signed, scanned or faxed copy, while awaiting hard-copy to begin study procedures. We will conduct the consent interview by telephone/other secure platform when the subject and/or guardian can read the consent as it is discussed. We will also accept eConsent obtained via MGH-compliant electronic platforms (i.e. REDCap). The consent process will not be different for virtual and in-person consenting.

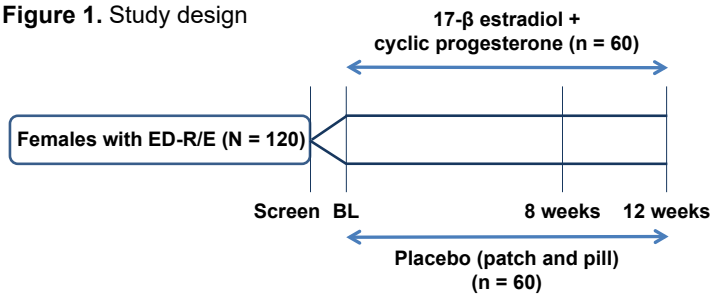
In addition to the informed consent requirements, per the Privacy Rule (HIPAA) regulations, research participants will provide a written "authorization" to use their Protected Health Information in connection with research. Investigators will specify how the information will be used and how the privacy of that information will be protected. Importantly, no subject will be requested to delay treatment for the purpose of participating in this study. Participants who reach the age of majority (18 years) and have the cognitive capacity to read and understand the

consent form while participating in this study will be re-approached by one of the physician investigators. The investigator will continue the informed consent process acknowledging that the individual has reached the age of majority and will ask the participant to sign the informed consent document and a new HIPAA authorization if they voluntarily choose to continue participating in the study. If the individual chooses to withdraw from participating in the study, they will be removed from the study just as any other individual who chooses to withdraw consent from participation. In the event that a study clinician is unable to reconsent a participant at a follow up visit, a trained study coordinator will review the consent form with the participant and/or their parent and provide an opportunity to speak to a nurse practitioner, research nurse, or licensed physician investigator in line with any participant requests.

Randomization

ED-R/E will be randomized (double blind design) 1:1 for 12 weeks to: (i) 100 mcg 17-β estradiol transdermal patches with cyclic progesterone pills (200 mg Prometrium (micronized progesterone) daily in two to three 10-day courses during the 12-week study) or (ii) placebo patches and placebo pills. Participants allergic to peanuts will receive 5 mg of medroxyprogesterone acetate/Provera instead of Prometrium as an unblinded, active medication. This is because the Prometrium capsule contains peanut oil, while the Provera pill does not have this as a constituent. Further, the pharmacy is unable to blind Provera pills. Randomization will be performed based on a computer-generated template developed by the study statistician and maintained by the Research Pharmacy. Because the age range of 14-35 is wide in the context of neurodevelopment, we will use block randomization to ensure even randomization to active estrogen/progesterone or placebo; blocks will include the following age ranges: 14.0-20.9 years, 21.0-28.9 years, and 29.0-35.9 years.

Figure 1. Study design



V. STUDY PROCEDURES

Study overview

The proposed study will focus on testing estrogen as a probe in hypoestrogenemic adolescent/young adult females aged 14-35 years with ED-R/E. We are including this age range because this is a window when: (i) ED-R/E onset is common, (ii) most women have attained menarche and puberty is mostly complete with stable menstrual cycling in a healthy population, and (iii) brain connectivity and structure are still evolving. ED-R/E will be randomized (double-blind design) 1:1 for 12 weeks to: (i) 100 mcg 17-β estradiol transdermal patches with cyclic progesterone pills (200 mg Prometrium or 5 mg (active, unblinded) medroxyprogesterone acetate/Provera daily in two to three 10-day courses during the 12-week study) or (ii) placebo patches and placebo pills. Randomization will be performed based on a computer-generated template developed by the study statistician and maintained by the Research Pharmacy. Because the age range of 14-35 is wide in the context of neurodevelopment, we will use block randomization to ensure even randomization to active estrogen/progesterone or placebo; blocks will include the following age ranges: 14.0-20.9 years, 21.0-28.9 years, and 29.0-35.9 years. Randomization will prevent biasing the study in favor of any arm. For the estradiol patch, we will use a generic brand patch, while for micronized progesterone we will use Prometrium. For participants with a peanut allergy, we will use active/unblinded medroxyprogesterone acetate/Provera. All medications will be dispensed by the Research Pharmacy. For active drug patches, the study coordinator (who will be unblinded) will affix Avery Labels to the pouch of the

active patches (using an SOP developed by the Research Pharmacy). For the placebo patches, we will use similar transparent patches obtained from retail pharmacies. These will also have Avery labels fixed on them by the unblinded study coordinator. Active prometrium pills will be blinded by the Research Pharmacy, which will also prepare the placebo pills. For participants with peanut allergy, medroxyprogesterone acetate/Provera tablets will be provided in active form and will be unblinded. All of this has been discussed with the Research Pharmacy. Although ED-R/E randomized to estrogen/progesterone might have cyclic menses, this is not uniform, and ED-R/E randomized to placebo may have intermittent menses. Thus, menses will not unmask group assignment. Participants will be counseled at the screening and subsequent visits that the combination of the transdermal 17β-estradiol patch with cyclic progesterone has no contraceptive efficacy and will be provided with information regarding acceptable methods of non-hormonal contraception during the study period. Participants will undergo a screen followed by a baseline visit, and follow-up visits at 8 and 12 weeks (Figure 1).

Use of estrogen as a physiological probe

ED-R/E will be evaluated for study endpoints after 8 and 12 weeks of the physiological probe (estrogen). Estrogen therapy is standard of care in certain estrogen-deficient states in adolescence (e.g., Turner syndrome, POI) (42, 43) and is also used for contraception (44) in adolescents and is thus feasible in this age group. However, it is not yet standard of care in ED-R/E with amenorrhea or oligomenorrhea.(45) Thus, a randomized design is justified and does not violate equipoise. We will use transdermal 17-β estradiol at a dose of 100 mcg daily (patch will be replaced twice weekly). This is the natural form of estradiol, and the transdermal route avoids hepatic first pass effects and suppression of IGF-1, which is associated with cognitive decline in older individuals.(46, 47) It is associated with fewer side effects than equivalent doses of oral estradiol.(48) We will use a fixed 17-β estradiol dose rather than phasic doses to reduce variability in study endpoints from changing estradiol levels and because physicians typically use a fixed estradiol replacement dose in hypoestrogenic girls.(49, 50) These doses aim to mimic physiological levels.

Progesterone

Full replacement of estrogen can cause endometrial hyperplasia and in the long-term increase cancer risk.(51) Cyclic progesterone protects against this risk. Therefore, ED-R/E randomized to 17-β estradiol will take 200 mg of micronized progesterone (natural progesterone) in two 10-day courses: the first course occurring five weeks after the first day of estrogen, and the second course of occurring after the 8-week study visit, given that this visit occurs at least four weeks after the five-week progesterone course. A third course may be administered if needed for subjects remaining in the study for > 12 weeks. This mimics the higher progesterone levels in the luteal phase of the cycle. Subjects will be on progesterone for any 10 consecutive days within a month based on baseline date. This will optimize timing of study visits and avoid all study visits taking place in the same part of the month. To minimize progesterone effects on study endpoints, we will time study visits such that they occur ≥ 4 days after last progesterone dose. As previously noted, effects of progesterone on study endpoints are either minimal and/or change within days of changing progesterone levels.(26, 52) Thus, follow-up visits will occur with a 2-week window. For participants with peanut allergy, 5 mg of active, unblinded medroxyprogesterone acetate/Provera will be administered in lieu of 200 mg of Prometrium.

Optimizing and assessing compliance with hormone replacement

The following strategies will be used to improve and assess study compliance:

- Self-report medication diaries with calendar check boxes and/or REDCap survey to complete to check off following patch application and progesterone/ placebo pill ingestion
- Return of empty packaging
- Phone calls/study visits at 4 weeks post-randomization to encourage compliance and check on side effects
- Measurement of serum estradiol levels.

We will also work on optimizing compliance by implementing incentives and striving to maintain morale with interactions between subjects and research staff. We will build a sense of teamwork and loyalty among the subjects.

Study visits

Screening**: a.** Written consent from subjects ≥18 years or consent from parents and assent from subjects <18 years. **b.** History, pulse, height, and weight measurements; with optional physical exam and/or vital signs measurement at clinician's discretion (H&P) including height on a single stadiometer, weight using an electronic scale, Tanner staging, and menstrual status. BMI SDS will be calculated from the 2000 CDC charts.(40). **c.** Urine or Serum pregnancy test. (If the test cannot be administered at the screen visit due to subject's treatment status but the subject is otherwise eligible for the study, we will accept hCG records from residential treatment admission and re-test for pregnancy at onset of baseline visit. For other subjects unable to present in-person (e.g. due to COVID-19 quarantine), we will test for pregnancy at or before the baseline visit. In any case, a subject will not begin study medication until subject is confirmed to not be pregnant.) **d.** Blood for laboratory tests to rule out other causes of amenorrhea (thyroid-stimulating hormone, FSH, and prolactin) (In cases where the participant can provide records of TSH, FSH, and prolactin performed within two months of the screening visit, we will not repeat those labs.) Estradiol labs may also be drawn at multiple timepoints pre-baseline at study physician's discretion, e.g. if a subject has a hormonal IUD or has recently ceased taking an oral contraceptive or also has a diagnosis of PCOS/thyroid dysfunction/mild hyperprolactinemia.*(i. Blood laboratory tests can be done at baseline visit if unable to obtain at screening visit. If blood laboratory tests are done at baseline, medications will be withheld until laboratory results are received and assessed. Blood laboratory tests can also be done at a local Quest lab or clinician's office if a subject is unable to present in-person to MGH (e.g. due to COVID-19 travel restrictions).* **e.** Bone age (only for participants < 16 years old) estimated using an x-ray of the left hand and wrist by standards of Greulich and Pyle (53). (In cases where the participant can provide a record of a bone age ≥ 13.5 years, we will not repeat the x-ray). **f.** Clinical interviews and self-reported ED pathology: **SCID**-5-RV, EDI-3 (Drive for Thinness Subscale only), Compulsive Exercise Test (**CET**), and **M.I.N.I.** to confirm eligibility and assess comorbidity. f. **Chapman Handedness** Questionnaire. Portions of the screen visit may be omitted if a subject is highly unlikely to be eligible.

Baseline**: a.** History, pulse, blood pressure, and weight measurements; with optional physical exam and/or height and/or additional vital signs measurement at clinician's discretion **b.** Urine or Serum pregnancy and drug test. **c.** Blood for estradiol, testosterone, and sex hormone-binding globulin (SHBG). SHBG to assess bioavailable testosterone using established formulas. Estradiol levels serve as a measure of compliance with physiological estradiol replacement. **d.** ED pathology: EDE, EDI-3 (Drive for Thinness, Bulimia, and Body Dissatisfaction subscales only), EPSI, CET, and LIFE EAT-3. **e.** Functional MRI paradigms: Task switching, monetary reward task, and delay discounting task. Tasks may be performed outside of the scanner for participants with contraindication to MRI. **f.** Behavioral tests: D-KEFS CWIT, Implicit Association Test-Drive for Thinness (IAT-DFT) (optional), modified Go/No-Go task (G/NG) (optional), Monetary Choice Questionnaire, Wechsler Abbreviated Scale of

Intelligence (WASI); **g.** Other questionnaires: Autoimmune History, Beck Depression Inventory-II (BDI-II), The CoRonavIruS Health Impact Survey (CRISIS; Adult or Youth Version), Obsessive-Compulsive Inventory (OCI), Parent Eating Disorder Examination Questionnaire (P-EDE-Q; optional), Positive and Negative Affect Schedule (PANAS*), Snaith-Hamilton Pleasure Scale (SHAPS), State-Trait Anxiety Inventory (STAI*), TAS-20, TEPS, VAS*. h. Four-day food diary, 24-hour food recall, Food Frequency Questionnaire (FFQ**), and International Physical Activity Questionnaire (IPAQ, short or long form***) **i.** Stool Collection: We will attempt to collect stool samples during main study visits (Baseline, 12-Week) or by using at-home stool collection kits that will be delivered or mailed to study staff. Additionally, participants will report any recent antibiotic and/or probiotic use, recently consumed foods, and stool quality. The collection of these samples will be optional, and consent will be obtained. Subjects will be paid up to $75 ($25 at baseline, $50 at 12-week visit) for completion of stool collection. Stool samples will be assayed for microbiome profiling. **j.** Hair sample collection: Small hair strands (~3 mm diameter) will be taken near the scalp from a posterior vertex position at the Baseline, 8-week, and 12-week visits. Cortisol concentrations in the proximal 3-cm hair segment will be determined as measure of the integrated cortisol secretion over the three month-period prior to hair sampling (54, 55) The collection of these samples will be optional. **k.** Grip strength collection: Hand grip strength will be measured using a Jamar Hydraulic Hand Dynamometer. Grip strength force will be recorded for three trials per hand. This collection will be optional.

