Document Title:	Study Protocol
Protocol Title:	Menopausal Sleep Fragmentation: Impact on Body Fat Gain Biomarkers in Women
Protocol #:	MGB Human Research Committee #2016P002821
NCT:	03047330
Document date:	January 3, 2024

PARTNERS HUMAN RESEARCH COMMITTEE
PROTOCOL SUMMARY

Answer all questions accurately and completely in order to provide the PHRC with the relevant information to assess the risk-benefit ratio for the study. Do not leave sections blank.

PRINCIPAL/OVERALL INVESTIGATOR

Hadine Joffe, MD, MSc

PROTOCOL TITLE

Menopausal Sleep Fragmentation: Impact on Body Fat Gain Biomarkers in Women

FUNDING

National Institute of Health – National Institute on Aging

VERSION DATE

1/3/2024

SPECIFIC AIMS

Concisely state the objectives of the study and the hypothesis being tested.

Aim #1: To determine the impact of menopause-related sleep fragmentation on metabolic and behavioral biomarkers of body fat gain using an experimental sleep disruption paradigm to fragment sleep in healthy female volunteers

Hypothesis 1a: Experimental sleep fragmentation will result in an adverse adipokine profile as a metabolic biomarker for body fat gain

Hypothesis 1b: Experimental sleep fragmentation will result in increased hunger and caloric intake

Hypothesis 1c: Sleep disruption due to hot flashes will result in an adverse adipokine profile and increased hunger and caloric intake

Aim #2: To examine if hot flashes are associated with metabolic and behavioral biomarkers of body fat gain using an experimental hormone suppression paradigm in healthy women

Hypothesis 2: Hot flash frequency will correlate with a worse adipokine profile and increased hunger and caloric intake

Aim #3: To determine if estradiol withdrawal adversely affects metabolic and behavioral biomarkers of body fat gain using an experimental hormone suppression paradigm in healthy women

Hypothesis 3: Experimental withdrawal of estradiol will correlate with a worse adipokine profile and increased hunger and caloric intake

Aims 1–3 Exploratory Hypotheses:

1) Experimental sleep fragmentation mimicking menopause-related sleep disruption will reduce resting energy expenditure. 2) Baseline levels of total body fat and visceral adiposity will influence

the metabolic and behavioral response to sleep fragmentation, hot flashes, and estradiol withdrawal. 3) Negative mood state will correlate independently with an adverse adipokine profile and increased hunger and caloric intake.

BACKGROUND AND SIGNIFICANCE

Provide a brief paragraph summarizing prior experience important for understanding the proposed study and procedures.

Obesity is highly prevalent in midlife and older women. Over 50% of women gain body fat during the menopause transition, unrelated to chronological aging, increasing the likelihood of cardio-metabolic diseases after menopause. While these body composition changes are attributed to E2 withdrawal, other menopause-related factors likely contribute to adiposity because: 1) body fat gains are not uniform despite universal progression to hypo-estrogenism, and 2) body fat accrues during the MT, when E2 continues to be produced intermittently. Sleep fragmentation related to HF occurs in almost half of midlife women and may be an important factor driving risk for body fat gain, but its impact on metabolic and behavioral biomarkers of body fat gain has not been investigated. This sleep fragmentation results commonly from HF, which occur in 2/3 of midlife women. In other populations, individuals who sleep less are heavier and have adverse adipokine profiles. Experimental studies restricting TST show that sleep reduction lowers leptin, and increases ghrelin, appetitive drive, and caloric intake, key biomarkers of adiposity, with some sex differences in effects. However, TST is not reduced in HF-related sleep fragmentation, and metabolic effects of sleep fragmentation have received limited investigation in humans, although animal studies show that sleep fragmentation adversely effects adipokines and eating behaviors. The goal of the current proposal is to determine the impact of HF-related sleep fragmentation, as well as that of HF and E2 withdrawal, on body fat gain in women. ***Given the prevalence of HF and associated sleep disruption, identifying the impact of these core menopause symptoms on body fat gain will inform health promotion strategies to prevent gains in body fat in midlife women, thereby reducing cardio-metabolic diseases in aging women.***

RESEARCH DESIGN AND METHODS

Briefly describe study design and anticipated enrollment, i.e., number of subjects to be enrolled by researchers study-wide and by Partners researchers. Provide a brief summary of the eligibility criteria (for example, age range, gender, medical condition). Include any local site restrictions, for example, "Enrollment at Partners will be limited to adults although the sponsor's protocol is open to both children and adults."