8-week follow-up**: a.** History, pulse, and weight measurements; with optional physical exam and/or height and/or vital signs measurement at clinician's discretion **b.** Urine or Serum pregnancy and drug test. **c.** Blood for estradiol, testosterone, and SHBG (may be collected at an external laboratory). **d.** Functional MRI paradigms (administered outside of the scanner for participants unable to participate in the MRI). **e.** Behavioral tests: D-KEFS CWIT, Monetary Choice Questionnaire; **f.** Self-reported ED pathology: EDI-3, EPSI, CET; **g.** Other questionnaires; **h.** Hair sample (optional). **i.** Grip strength (optional)

12-week follow-up**;*****:** All baseline procedures will be repeated except fMRI paradigms, urine drug test, G/NG, pre and post-MRI PANAS, WASI and the FFQ. (The autoimmune questionnaire and/or WASI may be administered at the 8 or 12-week visit if needed due to participant scheduling needs. These measures only need to be completed once. The autoimmune questionnaire may also be asked after completion of the study.) Like the 8-week follow-up visit, blood may be collected at an external laboratory. Side effects will be reviewed at every visit.

Baseline, 8-week and 12-week follow-up visits are expected to take 7, 4, and 3.5 h, respectively (including breaks). Screening and baseline visits may be combined for certain participants who are highly likely to be eligible based on the phone screen and whose schedule necessitates a combined visit. Study components (i.e. CET, EDI-3) do not need to be completed twice. Additionally, if all screening visit components cannot be completed in one sitting due to time constraints but the subject is highly likely to be eligible for the study, we will complete unfinished screening activities before or at the baseline visit, when the participant must present to MGH and McLean in-person. Baseline and 8-week follow-up visits are split into two parts (neuroimaging vs. other assessments) to be completed on the same day or different days as close to each other as possible based on convenience for the participant and availability of facilities. All neuroimaging will be completed at McLean Hospital, which is a 30-min drive from MGH, while enrollment of study participants, screening visit, as well as H&P, blood draw, clinical interviews, behavioral tasks, and questionnaires at baseline, 8 and 12-week visits can be completed virtually, at McLean, or on the MGH main campus. Study staff may escort the participant and parent (if the participant is a minor) between sites depending on participant preference. Study staff will escort any participant who is a minor between sites (if a

parent/guardian is not present) Minors may complete study visits without a parent present once the parent has given verbal and/or written consent.

*PANAS, STAI, VAS. PANAS will be performed up to four times at the Baseline and 8-week follow up visits prior to and following both the fMRI and cognitive tasks, and up to two times at the 12-week follow up visit prior to and following the cognitive tasks. STAI trait will be performed once at each Baseline, 8 and 12-week follow up visit. STAI state will be performed up to four times at each Baseline, 8 and 12-week follow up visit prior to and following both the fMRI and cognitive tasks. VAS will be performed up to two times at each Baseline, 8 and 12-week follow up visit prior to both the fMRI and cognitive tasks.

**If a subject is unable to complete the FFQ during the baseline visit, this measure may be completed at the 12-week visit.

***If a subject is unable to complete a long-form IPAQ, a short-form IPAQ may be completed instead. The subject will complete the same IPAQ version (short or long) at both the baseline and 12-week visits.

**** In the case that a participant is unable to physically present for a visit (e.g. due to COVID-19), we may conduct visit components virtually/over the phone, including medical history, nutrition measures, questionnaires, cognitive measures, and clinical interviews, with subject permission. Components that require subjects be physically present may be collected in the following ways: Weight: self-reported or provider records. Blood draw: Add on to clinical lab draw, charges for study related labs will be directed to study's fund. Labs may be omitted if no clinical labs are to be drawn. Urine hCG: participants will receive urine hCG test kit by mail and will show the completed test to the study coordinator. Pulse: participants will be instructed on how to take their pulse and will do so while being speaking with study NP. 8-week fMRI: In the case that a participant is unable to physically present for her 8-week visit, all measures except for the fMRI will be collected virtually. If the second fMRI is totally unable to be obtained, subjects will be paid $75 for completing other non-imaging 8-week study activities. All reasonable attempts will be made to complete the second fMRI: ideally within the next 4-5 weeks, around the time of the 12-week visit,along with typical study activities. If the fMRI is delayed to 12-weeks, the patch will be worn for an additional 4-5 weeks and typical 12-week data (including ED pathology) will be collected around 16-weeks. An additional course of cyclic progesterone/placebo will be administered per protocol 4 weeks after last course. Moreover, if a subject wishes to complete the study but is unable to within 12 weeks, we may extend her duration on the study medication up until 20 weeks post-baseline and collect final study measures by this timepoint.

***** In the case that a participant is unable to complete a full 12-week visit, we will collect as many measures as possible in an abbreviated visit. In order of importance, these measures shall be the EDI-3, EDE Dietary Restraint items, 4-day food record, 24-hour recall, or other measure of recent caloric intake, CET, any other measures that the participant can complete. Compensation to the subject will be prorated up to the typical 12-week payment depending on how many visit components are completed.

****** In the case that a participant completes the 12-week visit virtually instead of completing in-person, Compensation to the subject will be reduced to $75 instead of the typical $125 payment.

Subjects who are in residential treatment can have their screen or portions of the main study visits at the treatment center with permission from the center and the subject. If a subject currently resides in a treatment facility and is unable to complete the entire visit due to time constraints, we will request that the study visit be completed over the course of multiple days, following the same task order and structure as the other applicable visits. The consent, history,

and optional physical, and any non-time specific study interviews/questionnaires will be done by study staff. Study staff will travel to these centers to conduct study procedures. Blood will not be drawn at the treatment center by study staff. If lab results are available for any screening labs that we need for the visit, we will use those records. A release of information will be signed by the subject prior to obtaining any health information. This split screening visit may also apply to subjects who live outside of Boston and are unable to commute to MGH, such as in the case of COVID-19 quarantine, or who are unable to complete a screening visit in one sitting due to time constraints.

Hormonal assessment

A blood sample will be drawn for estradiol, testosterone and SHBG levels. This may occur at the study visit or at an external laboratory. Samples from both sites will be run by the Brigham Research Assay Core Laboratory or Quest Laboratory using their standard assays, to avoid bias introduced by assays at different labs. Medication logs and unused patches/medications at 8 and 12 weeks, and serum estradiol levels will be assessed to determine hormone replacement adherence.

Functional MRI

Data acquisition: Data will be acquired on a 3T Prisma scanner with a 64-channel head-neck coil at McLean Imaging Center. **Structural MRI:** High-resolution anatomical data (T1- and T2-weighted) will be collected for anatomic localization of fMRI data using the Human Connectome Project recommended acquisition protocol for 3T scanners with 0.8mm isotropic resolution; T1w MPRAGE (TR/TI = 2400/1000 ms, GRAPPA = 2); T2w SPACE (TR/TE = 3200/565 ms, GRAPPA = 2). High resolution diffusion weighted images (DWI) will be collected either at the baseline or 8-week scan (optional). **Functional MRI:** Whole brain gradient echo T2*-weighted echo planar images (EPI) will be acquired using the University of Minnesota multi-band BOLD EPI sequence,(56, 57) which achieves excellent temporal and spatial resolution (TR/TE = 1300/35 ms; voxels 2 x 2 x 2 mm, multiband factor 6, phase encoding (PE) direction P->A). **Fieldmap:** Reversed PE blip spin-echo EPI based field map scan will be acquired for distortion correction.(58) **Resting and Perfusion:** In addition, we will also collect 6min resting and perfusion scans. **Peripheral Psychophysiology:** Peripheral psychophysiological measures (e.g. heart rate and respiration) will be collected throughout the scanning session using MRI-compatible equipment. Heart rate will be collected using a non-invasive wireless physiological monitor that clips on to the participant's pointer finger. Respiration will be collected by attaching a respiratory cushion to the participant using a belt fastened to the lower chest. Both the sensor and belt can be easily applied and removed. The experimenter will ensure that the clip and belt are securely attached to the participant and that the participant is comfortable. This wireless monitoring system is a standard Siemens system, which comes with the FDA-approved Siemens 3.0 magnetic resonance scanner and is MRI compatible. **Functional MRI paradigms:** **a.** Cognitive Flexibility: **Task switching:** Suggested by the RDoC matrix; it robustly activates the targeted brain network underlying cognitive flexibility. Subjects will randomly switch between performing two choice reaction tasks, namely, categorizing visually presented letters as vowels/consonants or upper/lower case, by providing manual responses (button presses).(59) The color of the letter will indicate the task (red: vowel-consonant discrimination; green: upper-lower case discrimination). As a control condition, subjects will perform the two tasks separately, and performance will be averaged across blocks with the vowel-consonant and upper/lower case discrimination conditions. This paradigm has shown sensitivity to detect changes in neural activation due to hormone replacement therapy in hypoestrogenemic postmenopausal women.(59) **b.** Initial Response to Reward: **Monetary reward paradigm:** Subjects will view images of slot machines with displays of magnitude and probability of a reward (presentation phase), followed by the spinners rotating (delay phase) and display of received monetary reward or lack thereof (outcome phase). Initial responsiveness to reward receipt will be

assessed by analyzing brain activation during the outcome phase, contrasting trials with reward receipt to those without reward delivery despite >0% chance of reward receipt.(26) This task has been successfully employed to document the effect of estrogen replacement on the neural response to reward receipt in hypoestrogenemic postmenopausal women.(29) **c.** Delay: **Delay discounting task:** In this fMRI paradigm, shown to detect differences in neurocircuitry underlying reward valuation in those with ED-R/E versus HC,(11) subjects will choose between smaller immediate vs. larger delayed monetary rewards. In 50% of trials, the immediate reward will be available now, in the other 50%, within two weeks.(11) All fMRI tasks will be practiced outside the scanner prior to scanning to familiarize subjects with the tasks and minimize scanner time. The total duration to complete all 3 tasks including repeated instructions to orient subjects to the task at hand and sufficient breaks within and between tasks will be 60 min. Total time in the scanner (including set-up and structural scans) will not exceed 120 min. **Anatomical image pre-processing:** To minimize potential biases associated with registration of longitudinal images, we will follow the strategy advocated by FreeSurfer and FSL, by registering each time-point to a robust (i.e., midway) within-participant template.(60) To improve the quality of between-participant registration while avoiding potential biases in group-level analysis, a study-specific template (61) will be generated in MNI space from the individual within-subject templates using ANTS (62) for its superior performance in normalization.(63) Gray matter density (GMD), volume (GMV), and mass (GMM) will be measured from T_1-weighted images segmented using an iterative approach without tissue priors with ANTs Atropos.(62, 64) **Functional MRI analyses:** fMRI data will be pre-processed with motion correction, EPI distortion correction, slice-timing correction, spatial smoothing (4 mm), grand-mean intensity normalization and high pass temporal filtering (0.01 Hz).(65-67) Functional MRI data at each time point will be registered to the high-resolution structural images in the within-participant midway template using boundary-based registration (68) for 1st and 2nd level fMRI analyses. For all tasks, at the individual level, analyses of fMRI data will be conducted using general linear models (GLM) with separate regressors. Each event will be modeled using a gamma function and constructed as a hemodynamic response function, convolved with the event onset times. Data will be analyzed using a region of interest (ROI) approach to evaluate *a priori* hypotheses. ROIs will be obtained from the Freesurfer parcellation of the individual midway template image. In the ROI approach, mean parameter estimates (β-weights) and their changes between two time points will be extracted from each ROI and analyzed with ANOVAs. Whole-brain psycho-physiological interaction (PPI) analyses will be implemented to probe task-related functional connectivity between confirmatory and further potential ROIs in the study-specific group template.(65, 69)

Clinical interviews

The **M.I.N.I.** is a structured clinical interview for DSM-5 diagnoses administered at screen to determine eligibility and characterize comorbidity.(70) The **EDE** is a semi-structured interview (71) with high inter-rater reliability and validity.(72) It features 4 subscales (Eating Concern, Shape Concern, Weight Concern, Dietary Restraint) and a global severity score. The Structured Clinical Interview for DSM-5 Research Version (**SCID-5-RV**) is a semi-structured interview performed at screen. The Eating and Feeding Disorders Module (Module I1-I12), Optional Feeding and Eating Disorders ARFID Module (Module Opt I1-I3) and ADHD Module (Module K1-K6) questions will be asked. The **Life-EAT 3** is a clinician-rated interview that is utilized to track an individual's psychiatric course and psychosocial functioning over time (99). Clinical interviews will be audio-recorded.

Questionnaires

The **EDI-3** is a 91-item self-report assessment of eating attitudes that includes subscales that robustly measure core ED-R/E features of drive for thinness and body dissatisfaction.(73)

The **CET** is a 24-item self-report assessment of core features of excessive exercise in eating disorders.(74) The 18-item **TEPS** assesses traits such as openness and flexibility with a variety of pleasurable stimuli/experiences for anticipatory and consummatory components of pleasure experience (10 and 8 items, respectively).(75) The 21-item **BDI-II** provides a dimensional rating of depressive symptom severity.(76) The 40-item **STAI** represents a dimensional measure of current and general anxiety.(77) The 20-item **PANAS** assesses current levels of positive and negative affect.(78) The 14-item **SHAPS** assesses current symptoms of anhedonia.(79) The **VAS** assesses current hunger levels.(80) The **EPSI** is a 45-question self-report assessment that measures restrictive eating habits and cognitive restraint (81) The **OCI** is a 42-item self-report assessment that measures current symptoms of obsessive compulsive disorder (82). The **P-EDE-Q** is administered to individuals' parents/guardians and assesses the same subscales as the EDE (71). The **TAS-20** is an assessment of alexithymia, or difficulty identifying, describing, or processing emotions (98). The CoRonavIruS Health Impact Survey (**CRISIS**) assesses changes in lifestyle and health due to the COVID-19 pandemic. All questionnaires will be administered using Research Electronic Data Capture (REDCap™).

Behavioral measures

D-KEFS CWIT: Subjects will complete the CWIT portion of this highly standardized and normed neuropsychological test battery.(83) Its Inhibition-Switching component assesses cognitive flexibility, with shorter completion time and higher accuracy indicating more flexibility. Subjects will name the color of words representing colors printed in dissonant ink colors (e.g., the word "blue" in red ink). However, if words are framed by a box, they will be instructed to read the word instead. **Implicit Association Test, Drive for Thinness (IAT-DFT):** The IAT-DFT assesses the correlation between the attribution of positive traits to underweight models and participants' eating disorder symptoms. In this computerized task, participants will press the same key for either positive or negative attributes paired with photos of either ultra-thin or normal-weight models. Stronger implicit associations to positive attributes for ultra-thin models have been correlated with increased drive for thinness in high school and college women (84). This measure is optional as time allows. **Modified Go/No-Go Task (G/NG):** The G/NG investigates the decision-making processes underlying active versus passive responses to escape aversive situations in individuals engaging in restrictive eating. In this computerized task, participants will learn contingencies between cues, responses, and outcomes in two conditions: an escape condition (cues presented with simultaneous onset of an aversive sound) and an avoid condition (cues presented without an aversive sound). After being presented with the cue, participants will then decide to either press the spacebar (Go) or withhold a response (No-Go). Participants will then receive feedback of either the aversive sound or silence, with the goal of learning which response (go vs. no-go) is more likely to result in silence (85). This measure is optional as time allows. **Monetary Choice Questionnaire:** In this computerized task/verbal administration of this widely used behavioral tool, we will assess an individual's extent of discounting the value of future rewards with increasing delay to reward receipt. Subjects will make 27 binary choices between smaller immediate and larger delayed monetary rewards of varying value and delay to reward receipt. From their responses, their Delay Discounting Parameter k is calculated and indicates the extent of discounting future rewards.(86, 87) **WASI:** To test (and control) for general intellectual ability: **a.** Matrix Reasoning: Subjects will complete gridded patterns by choosing the correct of 5 options (fluid intelligence); **b.** Vocabulary: Subjects will define given words, and responses will be scored based on definition match (crystallized intelligence).(88)

Four-day food diary

The four-day food diary includes 3 weekdays and 1 weekend day and is a validated tool for young women, including ED-R/E (89, 90). Records will be analyzed by dieticians in the Clinical Research Center using NDS-R software v2008 (Nutrition Coordinating Center (NCC),

University of Minnesota, Minneapolis, MN).(91-93). The 4-day food record or 24-hour recall will not be required for subjects who are currently in residential treatment centers, or for subjects who feel uncomfortable recording their food intake. For the FFQ, the nutritionist will ask questions about intake of foods and supplements that contain calcium and/or vitamin D.

Retention

If individuals are interested in the study and elect to enroll, we will utilize several strategies to retain participants in the proposed research that have been successful in our ongoing work:

- We will develop a comprehensive tracking database with multiple ways to reach participants, in case of an unexpected change in a participant's contact information.
- As always, we will develop a congenial relationship with participants to establish trust and to enable them to discuss limitations regarding returning for study visits. This will allow us the opportunity to work around these limitations and ensure successful return visits.
- A study physician or his/her delegate will be available to answer questions regarding study or hormone replacement-related adverse events 24 h a day.
- We will provide monetary compensation to defray the costs involved in completing research assessments: Participants will receive financial compensation as follows: $200 at the baseline and 8-week visits, $125 at the in-person 12-week visit (or in the case of an abbreviated visit, up to $100 prorated on how many activities the subject completes. In the case of a virtual visit, $75), and $50 at the 16-week visit, should a 16-week visit occur. Additionally, participants will receive monetary rewards (up to $210) based on two completed MRI tasks. For these extra payments, we will use an approved, secure, outside vendor (Forte Research) to make these payments via a reloadable credit card-based system, called Forte Payments. Participants will also receive up to $75 for completing the optional stool collection sub-study.

Following study conclusion, participants will be provided with referrals for treatment at either MGH/McLean or other treatment centers if they ask for one. For those who have insurance or can afford private care, we will work with them to identify an appropriate referral source. For those with no insurance and/or limited access to care, we have resources within MGH/McLean for referral.

Study discontinuation

Criteria for discontinuing the protocol are:

- Pregnancy: If a subject becomes pregnant during the course of the study, she will be withdrawn from the study. The requirement that subjects and their partners use effective non-hormonal forms of birth control during participation (the progesterone releasing intrauterine device may be used as there is little systemic absorption) will be stated in the consent form and strongly emphasized during recruitment and throughout the study.
- Therapeutic use of medications that may affect gonadal status during the study period (other than ongoing and stable thyroid hormone replacement)
- Serious protocol related side effects such as thromboembolism
- Active suicidality.

Participants may be withdrawn from the study:

- At participant or guardian request
- If severe adverse events occur that cannot be addressed
- At the study clinician's discretion.

V. Sub-Studies

CSF Sub-Study

Background and Significance

In healthy women, natural changes in estrogen status, and estrogen replacement in states of hypoestrogenism are associated with changes in inflammatory markers. Menopause is characterized by marked reductions in estrogen and progesterone levels and increases in peripheral inflammation.[1,2] Physiological estrogen replacement in menopause leads to a decrease in peripheral inflammation.[1] Further, estrogen drastically increases during pregnancy,[3,4] and these endocrine changes influence neural plasticity and impact brain structure in animals[5,6] and humans by strengthening the brain barrier,[7–9] thus blocking the passage of inflammatory cytokines. Immunoendocrine interactions have not been studied in restrictive eating disorders (R-EDs).

R-EDs are often associated with hypoestrogenism (~60%) secondary to low energy availability. The brain regulates estrogen secretion through activation or suppression of the hypothalamus-pituitary-gonadal (HPG) axis, and in turn estrogen regulates certain neuropsychiatric outcomes (such as cognitive function, anxiety, and eating behaviors). Adolescence represents both the window of greatest risk for R-ED onset, and is coincident with maturation of the HPG axis and increasing levels of the gonadal hormones, estrogen, progesterone, and testosterone. Estrogen dominates in girls, and pubertal maturation is associated with emergence of a complex cycling of estrogen/progesterone across the menstrual cycle. Our pilot data suggest that estrogen replacement may improve ED pathology, yet the mechanisms by which estrogen replacement improves R-ED pathology are unknown.

Specific Aims

The purpose of this substudy is to establish the presence of immunoendocrine alterations in R-EDs by assessing central and peripheral inflammation in R-EDs. Given the paucity of data on the relationship between peripheral blood markers of inflammation and central cerebrospinal fluid (CSF) markers of inflammation in patients with R-EDs, we will explore relationships between plasma and CSF concentrations of inflammatory cytokines and their receptors/antagonist in patients with R-EDs, and examine relationships with estrogen replacement, hypoestrogenemia, and core R-ED symptoms. Our central hypothesis is that estrogen deficiencies secondary to R-EDs may increase central and peripheral inflammation via alterations in the brain barrier in R-EDs, and thus, contribute to core symptoms of R-EDs (restriction/weight shape concerns). Specifically, we hypothesize that establishing physiological estrogen levels in R-EDs (vs. placebo) will stabilize inflammatory protein ratios (greater anti-inflammatory vs. pro-inflammatory) in plasma and CSF (Aim 1). Further, changes in the inflammation ratios (in CSF and plasma) will mediate estrogen effects on R-EDs symptoms at 12 weeks (Aim 2). Whole genome analyses, if performed on DNA samples, will allow researchers to gain understanding of links between many diseases or conditions.

Visit Procedures

Inclusion/Exclusion Criteria: The inclusion/exclusion criteria are the same as the criteria to be enrolled in the parent study *with the addition of*

Inclusion:

- Age ≥ 18 years

Exclusion:

- Coagulation abnormalities for lumbar puncture (LP), including: (1) possible raised intracranial pressure (ICP) with risk for cerebral herniation; (2) thrombocytopenia or other bleeding diathesis, including ongoing anticoagulant therapy; (3) suspected spinal epidural abscess. We will test safety labs prior to LP to confirm eligibility (see below).