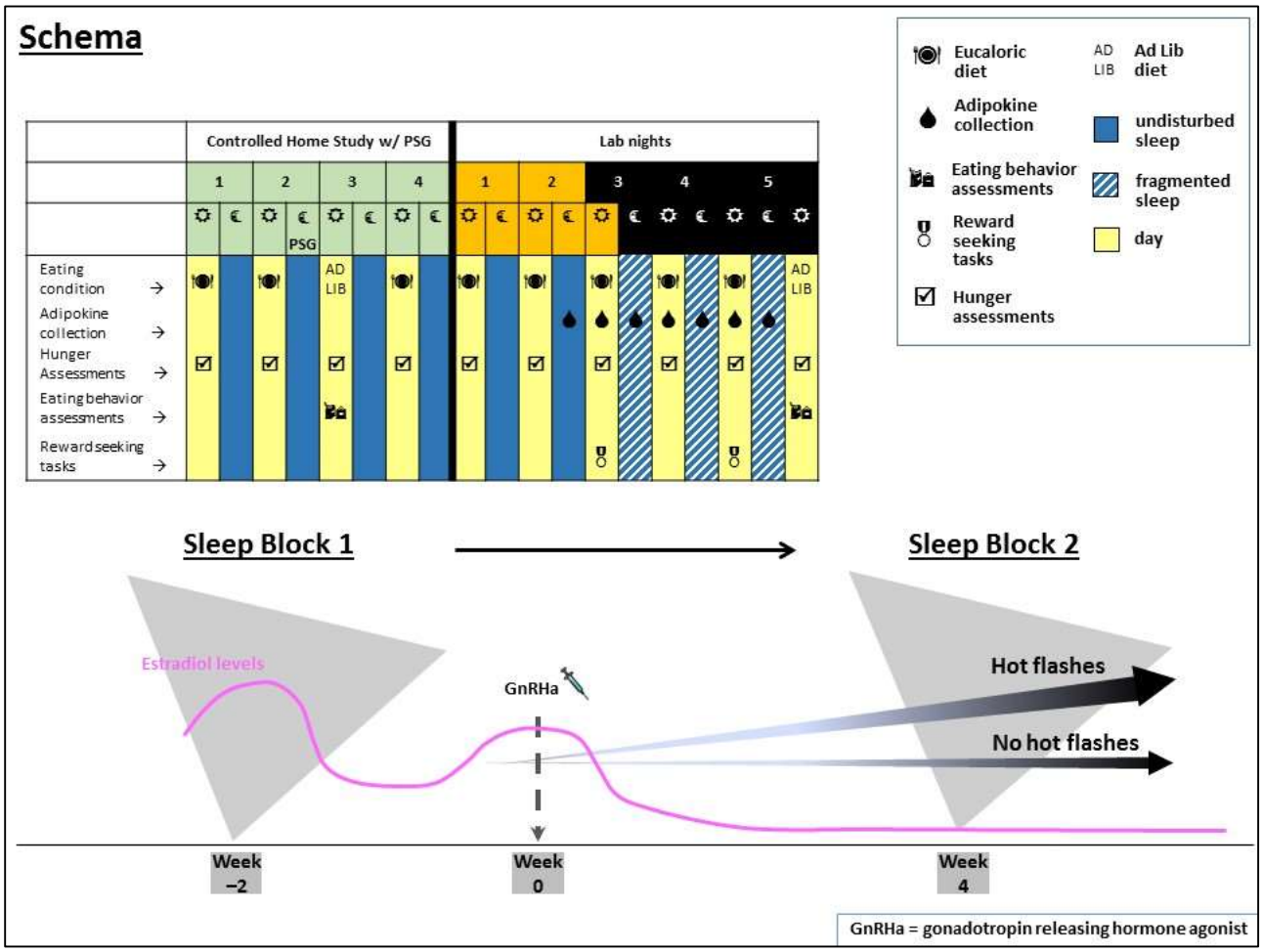
Overview

Forty women ages 18–45 years will complete a protocol involving open-label treatment with standard-dose depot leuprolide to induce hypo-E2 in all women and generate HF in approximately 2/3, thereby mimicking menopause (see **Schema**). As part of the protocol, subjects will be admitted to the BWH Clinical And Translational Science Center/Center for Clinical Investigation (CCI) inpatient unit for a total of 10 nights over a 2-month period (see **Schema**). Assessments will be divided into 2 sleep blocks. Each SB includes 2 nights during which sleep is undisturbed and 3 nights during which sleep is experimentally fragmented. Using PSG, sleep patterns will be experimentally interrupted to the same target level for all subjects during all fragmented nights using auditory procedures to mimic the menopausal hot flash-associated sleep maintenance problem. The in-lab sleep blocks will be conducted: 1) during the mid-to- late follicular phase of the menstrual

cycle when E2 is high; and 2) after at least 4 weeks on leuprolide when HF are prominent in a subset of subjects.

Participants will be given a standardized eucaloric diet for several days preceding the inpatient admission, as well as for the first four days of the inpatient visit. On one day before admission, and on the final day of inpatient admissions, subjects will have an ad lib diet. Fasting adipokine levels will be measured and leptin levels will be obtained to determine the 24-hour leptin profile under standardized eucaloric diet conditions. Food intake and macronutrient composition will be assessed as key behavioral biomarkers for body fat gain when the diet is ad lib. Hunger will be measured under both eucaloric and ad lib dietary conditions. Secondary and exploratory measures of objective HF using skin conductance monitors, negative mood/affect, reward seeking behaviors, and appetite/food preference will also be obtained. Behavioral measures will continue to be assessed at home after the 2nd SB is complete.

Protocol Schema



Study Sample and Criteria

Informed consent will be obtained from up to 130 healthy non-pregnant premenopausal women 18-45 years who do not have hot flashes in order to have 40 women complete study procedures.

Informed consent will be obtained from up to 25 healthy non-pregnant premenopausal women 21-45 years to complete the pilot study. Eligibility will be evaluated initially through a telephone screening process and then established in an in-person screening office visit. The following inclusion and exclusion criteria will then be applied:

A. **Inclusion/Exclusion Criteria for Full Study Participants**

Inclusion Criteria:

1. Healthy premenopausal women 18–45 years old
2. Habitually sleep 7–9 hours per night and usual bedtime between 9 pm and 2 am
3. Consume <2 alcoholic drinks per day
4. Consume <3 caffeinated drinks per day
5. Regular monthly menstrual cycles
6. No lifetime history of hot flashes
7. Willingness to use barrier methods of contraception or IUD during study and after completion of study until menses resume
8. Weight >110 pounds (given blood sampling plan; see Section 1c below)
9. Body mass index ≥18.5 and <35 kg/m^2 (to avoid possible under-dosing of leuprolide and eliminated confounding of adipokine effects by obesity)
10. Good general health
11. Ability to understand and willingness to sign written informed consent document

Exclusion Criteria:

1. Contraindication, hypersensitivity or previous adverse reaction to gonadotropin releasing hormone agonists
2. Pregnancy, confirmed by serum HCG at screening and prior to leuprolide administration
3. Breastfeeding
4. Tobacco use
5. Current or recent (1 month) use of centrally active medications, or a plan to initiate one of these medications during the study.
6. Current or recent (1 month) use of systemic doses of female reproductive hormone medications, except from intrauterine device initiated more than 6 months prior to study participation
7. Current or recent (1 month) use of over-the-counter (OTC) medications that may affect hot flashes, sleep, or mood (eg diphenhydramine, St. John's Wort, Black Cohosh, and other phytoestrogen supplements)
8. Shift workers or time zone travel within 4 weeks prior to beginning the study
9. Obstructive sleep apnea as indicated by an elevated score on the Berlin screening questionnaire or by history
10. Insomnia symptoms as indicated by a score on the Insomnia Severity Index suggesting subthreshold or clinically significant insomnia symptoms
11. Contraindications to CT or DXA scanning
12. Diagnosis of osteoporosis or osteopenia, or history of non-traumatic fractures
13. Hypothalamic-pituitary-adrenal axis disorders
14. Diabetes by history, or as indicated by an abnormal hemoglobin A1c
15. Gastric bypass, metabolic disorders, or other conditions that affect adipokines
16. Abnormalities on screening laboratory tests (hemoglobin A1c, complete blood count, thyroid,

hepatic and renal function) including specifically a hemoglobin <12 g/dl (given multiple blood draws) judged by the investigator to be medically relevant
17. Hearing loss interfering with ability to respond to auditory stimuli
18. Cardiovascular illness
19. Neurological illness
20. Psychiatric illness, defined by clinical assessment at baseline as:
    a. Current depression as indicated by an elevated score on the PHQ-8
    b. A lifetime history of major depressive disorder, bipolar disorder, eating disorder, or psychotic symptoms
    c. Current suicidal or homicidal ideation, or any prior suicide attempt or inpatient psychiatric hospitalization
21. Current medical or psychiatric conditions or medicine use judged by the investigator to interfere with study procedures or data collection
    a. An alcohol or substance-use disorder active within the past year

B. **Inclusion/Exclusion Criteria for Pilot Study Participants**

Inclusion Criteria for Pilot Study Participants:

1. Healthy premenopausal women 21–45 years old
2. Habitually sleep 7–9 hours per night and usual bedtime before 12 midnight
3. No lifetime history of hot flashes
4. Body mass index <35 kg/m^2
5. Good general health
6. Ability to understand and willingness to sign written informed consent document

Exclusion Criteria:

1. Current medical or psychiatric conditions or medicine use judged by the investigator to interfere with study procedures or data collection
2. Pregnancy
3. Breastfeeding
4. Regular tobacco use
5. Shift workers
6. Diagnosis of obstructive sleep apnea
7. Insomnia symptoms as indicated by a score on the Insomnia Severity Index suggesting subthreshold or clinically significant insomnia symptoms
8. Hearing loss interfering with ability to respond to auditory stimuli
9. Current depression, defined by a score >3 on the PHQ-2

Off-Study Criteria:

1. Emergence of significant psychiatric symptoms, suicidal or homicidal ideation
2. Development of any significant medical problem
3. Pregnancy
4. Significant deviations from study protocol, including enrollment in another research protocol that may influence the results of the current protocol.

Study Procedures Overview

Briefly describe study procedures. Include any local site restrictions, for example, "Subjects enrolled at Partners will not participate in the pharmacokinetic portion of the study." Describe study endpoints.

Screening Phase (1-2 months)

- **Telephone/Pre-screening**: Consent to screen will be obtained orally on the phone for the initial screening procedures and diaries. Women who verbally consent to screening and are interested in participating in this trial will answer some questions by phone to assess preliminary eligibility. The Berlin Questionnaire will be administered to screen for sleep apnea, and questions about general medical and mental health will be asked to screen for any major exclusion criteria. Those who are eligible and interested will be scheduled for an initial remote screening and research consent visit with a study clinician.

- **Screening visit 1a (remote)**: Screening visit 1a will be conducted remotely. Written informed consent will be obtained via electronic signature using REDCap e-consent template and initial eligibility criteria will be assessed by a clinician, including a medical and psychiatric interview, and sleep interview. All women who sign the consent form will be assigned a study identification number regardless of eligibility status. Eligible participants will start monitoring their menstrual cycles and tracking their sleep after this visit.

- **Screening Visit 1b (in-person):** At this visit, a clinician will conduct a physical exam, and a blood draw will be taken for screening assays (e.g., CBC, glucose, insulin, HCG, TSH), for baseline levels of adipokines, metabolic and reproductive hormones, and for measures of immune function related to sleep and menopause. Once lab results are reviewed by a study clinician, final eligibility will be assessed. Eligible participants will be scheduled for Sleep Block 1, leuprolide injection, and dual-energy x-ray absorptiometry (DXA) scan.

Sleep Block 1 (10 days – two weeks before GnRH Agonist injection)

- Subjects will be admitted for two five night stays within the BWH Clinical And Translational Science Center/Center for Clinical Investigation (CCI) inpatient unit. Subjects will also complete at-home procedures, including an at-home PSG procedure. Subjects may log meals before inpatient admission and for up to 7 days after discharge with the app MealLogger™

GnRH Agonist Injection (1 day)

- Subjects will come to one of the outpatient research units to be given an injection of leuprolide (GnRH agonist). The timing of ovulation will have been previously determined based on the collection of at least 3 menstrual cycle start dates. Leuprolide will be administered in the week prior to an expected menstrual cycle start date. If the subject starts her menstrual cycle early prior to receiving leuprolide, the protocol will be extended to the next cycle.On the day of the scheduled appointment for the injection, a urine pregnancy test will be performed to confirm that the subject is not pregnant and blood will be drawn to measure reproductive hormone levels.

Sleep Block 2 (10 days – 4 weeks after GnRH Agonist Injection)

- Subjects will be admitted for two five night stays within the BWH Clinical And Translational

Science Center/Center for Clinical Investigation (CCI) inpatient unit. Subjects will also complete at-home procedures, including an at-home PSG procedure. Subjects may log meals before inpatient admission and for up to 7 days after discharge with the app MealLogger™.

Follow-up Phase (4-12 weeks or until symptoms resolve)

- Follow-up phone call from the study coordinator 4 weeks after the final study visit and every 4 weeks thereafter to confirm resumption of menses and cessation of hot flashes (if they occurred).
- Study staff will also collect food and appetite measures that were recorded by subjects the end of Sleep Block 2
- Subjects who do not have resumption of menses or who continue to have hot flashes 4 months after receiving the leuprolide injection (3 months after final visit) will be referred to their routine medical provider for evaluation.
- A study summary letter will be sent to subjects once the final follow-up phone call has occurred. If there are any abnormal laboratory results listed in the letter that have not yet been discussed with the subject, a study clinician will call the subject to discuss prior to sending the letter.

Detailed Description of Sleep Blocks

Assessments will be divided into 2 sleep blocks, each of which lasts 9 nights/10 days and includes 5 nights of inpatient laboratory assessment. Each block includes 2 in-lab nights during which sleep is undisturbed and 3 nights during which sleep is experimentally fragmented. Subjects will also receive an actigraphy watch and record their sleep habits before entering the lab, and for up to 7 nights after. During one night of each sleep block, subjects will use an at-home PSG to record their sleep. Subjects will return to BWH the following morning to deposit the PSG device with study staff, and to eat an ad lib breakfast.

- **At-home procedures during the Sleep Block:** Subjects will complete four days of at-home procedures before their admission to the CCI inpatient unit. On the 1st, 2nd, and 4th days, subjects will eat eucaloric meals provided by the BWH Nutrition Core. On the 3rd day, subjects will be given food provided by the Nutrition Core for that entire day, and will be allowed to consume as much as desired at home while logging their meals, snacks and drinks. On the 2nd night of the sleep block, subjects will come to Brigham and Women's to have an at-home ambulatory PSG machine attached; this machine will be removed the following morning when subjects arrive for breakfast. Eucaloric diets will approximate the current American women's diet, with approximately 15% protein, 50% carbohydrates, and 30% fat. Subjects will select each eucaloric meal from options prepared by the Nutrition Core; this selection will be done in advance of sleep blocks. If a subject would like to change their meal preferences, they will be permitted to do so as long as the change can be accommodated by the BWH Nutrition Core. They will be asked to finish meals and log any deviations of the eucaloric diet. For each day with an ad lib diet, caloric intake and macronutrient composition will be calculated and analyzed with Nutrition Data System for Research software after each meal and snack. Subjects will order these meals from a prepared ad lib menu.
- **Procedures to occur in both SB_{UD} and SB_{SF}:** Subjects will remain indoors during each admission. Technicians will hook subjects up to a PSG monitor each night. Participants will be given a standardized eucaloric diet for the first 5 days of their visit, and will have an ad lib menu on the 6th day of their in-lab stay. On the 6th day, subjects will be given food provided by the Nutrition Core for that entire day, and will be allowed to consume as much as desired at home while logging their meals, snacks and drinks with the app MealLogger™. Subjects will be expected to finish these meals. Caloric and macronutrient intake will be quantified. Fasting adipokine levels, other

metabolic and stress markers will be collected during each visit. Blood samples will be collected repeatedly up to every 30 minutes during each visit. Blood will be drawn without interrupting sleep using an approximately 72" long IV line from outside the room and a blood-sparing technique to minimize blood loss. If issues with the IV occur, then select samples, up to 2 per wake episode may be drawn via venipuncture. Core Body Temperature will be measured with an oral thermometer. Hunger will be measured under both eucaloric and ad lib dietary conditions by administering Hunger Visual Analog Scales. On the day of admission, reproductive hormone levels will be tested, a urine pregnancy test will be performed, and morphometric measures will be gathered. During the inpatient stay, subjects will complete various questionnaires and tasks (e.g. Food Preference Questions, the Dutch Eating Behavior Scale, the Positive and Negative Affect Scale, hot flash diaries, reward seeking behavior, PSQI, ISI, PHQ-8). After waking, subjects may complete the post-sleep questionnaire and the CCI IPM Exit Questionnaire, which are regularly administered by CCI inpatient unit staff. Subjects may also complete neurobehavioral testing administered automatically by the computers on the CCI inpatient unit. Throughout the inpatient portions of the study urine voids may be scheduled to be collected approximately every 3 hours when the subject is awake. Unscheduled urine voids during the day or overnight may also be collected. For each void, the urine volume will be measured, and aliquots may be saved for later hormonal, metabolite, or electrolyte analysis of analytes that may be affected by sleep fragmentation and/or changes in hormonal status. Urine samples will be collected and stored per standard techniques. Skin conductance may be measured using the Biolog skin conductance monitor. During the 4 days prior to admission, subjects will receive several days of eucaloric meals from the CCI Nutrition Service, and ad lib breakfast will take place at BWH in order to measure food intake. Subjects will be instructed to standardize their sleep patterns during the 4 days at home, and up to a week beforehand, to extend the standardized routine.

- **Undisturbed procedures only:** Bedtime and wake-up time will be fixed to an 8-hour standardized TST.
- **Fragmentation procedures only:** During SB_{SF} nights, subjects will be awakened using an auditory stimulus, adjusted to maintain the target number of awakenings and WASO observed in our most symptomatic subjects with leuprolide-induced HF (36 awakenings, 62 minutes WASO). TIB will be extended to maintain the 8-hour TST. Subjects will not be permitted to get out of bed or eat in the middle of the night. Auditory tones between 2 and 30-second duration and 60-110 dB will be used. Once awake, subjects will be required to stay awake for up to 2 minutes. If subjects fall back asleep during the 1-2 minute awakening, the auditory stimulus will be played again to elicit an awakening.

Pilot Study Procedures Overview

To determine the most effective methods for fragmenting sleep in this protocol, we will first conduct a pilot study with up to 25 healthy volunteers. This pilot does not include intravenous lines or blood sampling procedures. These subjects will complete a much abbreviated version of the protocol detailed in the next section:

A. Telephone screen: The Insomnia Severity Index (ISI) and the PHQ-2 will be asked over the phone. Those who meet initial eligibility criteria will be scheduled for an in-person screening visit.

B. Screening visit: At the screening visit, written informed consent will be obtained and final eligibility criteria will be assessed, including a medical and psychiatric interview, sleep

interview, vitals, and a urine pregnancy test. All women who sign the consent form will be assigned a study identification number regardless of eligibility status. Eligible and consented participants will be scheduled for either a 1-night or 3-night stay in the Center for Clinical Investigation inpatient unit.