Subjects will have a CSF visit at the 12-week final visit, or 6 weeks before or after the 12-week visit depending on the participant's schedule. The CSF visit will be conducted either on the same day as the 12-week visit or on a separate day within approximately 6 weeks before or after each subject's 12-week visit. If the subject elects to participate in this portion of the study, they will have consented at the screening visit to learn more about the CSF sub-study and consented for the sub-study separately prior to, at, or following the final 12-week visit (or at or following the final study visit up to 20 weeks if the participant remained on study medications and the study period was extended due to scheduling). The following will be conducted at the CSF sub-study visit and/or at the 12-week final visit:

A. H&P, including vital signs measurement and assessment of recent illness, weight/%IBW/BMI, 4-day food record, activity assessment (IPAQ), 24-hour food recall (These components do not need to be repeated if the CSF visit occurs close enough to the final 12-week visit to allow use of the data from the final 12-week visit). Height and optional physical exam at clinician's discretion
B. Phlebotomy: Approximately 30 cc (2 tablespoons) of blood will be drawn from the participants during the visit. Serum and plasma will be extracted from the sample and stored for use in future research studies. Tests for CBC and PT/INR which are required prior to performing a lumbar puncture may be performed via a finger stick using CLIA waived point of care testing using the Sysmex XW-100 CBC analyzer and the Roche CoaguChek XS Plus PT/INR analyzer or analyzed via a sample collected during phlebotomy.
C. Lumbar Punctures (LPs): CSF will be collected with LPs after a minimum of an 8 hour fast. The subject will be positioned seated or lying on his/her side on the examination table. Standard protocols will be used employing palpation to identify the L3-4, L4-5 or L5-S1 vertebral interspaces, sterile conditions, local lidocaine anesthesia, and use of the Sprotte 24 gauge needles. Lumbar punctures will be performed by qualified, experienced practitioners. Approximately 20 cc of CSF will be collected for analysis of various AD, redox, and metabolism biomarkers, including NfL, FABP3, 8-OHdG, and pyruvate/lactate, tau, phospho-tau, amyloid-$\beta_{42/40}$, and neurogranin.
D. Post LP survey: Subjects will also complete a brief REDCap survey after the LP in which they will answer questions about the tolerability of the procedure.
E. Post-Visit Phone Check
   Following the CSF visit, a review of symptoms will be performed over the phone by a member of the study team. The phone call will be reviewed by a licensed nurse practitioner and/or other qualified clinician.

   For completing the sub-study, participants will receive $150 in financial compensation.

Potential Risks

Lumbar puncture (LP): Pain may occur during the procedure. This is usually temporary, confined to the lower back, and minimized with the cutaneous and soft tissue administration of 1% lidocaine as a local anesthetic. Short-lived LP associated headaches occur in <1%-36% of subjects with an incidence that decreases with age.[38-40] Less commonly, a persistent low-

pressure headache may develop as a result of a post-LP CSF leakage. Lower rates of post-LP headache have been noted with the atraumatic (Sprotte) 24-gauge needle that will be used in this study. Potentially more serious, but very rare risks, include infection, damage to radicular nerves and bleeding into the lumbar CSF space. The risk of these procedure related complications is much less than 1%. There is also a very rare risk of an allergic reaction to the lidocaine.

VI. BIOSTATISTICAL ANALYSIS

Enrollment target

We plan to screen up to 200 oligo-amenorrheic young women 14-35 years old with eating disorders associated with restriction or excessive exercise (ED-R/E) and high drive for thinness to enroll 120 participants in the 12-week randomized controlled trial of physiologic estrogen replacement versus placebo. We expect 102 completers (15% drop-out rate) for this 12-week study. Data generated will be longitudinal over 8 weeks (most measures) and 12 weeks (EDE, EDI-2, and caloric intake) (Table 1). Study groups will be ED-R/E randomized to estrogen versus placebo. Baseline characteristics will be assessed by randomization group using the independent samples t-test or Wilcoxon rank sum test for continuous variables (depending on data distribution) or the Chi-square test for comparison of proportions.

Study endpoints

Primary endpoint: 8-week change across randomization groups in Cognitive Flexibility (improved inhibition-switching performance on the D-KEFS CWIT) (Aim 1). We selected this as the primary outcome, because we have shown this measure is sensitive to changes in cognitive flexibility following estrogen replacement in adolescent and young adults with amenorrhea and high drive for thinness. **Co-primary endpoints:** 8-week change across groups in: (i) TEPS Consummatory Pleasure scores, and (ii) Amount of discounting the value of rewards with increasing delay to reward receipt (assessed with the delay discounting parameter k in the Monetary Choice Questionnaire) (Aim 1); 12-week change across randomization groups in: EDI-3 Drive for Thinness and Body Dissatisfaction scores (Aim 2). **Secondary endpoints:** 8-week change across groups in: (i) DLPFC and ACC activation in response to task switching paradigm, and (ii) VMPFC and striatum activation in response to reward receipt and during delay discounting (Aim 1); 12-week change across groups in: (i) EDE Dietary Restraint subscale, (ii) Caloric intake (4-day food diary) (Aim 2); Mediation effect of 8-week change (Δ) in Cognitive Flexibility, Initial Response to Reward, and Delay on 12-week change in ED symptoms (Aim 3). **Covariates of interest:** To assess the impact of associated clinical conditions and ongoing treatment on the response of ED-R/E to study intervention, we will consider accounting for obsessive-compulsive disorder, anxiety and depressive symptoms, and treatment (i.e., type [psychotherapy, medication] and intensity of treatment received: level of care, h/week) concurrent to estrogen replacement in all analyses. Although this study is designed as a randomized controlled trial, if differences are observed between randomization groups in covariates that may possibly confound treatment effects, such as age, amenorrhea duration, baseline ED symptoms, and BMI changes (even though chances are small), these will be included in adjusted analyses.

Sample size

We determined the sample size to target the minimum detectable statistically significant effect size of between-group differences for endpoints related to Aims 1 and 2 of no larger than 65% of the clinically meaningful effect size for all endpoints that have been shown by pilot or preliminary studies as Cohen's $d \geq 0.92$. We thus targeted a detectable effect size of $d \geq 0.56$ ($p < 0.05$) or $d \geq 0.62$ ($p < 0.025$) depending on the relatedness of the endpoints in question (see

Table 1). Further adjustment for multiplicity of statistical comparisons for all endpoints was not performed because it is desirable that individual statistical test results be interpreted marginally, and such interpretations will have implications for different aspects of those endpoints.(94) 102 trial completers (i.e., 51 completers/arm) are required to detect these effect sizes using a two-sided test at a 2.5-5% type-1 error rate and with 80% power. Assuming an attrition rate of up to 15%, we determined that the total enrollment size needs to be 120 subjects for the 1:1 randomization. This study is adequately powered to detect not only a conservative target effect size for the primary analysis but also will attain sufficient power for the Sobel test for mediation. Our preliminary data from a randomized controlled trials at MGH showed a Cohen's effect size d = 1.2 (with p = 0.0009) for a 12-month estrogen effect on body dissatisfaction score change and a Pearson's correlation of 0.6, which is equivalent to the total effect (τ). While the Sobel test will resort to the bootstrap sampling approach, we present here the detectable sizes of α and β for the parametric test at a two-sided 5% type-1 error rate with 80% power. With 102 observations, $\alpha > 0.27$ can be detected.(95) If the multivariable model includes not only M and CE12 but also an additional covariate (e.g., fMRI variable) assuming that the R^2 attributable to CE12 and the third controlling covariate is less than 0.2, then the detectable size of β with $p < 0.05$ will be at least 0.25. Thus, the smallest effect size of $\alpha \cdot \beta$ will be 0.07.

Table 1. Sensitivity Analysis for Primary and Secondary Endpoints (n = 51 per group)

Endpoint	Outcome Measure	SD	Estimated effect size for p < 0.05 (d = 0.56)	Estimated effect size for p < 0.025 (d = 0.62)	Observed effect size (prelimina-ry data)
Primary	8-wk Δ Inhibition-Switching completion time	7.5	4.20	4.65	5.3
Co-Primary	8-wk Δ TEPS Consummatory Pleasure scores	0.54	0.27	0.30	0.31
	8-wk Δ delay discounting performance	1.79	1.00	1.11	1.17
	12-wk Δ EDI-3 Drive for Thinness scores	2.8	1.57	1.74	2.08
	12-wk Δ EDI-3 Body Dissatisfaction scores	3	1.68	1.86	3.92
Secondary	8-wk Δ in fMRI activation in the DLPFC and ACC in response to task switching	1.04	0.582	0.645	0.8
	8-wk Δ in fMRI activation in the VMPFC and striatum in response to reward receipt and during delay of reward	0.104	0.058	0.064	0.11
	12-wk Δ EDE Dietary Restraint subscale	1.54	0.862	0.955	2.08
	12-wk Δ caloric intake (4-d food diary)	560	313.6	347.2	439.8

Analytical plan

Hang Lee, Ph.D., Associate Professor of Medicine at Harvard Medical School and co-investigator on this study is our study statistician who has been instrumental in our study design and will analyze the data. **Aim 1:** Evaluates effects of 8-week estrogen replacement on Cognitive Flexibility, Initial Response to Reward and Delay. Longitudinal changes will first be compared across randomization groups stratified by age (14-20.9 and 21-25.9 years); if no differences are found across the age groups, we will combine the groups. Analyses of primary endpoints will include all randomized subjects (intent to treat). We will perform a paired samples t-test or Wilcoxon signed rank test (per data distribution) to determine if within-group changes differ from 0. The between-group difference of the within-subject mean longitudinal change will be tested with the independent samples t-test or Wilcoxon rank sum test (per data distribution for within-subject change). **Missing data:** We anticipate missing data will be independent of the unobserved true value (i.e., missing at random [MAR]). Based on the intention to treat (ITT) analysis principle, the difference in response over time between treatment arms will be assessed using a longitudinal mixed effects model utilizing completers and non-completers using all available observations, as treatment estimates will be consistent provided data are MAR. If needed, we will consider extending the mixed effects model analysis using multiple

imputation. We will also perform a completers' analysis for all endpoints, and adjust for covariates as described above. **Aim 2:** Evaluates effects of 12 weeks of estrogen replacement on (i) ED symptoms using the EDI-2 and EDE and (ii) total caloric intake (mean of data over four days using the 4-day food dairy). **Aim 3**: Tests whether improved Cognitive Flexibility, Initial Response to Reward, and Delay at 8 weeks are associated with ED symptom improvement from 0-12 weeks. Longitudinal Structural Equation Models (SEM) can be instrumental in confirmatory testing of a complex longitudinal pathway between variables. However, the problem of overfitting may be unavoidable with this approach. As an alternative, we will use general linear models for inference of the estrogen (each for Cognitive Flexibility, Initial Response to Reward, and Delay) in the following manner. We will first fit a univariable model to regress M on the circulating estradiol level at 12 weeks (CE12) to estimate the first effect (α) of CE12 on M. We will also estimate the total direct estrogen effect (τ) on ΔED. Then we will fit a multivariable model to regress ΔED on M and CE12 by which the partial effect (β) of M on ΔED can be estimated. α and τ are the signed square roots of univariable regression's R^2, and β is the signed square root of the multivariable regression's partial R^2. Next, we will estimate the Sobel's product coefficient ($\alpha \cdot \beta$) and its SE, and the statistical inference testing of whether $0 < |\alpha \cdot \beta| < \tau$. We will construct the 95% confidence interval for $\alpha \cdot \beta$ using a bootstrap sampling approach.(95)

VII. RISKS AND DISCOMFORTS

Screening and evaluation

The risks and discomforts are minimal. No discomfort is expected to be associated with the physical examination or the clinical interview other than potential stress of answering personal questions.

Collection of blood samples

Blood draws will be performed by trained staff who are certified to draw blood. Blood will be drawn with the subject in a reclining position and with sterile techniques. Thirty mL of blood will be drawn at each study visit. There is a minor risk of infection, bruising or syncope during a blood draw. Trained and certified phlebotomists will perform all blood draws with the participant in a reclining position to minimize syncopal episodes. We have had over 15 years of experience in collecting and storing these samples. For participants who elect to do so, we will provide topical ELA-Max cream (4% liposomal lidocaine) (to the areas of the skin where blood will be drawn from at each study visit 30-60 minutes prior to the blood collection.

Collection of stool samples

Collecting stool is not associated with any risks but may feel slightly uncomfortable. The subject may choose to not provide stool sample.

Collection of hair samples

Taking a hair sample may make the subject feel uncomfortable or concerned with their appearance. The subject may decide not to provide hair samples.