C. Inpatient visit: Subjects will be admitted for a one-night or three-night stay within the BWH Clinical and Translational Science Center/Center for Clinical Investigation (CCI) inpatient unit. Subjects will be admitted in the afternoon and discharged mid-morning. During the stay, we will conduct the following abbreviated protocol:

- On the day of admission, a urine pregnancy test will be performed, and vitals and morphometric measures will be gathered. Subjects will remain on the 9B floor during the stay. Nursing staff will hook subjects up to a PSG monitor each night, and 24-hour skin conductance will be measured during the night using the Biolog skin conductance monitor.
- Subject will be awakened during the night through auditory stimulus broadcast manually by sleep technicians throughout the night to achieve the target number of awakenings and minutes of Wake After Sleep Onset (WASO) (36 awakenings, 62 minutes WASO). Time In Bed (TIB) will be extended to maintain the 8-hour Total Sleep Time (TST). Auditory tones will be between 2- and 30-seconds duration, beginning at 60 dB and increased to a maximum of 1100db if an EEG awakening is not elicited. Once awake, subjects will be required to stay awake for up to 2 minutes. Subjects will not be permitted to get out of bed or eat in the middle of the night.
- Subjects will be able to order meals and snacks off a provided menu. There will be no dietary restrictions.

Nurses and technicians will be present 24 hours a day, so participants will have frequent social interactions in this controlled setting. Medical and psychologist/psychiatrist evaluation and treatment is available at all times.

Table of Procedures – Study overview

Table of Procedures - All Visits (revised 6/26/2020)							
Study Phase		Pre-Treatment				Post-treatment	
Visit Type		Phone/Pre-screen	Screening Visit 1a	Screening Visit 1b	Sleep Block 1	GnRH-a Injection 1	Sleep Block 2
		remote	remote	in-person	in-person	in-person	in-person
Time from Medication		-2-3 months	-1-2 months	-1-2 months	-2 weeks	0	1 week
General	Informed Consent	x	x				
	Medical and Psychiatric Interview		x				
	Sleep Interview		x				
	Vitals, exam, & Morphometric measures			x	x	x	x
	Screening Labs			x			
	Questionnaires (via REDCap survey link)			x			
Study Medication Administered						x	
DXA	DXA Scan				x		
Blood tests	Adipokines				x		x
	Reproductive Hormones			x	x	x	x
	Metabolic Markers			x	x		x
	Stress			x	x		x
Monitored Diet					x		x
Sleep Lab Assessments	Food intake/caloric consumption				x		x
	Hunger and Mood Ratings				x		x
	Energy Expenditure				x		x
	Polysomnography				x		x
	Skin Conductance						x
	Body Temperature				x		x
	Reward Behaviors				x		x
Hot Flash Diaries					x		x
Eligibility Assessments	Sleep diaries				x		x
	Actigraphy Watch				X		X
	Berlin Questionnaire	x					
	PSQI	x			x		x
	ISI	x			x		x
	PHQ-8		x		x		x
	Urine Pregnancy Test				x	x	x
	PHQ-2	x					
AE Monitoring			x	x	x	x	x

Study Endpoints

Metabolic endpoints are: 1) fasting morning levels of leptin (primary) adipokines, ghrelin (total, acetylated) (secondary), and 2) the circadian leptin profile (exploratory), calculated as area under curve (AUC) to detect whether the overall profile is lower, as seen in sleep restriction studies, and morning fasting AD levels. Behavioral endpoints are: 1) satiety (primary) and hunger (secondary) on a VAS scale, and 2) daily caloric intake during ad lib meals (exploratory). Analyses of each endpoint will be conducted in parallel as we seek to detect coherence among endpoints and focus on consistency across different outcomes in each hormonal and HF context.

For studies involving treatment or diagnosis, provide information about standard of care at Partners (e.g., BWH, MGH) and indicate how the study procedures differ from standard care. Provide information on available alternative treatments, procedures, or methods of diagnosis.

This study involves the administration of leuprolide to healthy individuals in order to better understand experimentally induced hot flashes. Subjects are healthy individuals who do not have any specific medical diagnosis. The DXA scan is not clinically indicated for women participating in the protocol. Alternative imaging studies are therefore not indicated.

Describe how risks to subjects are minimized, for example, by using procedures which are consistent with sound research design and which do not unnecessarily expose subjects to risk or by using procedures already being performed on the subject for diagnostic or treatment purposes.

There will be a number of different procedures in place to minimize the risks to the participants. The PI's current IND for use of leuprolide in healthy volunteers to study hot flashes (#73144) has been amended to include the current protocol. All study procedures will be approved by our IRB before being initiated. All adverse event reports will be reviewed by the PI. Serious and non-serious adverse events will also be reported to the Safety Monitoring Group (see DSMG form), FDA (for IND #73144) and our IRB in accordance with the respective reporting guidelines. Unanticipated problems involving risks to subjects or others including adverse events will be reported to the PHRC in accordance with PHRC unanticipated problems reporting guidelines**.** In the case of a serious adverse event, study procedures will be discontinued and appropriate diagnostic and therapeutic measures will be taken, as decided by the investigators.

Thorough screening procedures will be implemented to exclude women in whom study treatments and procedures are contraindicated. Subjects participating in the study protocol will be monitored closely for the development of any adverse events. Criteria for withdrawing participants from the protocol in the event of an adverse event or serious health concern will be implemented. Women who develop a serious health concern or adverse event will be withdrawn from the protocol and referred for appropriate treatment. Potential risks to study subjects will be further minimized by the close monitoring and contact with study staff as required by the protocol design.

All research records will be kept with maximum possible confidentiality. Results, including biological specimens, will be coded and the key to the code will be assigned by the PI and maintained in a locked file. No names will be used in presentation of any data. Source documents will be reviewed by study personnel. All HIPAA regulations will be strictly followed.

The PI will meet with the project manager and research coordinator on a frequent basis to review and monitor the validity and integrity of all data in study binders and adherence to the IRB regulations. A checklist will also be included in the binders for each subject at each study visit.

This checklist will be completed by the research coordinator and reviewed by the PI to confirm that all data are correctly obtained. In addition, all study personnel will review study data and discuss QA issues related to protocol adherence. Forms available on our IRB website will be used to keep track of adverse events and minor protocol violations.