Study laboratory results and research imaging

Any incidental findings from research imaging or screening tests will be shared with adult study participants and parents of those <18 years old, as well as the participant's caregivers (after obtaining a release from the participant/parent/guardian to share this information with the caregiver). An incidental finding of pregnancy in a participant <18 years old will be handled per IRB guidelines. Specifically, if the minor participant agrees that the parents be informed, we will share this information with the parent/s. If she does not agree to our sharing the information with

the parent/s, the study physician may decide based on age, maturity, or associated medical condition to inform the parents anyway. For participants who are emancipated minors, parents will not be informed unless the participant permits this. Bone age evaluation involves minimal radiation (~0.2 μSv for entire study) - less than 1% of the background radiation received/person/year in the US.

Estrogen patch

Side effects associated with estrogen medications are minor and few when administered transdermally. The most common side effects include headaches and irritation at the patch site. Adverse reactions that have been reported with estrogen therapy in general include abnormal bleeding patterns, changes in nature and quantity of vaginal discharge, tenderness and enlargement of breasts, nausea, vomiting, abdominal pain, bloating, gall bladder disease, rashes, intolerance to contact lenses, migraines, dizziness, depression, abnormal movements, and changes in weight. These adverse effects are however rare and seen more commonly with estrogen taken orally rather than transdermally. The major complications include increased risk of venous thrombo-embolism, hypertension, lipid disorders, and increased risk of breast and endometrial cancer. These are more commonly seen in older women and in women with a family history of clotting disorders and cancer.

However, we plan on administering estrogen to ED-R/E participants who are hypogonadal and who are at an age when estrogen levels are steadily rising in healthy adolescents and young adults. Estrogen will be administered in physiologic replacement doses to mimic normal levels of estrogen at this age. These adolescents and young adults are thus different from postmenopausal women with low estrogen levels. Importantly, in our randomized controlled trial of transdermal estradiol (100 mcg) vs. placebo in adolescent girls with AN, adverse events possibly/probably/likely related to study drug did not differ in the estrogen replacement vs. placebo groups.(96) We have similar findings in our study of transdermal versus oral versus no estrogen in 14-25 year old amenorrheic athletes.(34)

Since continuous estrogen administration increases the risk of uterine cancer, we will administer cyclic progesterone as well. ED-R/E participants who receive transdermal estrogen will receive micronized progesterone (200 mg pills orally daily) in two 10 day courses during the 12 week study. We will monitor for side effects at the 8- and 12-week follow-up visits.

Subjects will be instructed to call us if they develop any side effects. All participants will be provided with a site-specific emergency 24-h phone number to call in the case of an immediate medical concern. A study physician will return the call as soon as possible.

Use of higher doses of estrogen before epiphyseal closure could accelerate growth plate closure resulting in an inability to reach the full potential for adult height. To avoid this possible side effect, we plan to administer replacement estrogen doses transdermally to girls with a bone age of 13.5 or more years, who have achieved more than 97.4% of their adult height.

Progesterone

Progesterone has been associated with clotting abnormalities, dizziness, nausea, abdominal pain, fatigue, headaches, insomnia, nervousness, sleepiness, and breast tenderness and secretion. Rare cases of breast cancer have been reported in women taking estrogen and progesterone. We will thus not include participants with a history of estrogen responsive breast cancer in a first degree relative unless they have consulted with their primary provider and the provider is unopposed to the subject’s participation. Rare instances of abnormal liver function have also been reported. Elderly post-menopausal females were diagnosed with a higher rate of dementia with prescribed combined estrogen/Provera. It is not known if the findings apply to a younger population. The doses of progesterone that will be given are small and are unlikely to

cause these side effects. Particularly micronized progesterone (a natural form of progesterone) is associated with fewer adverse effects compared to synthetic progestogens such as medroxyprogesterone acetate.(97) Possible side effects of medroxyprogesterone acetate/Provera are similar to those of micronized progesterone.

Functional MRI

Functional MRI will be performed at McLean Hospital using FDA-approved devices and pulse sequences. There are no known foreseeable risks associated with exposure to MRI, provided there are no metal or magnetic implants (i.e., vascular clamps, metal implants, or pacemakers). Participants who are pregnant or have metallic implants will be excluded from participation. A pregnancy test will be administered at the beginning of the MRI session. All potential subjects will be screened for the presence of any MRI contraindication prior to each MRI visit. Some subjects report claustrophobia or discomfort to noise during MRI scans. We will provide access to earplugs to minimize noise exposure. If a participant expresses any discomfort during the scan, the procedure will be aborted and will not be repeated without her full consent. If an adverse event occurs during the imaging protocols, appropriate medical intervention will be provided. Before the baseline imaging session, participants may choose to do a mock scan that provides a similar experience as in our 3T scanner (including exposure to sounds of the scanner through headphones). The mock scanner at the McLean Imaging Center is equipped with SimFx software (which simulates the ambient scanner sound and the active scanning noise) and MoTrak software (which tracks head movement through a sensor attached to the subject's head). The goal of this session will be to familiarize participants with the MRI environment, address potential anxious reactions to MRIs, and help train participants to minimize head motion during the real MRI session. We have chosen to make the mock scanner available for the proposed study given the age range of subjects and the fact that most of them are likely to be scanner-naive.

For MRI research screening purposes, subjects are required to have a structural brain MRI scan of clinical quality at least once per year at McLean Hospital that is reviewed by a board-certified radiologist or neurologist and a report is generated usually within 2-4 days. If the subject has not received a screening scan in the past year, one will be obtained for the present study. Subjects will not receive more than one screening scan within a given 12-month period unless a follow-up scan is recommended by the radiologist who reads the scan.
Abnormal reports are reviewed by the Clinical Director of the McLean Imaging Center (MIC), Dr. David Olson and forwarded to the Principal Investigator, in this case, Dr. Kumar. If an abnormality is present, the PI will coordinate the process of contacting the subject so that an appointment can be made to discuss the findings. The MIC Clinical Director may also assist with this process when requested and appropriate. If a follow-up scan is recommended by the radiologist, we will offer the subject a cost-free repeat scan (with contrast if indicated). A copy of both the clinical report and the MRI scan is offered to subjects to take to their primary care or other consulting physicians, at no cost.

Clinical interviews and questionnaires

Some questions in the clinical interviews or on the questionnaires may be difficult to answer or may make the subject uncomfortable. Subjects will have the choice of not answering a particular question or questionnaire.

Behavioral tasks

Behavioral tasks might be tiring. Subjects will be provided with regular breaks that they can take as needed. They will also have the choice of not performing a certain task/certain tasks, if

there is any associated discomfort. The computerized tasks require responding by pressing buttons on a keyboard, which could lead to physical strain and tiredness of the hand. In this case, the study staff will suggest a more comfortable position or to take a break. Additionally, in the G/NG, we are playing aversive sounds to participants that could be distressing. However, our colleagues at Harvard University's Nock Laboratory have conducted this identical task in over 100 participants with a psychiatric history (including those with current suicidal ideation or past suicide attempts) and over 100 healthy participants without incident or complaint. Therefore, although aversive, this task does not pose more than a minimal risk.

VIII. POTENTIAL BENEFITS

The study will generate important findings regarding neurobiological mechanisms responsible for the development and maintenance of ED-R/E with implications for (i) a novel treatment target and (ii) improved outcomes in this population. The results of this study will provide preliminary data for future studies that may provide specific recommendations for ED in the context of hormone replacement. There is no known direct benefit to the individual participating in the research study, and participants will be informed that participation in this study may not improve their physical or mental health.

IX. MONITORING AND QUALITY ASSURANCE

Monitoring by the principal investigators

Study initiation and subsequently study progress will be reviewed by study principal investigators Drs. Misra and Eddy once a week. The principal investigators will review any necessary study amendments, protocol deviations or violations, continuing review of the protocol, participant recruitment and retention, safety issues, and stopping rules. Unanticipated events, including adverse events, will be reviewed and managed based on institutional guidelines.

Monitoring by the data safety monitoring board (DSMB)

In addition, because study participants include adolescents and an intervention, an independent DSMB will be developed to provide initial review and ongoing study oversight. Progress will be reviewed twice annually (every 6 months) by the DSMB and safety concerns considered. The DSMB will evaluate study progress, participant recruitment, accrual and retention, participant risks versus benefits, and other factors as appropriate. DSMB review will also consider factors external to the study, such as scientific or therapeutic developments that may impact safety of participants or ethics or design of the study. The study will be subject to ongoing IRB oversight and monitoring. We will include the following personnel on the DSMB panel:

- A licensed Clinical Psychologist: Angela Fang
- A Pediatric Endocrinologist: Vibha Singhal
- A Biostatistician: Mark Vangel.

The DSMB will meet twice annually (every 6 months) either in person or by conference call. The first meeting will occur prior to study initiation to review study procedures and safety assessments (including the risks and benefits), and to review stopping/pause rules. Subsequent meetings will occur semi-annually.

Data to be reviewed at semi-annual meetings:

- Study progress, participant recruitment and retention
- Protocol amendments
- Protocol violations
- Study-related adverse events

- Scientific or therapeutic developments that may impact participant safety

An interim analysis is not planned. However, the DSMB will be able to ask for an interim analysis based on its review of the above if necessary. Proposed study hold or stopping points are:

- Severe study related adverse events that cannot be addressed satisfactorily
- Participant or guardian request
- Study clinician determination.

Data confidentiality and quality control

All information about participants will be treated confidentially. All identifiable data will be stored in a locked facility in the offices of Drs. Misra, Eddy, and Kumar or Pizzagalli. The following measures will be implemented for protection of materials and information: (i) assignment of unique study subject numbers to patients, (ii) use of these primary identifiers throughout the study, (iii) storage of information in a locked facility, and (iv) access limited to study personnel for these data. Any presentation or publication of the data from this research will not reveal the identities of the participants.

Data from this study will be submitted to the National Institutes of Health (NIH)/National Institutes of Mental Health (NIMH) Data Repositories. The NIH/NIMH Data Repositories are informatics systems run by the NIMH that allow researchers to review and use data from this study for their own research. During and after the study, we will send information about participants' health and behavior to the NIH/NIMH Data Repositories. However, before we send it, we will remove identifiable information and replace that information with a code number. Other researchers nationwide can then file an application with the NIMH to obtain access to study data for research purposes.

A DUA will be fully executed to share de-identified data with Madhusmita Misra, MD, MPH. Dr. Misra is the former PI of this protocol and has since departed MGH. Dr. Misra is currently the Chair of Pediatrics at the University of Virginia, VA, USA. Study data will be fully de-identified (stripped of all 18 HIPAA identifiers) prior to sending. Data will be shared via MGB DropBox and/or LabArchives.

A DUA will be fully executed to share de-identified data with Sonakshi Negi. Ms. Negi is a doctoral candidate at the University of Kansas, KS, USA. Study data will be fully de-identified (stripped of all 18 HIPAA identifiers) prior to sending. Data will be shared via MGB DropBox and/or LabArchives. We are collaborating with Ms. Negi because her training with restrictive eating disorders will allow us to further explore research questions directly related to our study aims.

Accuracy of data entry will be checked as follows: The majority of data collection will be carried out using REDCap™, which ensures fewer data entry errors. Data may be directly entered or entered by two separate individuals in REDCap™. REDCap™ automatically compares the two records and flags any data points that do not match, allowing a third person to select the correct entry or input a new one. Quality control will be applied to all stages of data handling. In order to ensure that the trial is performed and data are documented and reported in compliance with good clinical practice and regulatory requirements, written standard operating procedures will be implemented and shared with all participating sites.

Adverse events reporting

All adverse effects will be reported as per Partners HealthCare IRB requirements. All protocol related serious adverse events (SAEs) will be documented and reported as soon as we become aware of the event to the IRB at Partners HealthCare (per institutional guidelines) as well as to the DSMB. An event that is serious must be recorded on the case record and requires expeditious handling to comply with regulatory requirements. In the event that a participant becomes ill or injured as a direct result of participation in the research study, necessary medical

care will be made available. Potentially SAEs will be followed to resolution or stabilization and reported as SAEs if they become serious. A serious adverse event is one that meets any one of the following criteria:

- Fatal or life threatening
- Requires inpatient hospitalization
- Results in persistent or significant disability or incapacity
- Congenital anomaly
- Important medical event that may jeopardize the patient or require intervention to prevent serious outcome
- Cancer
- Overdose
- Results in development of drug dependency or drug abuse.

Reports of unanticipated problems involving risks to subjects or others are to be submitted through eIRB within 5 working days/7 calendar days of the date the investigator first becomes aware of the problem. **Reporting unanticipated problems that are adverse events:** Any unanticipated untoward or unfavorable medical occurrence, including abnormal signs (for example, abnormal physical exam or laboratory finding), symptoms, or diseases, that indicate that the research places subjects at increased risk of physical or psychological harm than previously known or recognized are to be submitted through Insight/eIRB as an Other Event, Adverse Event. The investigator must provide the following information in the report:

- A detailed description of the adverse event
- The basis for determining that the event is unexpected in nature, severity, or frequency
- The basis for determining that the event is related or possibly related to the research procedures
- The basis for determining that the research places subjects at an increased risk of harm (i.e., an SAE)
- Whether any changes to the research or other corrective actions are warranted.

The IRB does not accept sponsor IND/IDE safety reports describing adverse events that have occurred at sites other than those subject to this policy unless the report is of an incident that is:

- Serious
- Unexpected or unanticipated
- Related to the investigational drug/device
- Suggests that subjects are at an increased risk of harm and as such warrants changes in the research, consent process, or informing subjects. IND/IDE safety reports that warrant changes are to be submitted through Insight/eIRB as an Amendment.

Reporting unanticipated problems that are not adverse events: All other unanticipated incidents, experiences, information, outcomes, or other problems that indicate that the research places subjects at an increased risk of physical, psychological, economic, legal, or social harm than was previously known or recognized are to be submitted through Insight/eIRB as an Other Event. The investigator must provide the following information in the report:

- A detailed description of the unanticipated problem
- The basis for determining that the problem is unexpected
- The basis for determining that the problem indicates that the research places subjects at an increased risk of harm
- Whether any changes to the research or other corrective actions are warranted.

Plans for dealing with at risk individuals, suicidality, and referral for psychiatric treatment

After consent is obtained, a full medical and psychiatric history will be taken. All clinical assessors (e.g., licensed clinical psychologists) have extensive experience in clinical psychiatric assessment with currently and previously depressed populations and will make every effort to implement protocol procedures in a sensitive and supportive manner. Research interviews or experimental assessments will be interrupted if participants become distressed or object to answering questions. If, at any point during the study, a participant becomes suicidal, homicidal, manic or psychotic, she will be withdrawn from the study and appropriate care will be provided. Both testing sites, the Neuroendocrine Unit/EDCRP at MGH and the McLean Imaging Center (Directors: Drs. Lukas and Pizzagalli), are located on hospital grounds (i.e., MGH and McLean Hospital, respectively), which ensures immediate access to crisis services if needed. In the unlikely event that any of the instruments administered are highly distressing to the subject the clinical interviewer will make a further assessment of current suicidal intent and planning. If the interviewer judges that the participant is at imminent risk of self-harm, the interviewer will walk the person to the nearby McLean Emergency Services (Clinical Evaluation Center) or MGH Emergency Department (Acute Psychiatry Service). The principal investigators will also be informed of the situation. If the participant refuses to take part in the plan above, the investigators will access the 24-h McLean Crisis Mental Health Care service (800-333-0338) or the 24-h MGH Acute Psychiatry Services at MGH (617-726-2712) for immediate assistance and evaluation for possible hospitalization and inform the staff of the subject's location and risk level. If risk is judged to be serious but not imminent, the project protocol calls for the interviewer, in consultation with the principal investigators, to provide crisis phone numbers as well as relevant referrals for therapy, and to verify that the subject understands how best to access these resources.

X. REFERENCES

1. Galimberti E, Martoni RM, Cavallini MC, Erzegovesi S, Bellodi L. Motor inhibition and cognitive flexibility in eating disorder subtypes. Prog Neuropsychopharmacol Biol Psychiatry. 2012;36(2):307-12. doi: 10.1016/j.pnpbp.2011.10.017. PubMed PMID: 22079108.
2. McAnarney ER, Zarcone J, Singh P, Michels J, Welsh S, Litteer T, et al. Restrictive anorexia nervosa and set-shifting in adolescents: A biobehavioral interface. The Journal of Adolescent Health. 2011;49(1):99-101. doi: 10.1016/j.jadohealth.2010.11.259. PubMed PMID: PMC3286875.
3. Roberts ME, Tchanturia K, Stahl D, Southgate L, Treasure J. A systematic review and meta-analysis of set-shifting ability in eating disorders. Psychological medicine. 2007;37(8):1075-84. doi: 10.1017/S0033291707009877. PubMed PMID: 17261218.
4. Sato Y, Saito N, Utsumi A, Aizawa E, Shoji T, Izumiyama M, et al. Neural basis of impaired cognitive flexibility in patients with anorexia nervosa. PLOS ONE. 2013;8(5):e61108. doi: 10.1371/journal.pone.0061108.
5. Tchanturia K, Davies H, Roberts M, Harrison A, Nakazato M, Schmidt U, et al. Poor Cognitive Flexibility in Eating Disorders: Examining the Evidence using the Wisconsin Card Sorting Task. PLoS ONE. 2012;7(1):e28331. doi: 10.1371/journal.pone.0028331. PubMed PMID: PMC3257222.
6. Bischoff-Grethe A, McCurdy D, Grenesko-Stevens E, Irvine LE, Wagner A, Yau WY, et al. Altered brain response to reward and punishment in adolescents with anorexia nervosa. Psychiatry Res. 2013;214(3):331-40. doi: 10.1016/j.pscychresns.2013.07.004. PubMed PMID: 24148909; PubMed Central PMCID: PMCPMC3880152.
7. Eddy KT, Plessow F. The paradoxes of unexpected reward in anorexia nervosa. Am J Psychiatry. 2017;174(6):508-9. doi: 10.1176/appi.ajp.2017.17030333. PubMed PMID: 28565954.

8. Harrison A, O'Brien N, Lopez C, Treasure J. Sensitivity to reward and punishment in eating disorders. Psychiatry Res. 2010;177(1-2):1-11. doi: 10.1016/j.psychres.2009.06.010. PubMed PMID: 20381877.
9. Holsen LM, Lawson EA, Blum J, Ko E, Makris N, Fazeli PK, et al. Food motivation circuitry hypoactivation related to hedonic and nonhedonic aspects of hunger and satiety in women with active anorexia nervosa and weight-restored women with anorexia nervosa. Journal of psychiatry & neuroscience : JPN. 2012;37(5):322-32. doi: 10.1503/jpn.110156. PubMed PMID: 22498079; PubMed Central PMCID: PMC3447131.
10. Wagner A, Aizenstein H, Venkatraman VK, Fudge J, May JC, Mazurkewicz L, et al. Altered reward processing in women recovered from anorexia nervosa. Am J Psychiatry. 2007;164(12):1842-9. doi: 10.1176/appi.ajp.2007.07040575. PubMed PMID: 18056239.
11. Decker JH, Figner B, Steinglass JE. On weight and waiting: Delay discounting in anorexia nervosa pretreatment and posttreatment. Biol Psychiatry. 2015;78(9):606-14. doi: 10.1016/j.biopsych.2014.12.016. PubMed PMID: 25641636; PubMed Central PMCID: PMCPMC4478277.
12. Steinglass JE, Figner B, Berkowitz S, Simpson HB, Weber EU, Walsh BT. Increased capacity to delay reward in anorexia nervosa. J Int Neuropsychol Soc. 2012;18(4):773-80. Epub 2012/05/18. doi: 10.1017/S1355617712000446
S1355617712000446 [pii]. PubMed PMID: 22591835; PubMed Central PMCID: PMC3638253.
13. Steinglass JE, Lempert KM, Choo TH, Kimeldorf MB, Wall M, Walsh BT, et al. Temporal discounting across three psychiatric disorders: Anorexia nervosa, obsessive compulsive disorder, and social anxiety disorder. Depress Anxiety. 2017;34(5):463-70. Epub 2016/12/24. doi: 10.1002/da.22586. PubMed PMID: 28009473.
14. Wu M, Brockmeyer T, Hartmann M, Skunde M, Herzog W, 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;61:177-96. doi: 10.1016/j.neubiorev.2015.11.017. PubMed PMID: 26698021.
15. Treasure J, Schmidt U. The cognitive-interpersonal maintenance model of anorexia nervosa revisited: A summary of the evidence for cognitive, socio-emotional and interpersonal predisposing and perpetuating factors. J Eat Disord. 2013;1:13. doi: 10.1186/2050-2974-1-13. PubMed PMID: 24999394; PubMed Central PMCID: PMC4081714.
16. Martini MG, Solmi F, Krug I, Karwautz A, Wagner G, Fernandez-Aranda F, et al. Associations between eating disorder diagnoses, behaviors, and menstrual dysfunction in a clinical sample. Arch Womens Ment Health. 2016;19(3):553-7. doi: 10.1007/s00737-015-0576-2. PubMed PMID: 26399871.
17. Poyastro Pinheiro A, Thornton LM, Plotonicov KH, Tozzi F, Klump KL, Berrettini WH, et al. Patterns of menstrual disturbance in eating disorders. Int J Eat Disord. 2007;40(5):424-34. doi: 10.1002/eat.20388. PubMed PMID: 17497704.
18. Baskaran C, Cunningham B, Plessow F, Singhal V, Woolley R, Ackerman KE, et al. Estrogen replacement improves verbal memory and executive control in oligomenorrheic/amenorrheic athletes in a randomized controlled trial. J Clin Psychiatry. 2017;78(5):e490-e7. doi: 10.4088/JCP.15m10544. PubMed PMID: 28297591.
19. Baskaran C, Plessow F, Ackerman KE, Singhal V, Eddy KT, Misra M. A cross-sectional analysis of verbal memory and executive control across athletes with varying menstrual status and non-athletes. Psychiatry Res. 2017;258:605-6. doi: 10.1016/j.psychres.2016.12.054. PubMed PMID: 28159335.
20. Colzato LS, Hertsig G, van den Wildenberg WP, Hommel B. Estrogen modulates inhibitory control in healthy human females: Evidence from the stop-signal paradigm. Neuroscience. 2010;167(3):709-15. doi: 10.1016/j.neuroscience.2010.02.029. PubMed PMID: 20219635.