Describe explicitly the methods for ensuring the safety of subjects. Provide objective criteria for removing a subject from the study, for example, objective criteria for worsening disease/lack of improvement and/or unacceptable adverse events. The inclusion of objective drop criteria is especially important in studies designed with placebo control groups.

Participants will have a serum pregnancy test at the screening visit to ensure that they are not pregnant, and an additional urine pregnancy test will be conducted immediately prior to leuprolide administration. Subjects will be required to use a barrier method of contraception for the duration of the study and after study completion until menses resume. These procedures will serve to minimize the likelihood that study participants become pregnant during study procedures and during

the period of medication exposure.
Participants will receive safety follow up calls regularly after study completion to ensure that menses resume and that, where applicable, that hot flashes resolve. If menstrual cycles do not return to normal or hot flashes do not resolve within several months of follow-up after the completion of all study procedures, participants be referred to an endocrinologist for further evaluation. We have used the same safety procedures in previous studies.

Once in the laboratory, the subject will be constantly supervised by laboratory personnel. The medical physician for the BWH Clinical And Translational Science Center/Center for Clinical Investigation (CCI) inpatient unit will also be available on-call to handle any medical emergency that might arise. The subject's vital signs (pulse rate, respiratory rate, systolic and diastolic blood pressure) will be checked at least once per day. An approximately 72" long intravenous line will be placed during each of the CCI inpatient unit admissions so that venous blood samples can be obtained using an IV line from outside the room without repeated venipuncture or sleep interruption related to the blood draw. If issues with the IV occur, then select samples, up to 2 per wake episode may be drawn via venipuncture. Prior to the insertion of the IV catheter, the skin around the insertion site will be washed with a Betadine solution followed by alcohol. An anti-bacterial ointment will be applied to the IV catheter insertion site prior to dressing the site. Hypoallergenic tape will be used on the IV dressing to minimize the development of contact dermatitis. The IV catheter insertion will be done while the subject is supine, and a staff member will remain in the suite with the subject for at least 10 minutes after the insertion to ensure that the subject does not feel light-headed or dizzy. Blood-sparing techniques will be used to minimize blood loss. The total amount of blood drawn will be up to 447.5 cc. Federal guidelines allow for up to 550 cc of venous blood per 2-month period in healthy non- pregnant adults. Anemic subjects will be excluded from participation and hemoglobin will be repeated at the completion of the study to confirm the absence of anemia. Oral ferrous sulfate supplementation and clinical follow up will be provided if anemia is present.

The PI has established additional monitoring procedures for specific adverse events related to use of leuprolide in healthy volunteers which will be implemented in the protocol. While there is a low likelihood (2% in our experience) that a subject will develop significant depressive symptoms, mood will be assessed repeatedly throughout the study as part of standard safety monitoring procedures. Women developing severe depression or who have suicidal or homicidal ideation will be withdrawn from the study and referred for appropriate care. If suicidal or homicidal ideation is of concern, subjects will be evaluated immediately. Dr. Joffe is an experienced psychiatrist with an expertise in women's mental health who will be available to evaluate and treat women with significant depression.

Off-Study Criteria:

1. Emergence of significant psychiatric symptoms, suicidal or homicidal ideation
2. Initiation of hormonal or centrally acting medications
3. Development of any significant medical problem
4. Pregnancy
5. Significant deviations from study protocol, including enrollment in another research protocol that may influence the results of the current protocol.

FORESEEABLE RISKS AND DISCOMFORTS

Provide a brief description of any foreseeable risks and discomforts to subjects. Include those related to drugs/devices/procedures being studied and/or administered/performed solely for research purposes. In addition, include psychosocial risks, and risks related to privacy and confidentiality. When applicable, describe risks to a developing fetus or nursing infant.

Potential risks associated with study medications:

Leuprolide in healthy volunteers: Leuprolide is a widely used gonadotropin releasing hormone (GnRH) agonist that is manufactured by Abbott. Leuprolide is indicated for treatment of endometriosis, uterine fibroids, precocious puberty, and prostate cancer, and has been used off-label for in-vitro fertilization and premenstrual syndrome. In the current protocol, leuprolide will be administered at a dose routinely used for treatment of endometriosis and uterine fibroids in premenopausal women. Leuprolide can induce amenorrhea for 2 to 3 months after a depot injection is given. However, unpredictable menstrual bleeding and spotting can occur during this time period. Menstrual-cycle patterns will be monitored closely during the study. Through its effects on female hormones, leuprolide temporarily reduces fertility for up to 3 months after a depot injection is given. Leuprolide may cause growth abnormalities and death in an unborn fetus.

Although long-term administration of any GnRH agonist (i.e., >6 months) is associated with bone loss, bone effects are not expected in this protocol because only a single dose of GnRH agonist therapy will be administered. Other side effects are related to suppression of ovarian steroid secretion, including:

Adverse reactions to short-term use:

- Common: Hot flashes, headache, vaginal bleeding, vaginal dryness, vaginitis, alopecia, body and joint pain, emotional lability, depression symptoms, memory problems, nausea, vomiting, peripheral edema
- Rare (<3%): Myalgia, hirsutism, acne, weight gain or weight loss, depression, decreased libido, dyspareunia, paresthesia, skin reactions, dizziness, breast tenderness and pain

Leuprolide is associated with emotional lability in 22–31% of women receiving leuprolide as part of large clinical trials for treatment of endometriosis and fibroids. These studies do not provide information about whether the mood symptoms that emerged were persistent or at a clinically significant level suggesting a depressive disorder. However, our experience, and that of other reproductive psychiatry investigators using close monitoring of mood in healthy volunteers given leuprolide, indicates that clinically significant depression is rare (2–3%). Additional information from the manufacturers of leuprolide also indicates that serious depression develops only rarely on GnRH agonists.***Potential risks associated with DXA scan:*** A dual-energy x-ray absorptiometry (DXA) scan will be performed to quantify total body fat mass. The total body DXA scan is non-invasive and takes less than 10 minutes to complete with minimal/no discomfort while lying down on a scan table under the supervision of the Bone Density staff. Participants will be exposed to a small amount of radiation from this scan. The amount of radiation exposure with DXA is even less than that of an abdominal CT scan. The exposure from the DXA scans is equal to less than 1% of the dose permissible during occupational exposure. The risk from radiation exposure of this level is too small to be measured directly and is considered to be negligible in comparison with other everyday risks. Pregnancy tests will be used to ensure that women undergoing the DXA scans are not pregnant.

Patients will be screened for contraindications to DXA as part of the eligibility/exclusion requirements so that they do not participate in this protocol.

It is possible that the DXA scan may coincidentally detect an abnormality that requires further evaluation. The participant and her physician (with the subject's permission) will be informed of the scan results so follow-up assessments and treatment can be undertaken if necessary.

Potential risks associated with Biolog Ambulatory Hot Flash Monitor: The Biolog ambulatory hot flash monitor (UFI, Morro Bay, CA) will be used to measure hot flashes objectively. There are no significant risks associated with skin-conductance methodologies. The input assembly wiring is fixed so that the subject is inherently isolated, making the risk of current leakage negligible. Skin conductance is measured by 2 adhesive gel electrodes that are attached to the sternum. There is a minor risk of skin irritation from the adhesive gel. The recorder and electrodes cannot get wet; therefore subjects will be instructed to not bathe while wearing the monitor.

Potential risks associated with inpatient sleep study procedures: Subjects will be admitted to the BWH Clinical And Translational Science Center/Center for Clinical Investigation (CCI) inpatient unit for 5 nights at a time. Admissions of this length may be psychologically stressful and inconvenient or disruptive to daily routines. Headache, nausea, dizziness, light headedness, excessive sleepiness, muscle soreness, and weakness may occur. During some of the admissions, subjects will experience experimentally induced sleep fragmentation which will reduce their quality of sleep, as a minor discomfort. Short term sleep fragmentation can cause drowsiness and increase the risk of accidents. While subjects will be monitored and accidents are unlikely in the controlled environment of the laboratory, we have less control when subjects leave the laboratory. Thus, we will ensure that the subject fully appreciates this risk and has safe transportation home from the unit after each of the sleep fragmentation sleep blocks. The subjects may become sleepy during some segments of the study but will be asked to remain awake during the entirety of their scheduled wake times. Should they feel unable to remain awake, they are free to withdraw consent to participate in this experiment and then go to sleep. After the sleep fragmentation procedures, subjects may have some difficulty sleeping and waking at their usual times for several days. Volunteer subjects are studied in the CCI inpatient unit within the main BWH campus. In the unlikely event that emergency treatment is required during the subject's stay in the research facility, treatment will be provided.

Polysomnography: PSG studies will be conducted according to standard clinical procedures, which have minimal risks. A minor rash may develop from the tape or adhesives used to attach electrodes, but this is expected to resolve within a few days. In addition, the monitoring equipment may make sleep somewhat more difficult than usual and the individual may therefore experience minimally reduced quality of sleep.

Ambulatory Polysomnography: The ambulatory polysomnography equipment is commonly used in clinical sleep testing and has minimal risk. A minor rash may develop from tape or adhesives used to attach the electrodes, but is expected to resolve within a few days. In addition, the monitoring equipment may make sleep somewhat more difficult than usual and the individual may therefore experience minimally reduced quality of sleep.

Potential risks associated with venous blood sampling: The risks associated with venipuncture and intravenous lines are minimal and include hematoma, pain, infection, and fainting spells. All blood draws and IV placements will be performed by trained personnel that use standard sterile techniques. Infection is unlikely since stringent aseptic techniques are followed during all

procedures. An approximately 72" long intravenous line will be placed for a 24-hour period during each of the BWH Clinical And Translational Science Center/Center for Clinical Investigation (CCI) inpatient unit so that venous blood samples can be obtained up to every hour using an IV line from outside the room without repeated venipuncture or sleep interruption related to the blood draw. Blood-sparing techniques will be used to minimize blood loss. If issues with the IV occur, then select samples, up to 2 per wake episode may be drawn via venipuncture. There may be some side effects from the use of heparin; such as bleeding and allergy.
There may be some discomfort or bruising on initial insertion of the catheter into a vein, but wearing the catheter should not be painful. Occasionally, mild discomfort may occur from the tube in the vein. If this happens, it can be repositioned or removed, asking the subject's permission before any subsequent reinsertion. Occasionally, there is a bruise at the site of the IV insertion, which may last a few weeks; and, rarely, a small scar may remain permanently at the venipuncture site. There may be a minor skin rash or reaction to the sterile tape (contact dermatitis) used to hold the catheter in position.
A total of up to 447.5 cc of venous blood will be drawn throughout the study. Federal guidelines allow for up to 550 cc of venous blood per 2-month period in healthy non-pregnant adults weighing >110 pounds. There is a risk of becoming anemic from frequent blood sampling.

Psychological risks: Answering questions on the research instruments and questionnaires used to evaluate psychological symptoms can be upsetting to some women. Subjects will be told that they can skip specific questions that make them uncomfortable. Any woman who develops severe depression during the study will be evaluated and referred for treatment, including emergency evaluation if appropriate.

Radiation exposure: Each set of DEXA scans result in an effective radiation dose of approximately 0.5 mrem so that both sets results in an effective dose of approximately 1 mrem. The total effective dose is therefore approximately 1 mrem.

Risks of MealLogger^{TM}: The research team will create credentials for patients using this app, so that MealLogger will not be able to associate the data with a real person. It is not necessary for the study staff to collect identifiable information through the app, so participants will be instructed that the only required information is their meals. MealLogger is easily installed on both Android and iPhone systems; the research team does not anticipate usability issues given that MealLogger is used both in research studies and in the general populations, but participants will be instructed to contact research coordinators with any questions about the technology. MealLogger has already been approved in other Brigham and Women's Hospital research studies (e.g., 2012P-001631).