21. Colzato LS, Pratt J, Hommel B. Estrogen modulates inhibition of return in healthy human females. Neuropsychologia. 2012;50(1):98-103. Epub 2011/11/19. doi: 10.1016/j.neuropsychologia.2011.11.003
S0028-3932(11)00503-3 [pii]. PubMed PMID: 22093437.
22. Girard R, Metereau E, Thomas J, Pugeat M, Qu C, Dreher JC. Hormone therapy at early post-menopause increases cognitive control-related prefrontal activity. Sci Rep. 2017;7:44917. doi: 10.1038/srep44917. PubMed PMID: 28322310; PubMed Central PMCID: PMCPMC5359606.
23. Bayer J, Bandurski P, Sommer T. Differential modulation of activity related to the anticipation of monetary gains and losses across the menstrual cycle. Eur J Neurosci. 2013;38(10):3519-26. Epub 2013/08/29. doi: 10.1111/ejn.12347. PubMed PMID: 23981052.
24. 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;74:186-93. Epub 2015/06/21. doi: 10.1016/j.yhbeh.2015.06.001
S0018-506X(15)00104-X [pii]. PubMed PMID: 26092059.
25. Diekhof EK, Ratnayake M. Menstrual cycle phase modulates reward sensitivity and performance monitoring in young women: Preliminary fMRI evidence. Neuropsychologia. 2016;84:70-80. Epub 2015/10/17. doi: 10.1016/j.neuropsychologia.2015.10.016
S0028-3932(15)30193-7 [pii]. PubMed PMID: 26471712.
26. Dreher JC, Schmidt PJ, Kohn P, Furman D, Rubinow D, Berman KF. Menstrual cycle phase modulates reward-related neural function in women. Proc Natl Acad Sci U S A. 2007;104(7):2465-70. Epub 2007/02/03. doi: 0605569104 [pii]
10.1073/pnas.0605569104. PubMed PMID: 17267613; PubMed Central PMCID: PMC1892961.
27. Macoveanu J, Henningsson S, Pinborg A, Jensen P, Knudsen GM, Frokjaer VG, et al. Sex-steroid hormone manipulation reduces brain response to reward. Neuropsychopharmacology. 2016;41(4):1057-65. Epub 2015/08/08. doi: 10.1038/npp.2015.236
npp2015236 [pii]. PubMed PMID: 26245498; PubMed Central PMCID: PMC4748430.
28. Smith CT, Sierra Y, Oppler SH, Boettiger CA. Ovarian cycle effects on immediate reward selection bias in humans: A role for estradiol. J Neurosci. 2014;34(16):5468-76. Epub 2014/04/18. doi: 10.1523/JNEUROSCI.0014-14.2014
34/16/5468 [pii]. PubMed PMID: 24741037; PubMed Central PMCID: PMC3988406.
29. Thomas J, Metereau E, Dechaud H, Pugeat M, Dreher JC. Hormonal treatment increases the response of the reward system at the menopause transition: A counterbalanced randomized placebo-controlled fMRI study. Psychoneuroendocrinology. 2014;50:167-80. doi: 10.1016/j.psyneuen.2014.08.012. PubMed PMID: 25222702.
30. Keel PK, Brown TA. Update on course and outcome in eating disorders. Int J Eat Disord. 2010;43(3):195-204. doi: 10.1002/eat.20810. PubMed PMID: 20186717.
31. Roberto CA, Steinglass J, Mayer LE, Attia E, Walsh BT. The clinical significance of amenorrhea as a diagnostic criterion for anorexia nervosa. Int J Eat Disord. 2008;41(6):559-63. doi: 10.1002/eat.20534. PubMed PMID: 18454485.
32. Plessow F, Baskaran C, Becker KR, Mancuso CJ, Izquierdo AM, Coniglio KA, et al. Estrogen status is associated with delay of gratification in girls and young women with low-weight eating disorders. 99th Annual Meeting of the Endocrine Society; Orlando, FL2017.
33. Misra M, Katzman DK, Estella NM, Eddy KT, Weigel T, Goldstein MA, et al. 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. J Clin Psychiatry. 2013;74(8):e765-71. doi: 10.4088/JCP.13m08365. PubMed PMID: 24021517; PubMed Central PMCID: PMCPMC4017248.
34. Plessow F, Singhal V, Toth AT, Micali N, Eddy KT, Misra M. Estrogen administration improves the trajectory of eating disorder pathology in oligo-amenorrheic athletes: A

randomized controlled trial. Psychoneuroendocrinology. 2018. doi: 10.1016/j.psyneuen.2018.11.013.
35. Treatment of postmenopausal woman: Basic and clinical aspects. Burlington, MA: Academic Press; 2007.
36. Abbate-Daga G, Piero A, Gramaglia C, Gandione M, Fassino S. An attempt to understand the paradox of anorexia nervosa without drive for thinness. Psychiatry Res. 2007;149(1-3):215-21. doi: 10.1016/j.psychres.2005.10.017. PubMed PMID: 17150258.
37. Ramacciotti CE, Dell'Osso L, Paoli RA, Ciapparelli A, Coli E, Kaplan AS, et al. Characteristics of eating disorder patients without a drive for thinness. Int J Eat Disord. 2002;32(2):206-12. doi: 10.1002/eat.10067. PubMed PMID: 12210663.
38. Vervaet M, Van Heeringen C, Audenaert K. Is drive for thinness in anorectic patients associated with personality characteristics? Eur Eat Disord Rev. 2004;12(6):375-9.
39. Klein DA, Poth MA. Amenorrhea: an approach to diagnosis and management. Am Fam Physician. 2013;87(11):781-8. Epub 2013/08/14. doi: d10456 [pii]. PubMed PMID: 23939500.
40. Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, Flegal KM, Guo SS, Wei R, et al. CDC growth charts: United States. Adv Data. 2000(314):1-27. Epub 2001/02/24. PubMed PMID: 11183293.
41. Chapman JP, Chapman LJ. Handedness of hypothetically psychosis-prone subjects. J Abnorm Psychol. 1987;96(2):89-93. Epub 1987/05/01. PubMed PMID: 3584671.
42. Papagianni V, Deligeoroglou E, Makrakis E, Botsis D, Creatsas G. Response to hormonal treatment of young females with primary or very premature ovarian failure. Gynecol Endocrinol. 2011;27(5):291-9. Epub 2011/03/09. doi: 10.3109/09513591003632274. PubMed PMID: 21381875.
43. Trolle C, Hjerrild B, Cleemann L, Mortensen KH, Gravholt CH. Sex hormone replacement in Turner syndrome. Endocrine. 2012;41(2):200-19. Epub 2011/12/08. doi: 10.1007/s12020-011-9569-8. PubMed PMID: 22147393.
44. Martinez G, Copen CE, Abma JC. Teenagers in the United States: Sexual activity, contraceptive use, and childbearing, 2006-2010 national survey of family growth. Vital Health Stat 23. 2011(31):1-35. Epub 2012/01/20. PubMed PMID: 22256688.
45. De Souza MJ, Nattiv A, Joy E, Misra M, Williams NI, Mallinson RJ, et al. 2014 Female Athlete Triad Coalition Consensus Statement on Treatment and Return to Play of the Female Athlete Triad: 1st International Conference held in San Francisco, California, May 2012 and 2nd International Conference held in Indianapolis, Indiana, May 2013. Br J Sports Med. 2014;48(4):289. Epub 2014/01/28. doi: 10.1136/bjsports-2013-093218 48/4/289 [pii]. PubMed PMID: 24463911.
46. Pardo J, Uriarte M, Console GM, Reggiani PC, Outeiro TF, Morel GR, et al. Insulin-like growth factor-i gene therapy increases hippocampal neurogenesis, astrocyte branching and improves spatial memory in female aging rats. Eur J Neurosci. 2016. Epub 2016/05/18. doi: 10.1111/ejn.13278. PubMed PMID: 27188415.
47. Vidal JS, Hanon O, Funalot B, Brunel N, Viollet C, Rigaud AS, et al. Low Serum Insulin-Like Growth Factor-I Predicts Cognitive Decline in Alzheimer's Disease. J Alzheimers Dis. 2016;52(2):641-9. Epub 2016/04/01. doi: 10.3233/JAD-151162 JAD151162 [pii]. PubMed PMID: 27031487.
48. Simon JA, Laliberte F, Duh MS, Pilon D, Kahler KH, Nyirady J, et al. Venous thromboembolism and cardiovascular disease complications in menopausal women using transdermal versus oral estrogen therapy. Menopause. 2016;23(6):600-10. Epub 2016/03/10. doi: 10.1097/GME.0000000000000590. PubMed PMID: 26953655.
49. Nabhan ZM, Dimeglio LA, Qi R, Perkins SM, Eugster EA. Conjugated oral versus transdermal estrogen replacement in girls with Turner syndrome: a pilot comparative study. J Clin Endocrinol Metab. 2009;94(6):2009-14. Epub 2009/03/26. doi: 10.1210/jc.2008-2123 jc.2008-2123 [pii]. PubMed PMID: 19318455.

50. Popat VB, Calis KA, Kalantaridou SN, Vanderhoof VH, Koziol D, Troendle JF, et al. Bone mineral density in young women with primary ovarian insufficiency: results of a three-year randomized controlled trial of physiological transdermal estradiol and testosterone replacement. J Clin Endocrinol Metab. 2014;99(9):3418-26. Epub 2014/06/07. doi: 10.1210/jc.2013-4145. PubMed PMID: 24905063; PubMed Central PMCID: PMC4154086.
51. Grady D, Gebretsadik T, Kerlikowske K, Ernster V, Petitti D. Hormone replacement therapy and endometrial cancer risk: a meta-analysis. Obstet Gynecol. 1995;85(2):304-13. Epub 1995/02/01. doi: 0029-7844(94)00383-O [pii]
10.1016/0029-7844(94)00383-O. PubMed PMID: 7824251.
52. Kromrey SA, Czoty PW, Nader MA. Relationship between estradiol and progesterone concentrations and cognitive performance in normally cycling female cynomolgus monkeys. Horm Behav. 2015;72:12-9. Epub 2015/04/30. doi: 10.1016/j.yhbeh.2015.04.017 S0018-506X(15)00068-9 [pii]. PubMed PMID: 25921587; PubMed Central PMCID: PMC4466063.
53. Greulich W, Pyle S. Radiographic atlas of skeletal development of the hand and wrist,. 2 ed. Stanford: Stanford University Press; 1959.
54. Stalder T, Kirschbaum C. Analysis of cortisol in hair--state of the art and future directions. Brain, behavior, and immunity. 2012;26(7):1019-29. Epub 2012/03/01. doi: 10.1016/j.bbi.2012.02.002. PubMed PMID: 22366690.
55. Wester VL, van Rossum EF. Clinical applications of cortisol measurements in hair. European journal of endocrinology. 2015;173(4):M1-10. Epub 2015/05/01. doi: 10.1530/eje-15-0313. PubMed PMID: 25924811.
56. Feinberg DA, Moeller S, Smith SM, Auerbach E, Ramanna S, Gunther M, et al. Multiplexed echo planar imaging for sub-second whole brain FMRI and fast diffusion imaging. PLoS One. 2010;5(12):e15710. Epub 2010/12/29. doi: 10.1371/journal.pone.0015710. PubMed PMID: 21187930; PubMed Central PMCID: PMC3004955.
57. Xu J, Moeller S, Auerbach EJ, Strupp J, Smith SM, Feinberg DA, et al. Evaluation of slice accelerations using multiband echo planar imaging at 3 T. Neuroimage. 2013;83:991-1001. Epub 2013/08/01. doi: 10.1016/j.neuroimage.2013.07.055 S1053-8119(13)00824-0 [pii]. PubMed PMID: 23899722; PubMed Central PMCID: PMC3815955.
58. Andersson JL, Skare S, Ashburner J. How to correct susceptibility distortions in spin-echo echo-planar images: application to diffusion tensor imaging. Neuroimage. 2003;20(2):870-88. doi: 10.1016/S1053-8119(03)00336-7. PubMed PMID: 14568458.
59. Girard R, Météreau E, Thomas J, Pugeat M, Qu C, Dreher JC. Hormone therapy at early post-menopause increases cognitive control-related prefrontal activity. Sci Rep. 2017. doi: 10.1038/srep44917.
60. Reuter M, Schmansky NJ, Rosas HD, Fischl B. Within-subject template estimation for unbiased longitudinal image analysis. Neuroimage. 2012;61(4):1402-18. Epub 2012/03/21. doi: 10.1016/j.neuroimage.2012.02.084. PubMed PMID: 22430496; PubMed Central PMCID: PMC3389460.
61. Huang CM, Lee SH, Hsiao IT, Kuan WC, Wai YY, Ko HJ, et al. Study-specific EPI template improves group analysis in functional MRI of young and older adults. Journal of neuroscience methods. 2010;189(2):257-66. Epub 2010/03/30. doi: 10.1016/j.jneumeth.2010.03.021. PubMed PMID: 20346979.
62. Avants BB, Epstein CL, Grossman M, Gee JC. Symmetric diffeomorphic image registration with cross-correlation: evaluating automated labeling of elderly and neurodegenerative brain. Medical image analysis. 2008;12(1):26-41. Epub 2007/07/31. doi: 10.1016/j.media.2007.06.004. PubMed PMID: 17659998; PubMed Central PMCID: PMC2276735.

63. Klein A, Andersson J, Ardekani BA, Ashburner J, Avants B, Chiang MC, et al. Evaluation of 14 nonlinear deformation algorithms applied to human brain MRI registration. Neuroimage. 2009;46(3):786-802. Epub 2009/02/07. doi: 10.1016/j.neuroimage.2008.12.037. PubMed PMID: 19195496; PubMed Central PMCID: PMC2747506.
64. Gennatas ED, Avants BB, Wolf DH, Satterthwaite TD, Ruparel K, Ciric R, et al. Age-Related Effects and Sex Differences in Gray Matter Density, Volume, Mass, and Cortical Thickness from Childhood to Young Adulthood. J Neurosci. 2017;37(20):5065-73. doi: 10.1523/JNEUROSCI.3550-16.2017. PubMed PMID: 28432144; PubMed Central PMCID: PMCPMC5444192.
65. Admon R, Nickerson LD, Dillon DG, Holmes AJ, Bogdan R, Kumar P, et al. Dissociable cortico-striatal connectivity abnormalities in major depression in response to monetary gains and penalties. Psychol Med. 2015;45(1):121-31. doi: 10.1017/S0033291714001123. PubMed PMID: 25055809; PubMed Central PMCID: PMCPMC4233014.
66. Dillon DG, Holmes AJ, Jahn AL, Bogdan R, Wald LL, Pizzagalli DA. Dissociation of neural regions associated with anticipatory versus consummatory phases of incentive processing. Psychophysiology. 2008;45(1):36-49. doi: 10.1111/j.1469-8986.2007.00594.x. PubMed PMID: 17850241; PubMed Central PMCID: PMCPMC2156200.
67. Pizzagalli DA, Holmes AJ, Dillon DG, Goetz EL, Birk JL, Bogdan R, et al. Reduced caudate and nucleus accumbens response to rewards in unmedicated individuals with major depressive disorder. Am J Psychiatry. 2009;166(6):702-10. doi: 10.1176/appi.ajp.2008.08081201. PubMed PMID: 19411368; PubMed Central PMCID: PMCPMC2735451.
68. Greve DN, Fischl B. Accurate and robust brain image alignment using boundary-based registration. Neuroimage. 2009;48(1):63-72. Epub 2009/07/04. doi: 10.1016/j.neuroimage.2009.06.060. PubMed PMID: 19573611; PubMed Central PMCID: PMC2733527.
69. Admon R, Holsen LM, Aizley H, Remington A, Whitfield-Gabrieli S, Goldstein JM, et al. Striatal hypersensitivity during stress in remitted individuals with recurrent depression. Biol Psychiatry. 2015;78(1):67-76. doi: 10.1016/j.biopsych.2014.09.019. PubMed PMID: 25483401; PubMed Central PMCID: PMCPMC4383718.
70. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): The development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry. 1998;59 Suppl 20:22-33;quiz 4-57. PubMed PMID: 9881538.
71. Black CM, Wilson GT. Assessment of eating disorders: Interview versus questionnaire. Int J Eat Disord. 1996;20(1):43-50. Epub 1996/07/01. doi: 10.1002/(SICI)1098-108X(199607)20:1<43::AID-EAT5>3.0.CO;2-4 [pii] 10.1002/(SICI)1098-108X(199607)20:1<43::AID-EAT5>3.0.CO;2-4. PubMed PMID: 8807351.
72. Byrne SM, Allen KL, Lampard AM, Dove ER, Fursland A. The factor structure of the eating disorder examination in clinical and community samples. Int J Eat Disord. 2010;43(3):260-5. Epub 2009/04/08. doi: 10.1002/eat.20681. PubMed PMID: 19350647.
73. Garner DM. Eating Disorder Inventory-3: Professional Manual. Lutz, FL: Psychological Assessment Resources; 2004.
74. Taranis L, Touyz S, Meyer C. Disordered eating and exercise: Development and preliminary validation of the compulsive exercise test (CET). Eur Eat Disord Rev. 2011;19(3):256-68. doi: 10.1002/erv.1108. PubMed PMID: 21584918.
75. Gard DE, Gard MG, Kring AM, John OP. Anticipatory and consummatory components of the experience of pleasure: A scale development study. J Res Personality. 2006;40:1086-102. doi: 10.1016/j.jrp.2005.11.001.
76. Beck AT, Steer, R.A., & Brown, G.K. . Manual for the Beck Depression Inventory-II. San Antonio, TX: Psychological Corporation; 1996.

77. Spielberger CD, Gorsuch RL, Lushene R, Vagg PR, Jacobs GA. Manual for the Stait-Trait Anxiety Scale: Consulting Psychologists; 1983.
78. Watson D, Clark LA, Tellegen A. Development and validation of brief measures of positive and negative affect: the PANAS scales. Journal of personality and social psychology. 1988;54(6):1063-70. PubMed PMID: 3397865.
79. Franken IH, Rassin E, Muris P. The assessment of anhedonia in clinical and non-clinical populations: further validation of the Snaith-Hamilton Pleasure Scale (SHAPS). Journal of affective disorders. 2007;99(1-3):83-9. doi: 10.1016/j.jad.2006.08.020. PubMed PMID: 16996138.
80. Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity. 2000;24(1):38-48. PubMed PMID: 10702749.
81. Forbush KT, Wildes JE, Pollack LO, Dunbar D, Luo J, Patterson K, et al. Development and validation of the Eating Pathology Symptoms Inventory (EPSI). Psychological assessment. 2013;25(3):859-78. doi: 10.1037/a0032639. PubMed PMID: 23815116.
82. Foa EB, Kozak MJ, Salkovskis PM, Coles ME, Amir N. The validation of a new obsessive–compulsive disorder scale: The Obsessive–Compulsive Inventory. Psychological assessment. 1998;10(3):206-14. doi: 10.1037/1040-3590.10.3.206.
83. Delis DC, Kaplan E, Kramer JH. Delis–Kaplan Executive Function System (D-KEFS): Examiner's manual. San Antonio, TX: The Psychological Corporation; 2001.
84. Ahern AL, Bennett KM, Hetherington MM. Internalization of the ultra-thin ideal: positive implicit associations with underweight fashion models are associated with drive for thinness in young women. Eating disorders. 2008;16(4):294-307. doi: 10.1080/10640260802115852. PubMed PMID: 18568920.
85. Millner AJ, den Ouden, H. E. M., Gershman, S. J., Glenn, C. R., Kearns, J. C., Bornstein, A. M., Marx,, B. P. K, T. M., & Nock, M. K. . Suicidal Thoughts and Behaviors Are Associated With an Increased Decision-Making Bias for Active Responses to Escape Aversive States. Journal of Abnormal Psychology. 2019;128(2):106-18. doi: http://dx.doi.org/10.1037/abn0000395.
86. Kirby KN. One-year temporal stability of delay-discount rates. Psychonomic Bulletin & Review. 2009;16(3):457-62. doi: 10.3758/PBR.16.3.457. PubMed PMID: 19451368.
87. Kirby KN, Marakovic NN. Delay-discounting probabilistic rewards: Rates decrease as amounts increase. Psychonomic Bulletin & Review. 1996;3(1):100-4. doi: 10.3758/BF03210748. PubMed PMID: 24214810.
88. Wechsler. PCD, editor. Wechsler abbreviated scale of intelligence San Antonio, Tex. : Psychological Corporation, ©2011; 2011.
89. Sawaya AL, Tucker K, Tsay R, Willett W, Saltzman E, Dallal GE, et al. Evaluation of four methods for determining energy intake in young and older women: Comparison with doubly labeled water measurements of total energy expenditure. Am J Clin Nutr. 1996;63(4):491-9. PubMed PMID: 8599311.
90. Taylor C, Lamparello B, Kruczek K, Anderson EJ, Hubbard J, Misra M. Validation of a food frequency questionnaire for determining calcium and vitamin D intake by adolescent girls with anorexia nervosa. J Am Diet Assoc. 2009;109(3):479-85, 85 e1-3. PubMed PMID: 19248866.
91. Schakel SF. Maintaining a nutrient database in a changing marketplace: Keeping pace with changing food products - A research perspective. J Food Comp and Anal. 2001;14:315-22.
92. Schakel SF, Buzzard IM, Gebhardt SE. Procedures for estimating nutrient values for food composition databases. J Food Comp and Anal. 1997;10:102-14.

93. Schakel SF, Sievert YA, Buzzard IM. Sources of data for developing and maintaining a nutrient database. J Am Diet Assoc. 1988;88(10):1268-71. Epub 1988/10/01. PubMed PMID: 3171020.
94. Cook RJ, Farewell VT. Multiplicity Considerations in the Design and Analysis of Clinical Trials. Journal of the Royal Statistical Society Series A (Statistics in Society). 1996;159(1):93-110.
95. Fritz MS, Mackinnon DP. Required sample size to detect the mediated effect. Psychol Sci. 2007;18(3):233-9. doi: 10.1111/j.1467-9280.2007.01882.x. PubMed PMID: 17444920; PubMed Central PMCID: PMCPMC2843527.
96. Misra M, Katzman D, Miller KK, Mendes N, Snelgrove D, Russell M, et al. Physiologic estrogen replacement increases bone density in adolescent girls with anorexia nervosa. J Bone Miner Res. 2011;26(10):2430-8. doi: 10.1002/jbmr.447. PubMed PMID: 21698665; PubMed Central PMCID: PMCPMC3304439.
97. Cummings JA, Brizendine L. Comparison of physical and emotional side effects of progesterone or medroxyprogesterone in early postmenopausal women. Menopause. 2002;9(4):253-63. Epub 2002/06/26. PubMed PMID: 12082361.
98. Bagby, RM, Parker, JD, and Taylor, GJ. (1994). The twenty-item Toronto Alexithymia Scale-I. Item selection and cross-validation of the factor structure. J. Psychosom. Res. 38, 23–32. doi: 10.1016/0022-3999(94)90005-1. PubMed PMID: 8126688.
99. Eddy KT, Murray HB, Thomas, JJ. (2015). Longitudinal Interval Follow-Up Evaluation Eating and Feeding Disorders Version. (LIFE-EAT 3). Adapted from Keller et al., 1987.

Sub Study References

1 Vural P, Akgul C, Canbaz M. Effects of hormone replacement therapy on plasma pro-inflammatory and anti-inflammatory cytokines and some bone turnover markers in postmenopausal women. *Pharmacol Res* 2006;**54**:298–302.
2 Malutan AM, Dan M, Nicolae C, Carmen M. Proinflammatory and anti-inflammatory cytokine changes related to menopause. *Prz Menopauzalny* 2014;**13**:162–8.
3 Brett M, Baxendale S. Motherhood and memory: a review. *Psychoneuroendocrinology* 2001;**26**:339–62.
4 Legacki EL, Bal BA, Jo Corbin C, Loux SC, Scoggin KE, Stanley SD, *et al.* Equine fetal adrenal, gonadal and placental steroidogenesis. *Reproduction* 2019:X1. https://doi.org/10.1530/rep-17-0239e.
5 Galea LAM, Qiu W, Duarte-Guterman P. Beyond sex differences: short and long-term implications of motherhood on women's health. *Curr Opin Physiol* 2018;**6**:82–8.
6 Duarte-Guterman P, Leuner B, Galea LAM. The long and short term effects of motherhood on the brain. *Front Neuroendocrinol* 2019;**53**:100740.
7 Hoekzema E, Barba-Müller E, Pozzobon C, Picado M, Lucco F, García-García D, *et al.* Pregnancy leads to long-lasting changes in human brain structure. *Nat Neurosci* 2017;**20**:287–96.
8 Taylor CM, Pritschet L, Yu S, Jacobs EG. Applying a Women's Health Lens to the Study of the Aging Brain. *Front Hum Neurosci* 2019;**13**:224.
9 Luders E, Kurth F, Gingnell M, Engman J, Yong E-L, Poromaa IS, *et al.* From baby brain to mommy brain: Widespread gray matter gain after giving birth. *Cortex* 2020;**126**:334–42.