Risks of Study Food: The study food provided by the BWH CCI Dietary Core is specific to each participant's preferences and restrictions. The Dietary staff are informed of participants' allergies, as well as preferences (e.g., vegetarian or vegan). There is a minor risk of gastrointestinal symptoms (e.g., stomach discomfort/ache, nausea, or abdominal bloating) as participants' diets at home may not include the same types of food consumed during the study diet.

EXPECTED BENEFITS

Describe both the expected benefits to individual subjects participating in the research and the importance of the knowledge that may reasonably be expected to result from the study. Provide a brief, realistic summary of potential benefits to subjects, for example, "It is hoped that the treatment will result in a partial reduction in tumor size in at least 25% of the enrolled subjects." Indicate how the results of the study will benefit future patients with the disease/condition being studied and/or society, e.g., through increased knowledge of human physiology or behavior, improved safety, or technological advances.

Benefits to subjects

As healthy volunteers, participants will not receive any direct benefit from this study protocol. However, the protocol is expected to provide a meaningful benefit to peri- and postmenopausal women through knowledge gained about the metabolic consequences of sleep disruption, hot flashes, and loss of circulating estradiol. For those participating in the study, the sleep studies, radiographic assessments, morphometric measures, metabolic and reproductive hormone levels may be of interest. If a participant requests routine health information obtained during the study, she will be provided with a copy after completing study procedures. If an abnormality is detected during the study, this information will be shared with the participant and her physician (with her permission). Our experience to date indicates that some women find the knowledge obtained through the experience of developing transient menopausal symptoms informative. Because there is no direct benefit of study procedures to these healthy volunteers, they will be compensated for their time, effort and willingness to undergo study procedures, including exposure to time-limited leuprolide therapy and time spent in the BWH Clinical And Translational Science Center/Center for Clinical Investigation (CCI) inpatient unit. However, financial compensation to subjects will not be presented as a benefit of participation in this research.

Importance of the knowledge to be gained

Obesity is highly prevalent in midlife and older women, with rates increasing markedly after age 40 and abdominal adiposity increasing in half of women during and after the menopause transition independent of age, but the factors that cause these changes are not well understood. Reduced total sleep time has been shown to adversely impact biomarkers of obesity, but the effect of menopause-related sleep fragmentation, which occurs in >40% of midlife women and is characterized by sleep interruption secondary to hot flashes, on metabolism and eating behaviors is not known. The broad goal of this proposal is to isolate the impact of menopause-related sleep disruption, as well as menopause-related hot flashes and estrogen withdrawal, on behavioral and metabolic biomarkers of body fat gain, results of which will inform strategies to prevent gains in body fat, thereby improving cardiovascular and metabolic health outcomes in women.

EQUITABLE SELECTION OF SUBJECTS

The risks and benefits of the research must be fairly distributed among the populations that stand to benefit from it. No group of persons, for example, men, women, pregnant women, children, and minorities, should be categorically excluded from the research without a good scientific or ethical reason to do so. Please provide the basis for concluding that the study population is representative of the population that stands to potentially benefit from this research.

The study population is restricted to non-pregnant premenopausal women, the population for whom

the information obtained in this study is relevant. Pregnant women are excluded because of risks with study medication to the unborn fetus. Children under 18 are excluded because the study is not relevant to pre-pubertal children and the hypothalamic-pituitary-ovarian axis is in flux in many post-pubertal teenage girls, as reflected by frequent menstrual-cycle abnormalities in this population. The age requirements are that women must be within 18-45 years of age in order to study experimentally induced hot flashes in women who are close to natural menopause. Men are excluded because the hormonal changes (e.g., estrogen) that occur with GnRH agonists differ between women and men and these outcomes are important components of this study. Minority populations are included.

When people who do not speak English are excluded from participation in the research, provide the scientific rationale for doing so. Individuals who do not speak English should not be denied participation in research simply because it is inconvenient to translate the consent form in different languages and to have an interpreter present.

Individuals who do not speak English will be excluded from participation in this study because many of the questionnaires administered during this study are not validated in other languages.

RECRUITMENT PROCEDURES

Explain in detail the specific methodology that will be used to recruit subjects. Specifically address how, when, where and by whom subjects will be identified and approached about participation. Include any specific recruitment methods used to enhance recruitment of women and minorities.

Participants will be recruited from outpatient primary care departments at BWH and affiliated community clinics as well as through the larger Boston community, using methods we have employed to successfully recruit healthy volunteers to similar studies. Advertising methods include using the Research Study Volunteer Program (RSVP for Health), an online system through which registered individuals receive information about research studies, recruitment postcards, and paid newspaper and radio advertisements to publicize the study. Study staff may also use other internet forums (e.g., Craigslist, Facebook) to recruit. Women inquiring about the study will be screened preliminarily over the phone by the research coordinator using an IRB-approved telephone script, and if appropriate for the study, will be invited for a 2-part screening visit: Visit 1a will be remote and will include electronic signatures for written consent via REDCap e-consent template obtained by a study clinician, and Visit 1b will be in-person for a brief physical exam and blood draw.

Provide details of remuneration, when applicable. Even when subjects may derive medical benefit from participation, it is often the case that extra hospital visits, meals at the hospital, parking fees or other inconveniences will result in additional out-of-pocket expenses related to study participation. Investigators may wish to consider providing reimbursement for such expenses when funding is available

Study participants will be compensated for the time, effort, and study procedure risks involved in this intensive protocol. Participants will receive up to $8,055 by check, upon completing the study. We anticipate that some women will not be eligible after completing screening procedures or will decline to continue to participate after Sleep Block 1.

Subjects will be paid at end of the study and will accumulate compensation according to the following schedule.

Screening visits: up to $100 ($25 for completing Visit 1a and $75 for completing Visit 1b)
DXA Scan: $55
Lupron Administration Visit: $250
Inpatient data collection: $275 per night spent in lab
Outpatient data collection: up to $700 per Sleep Block ($50/day; up to 7 days prior to and 7 days after each Sleep Block)
Bonus for completion of all study visits and procedures: $3500

If subjects do not complete the study, they will be paid for the portion of the study completed but will not receive any study bonus.

Subjects will not have to pay for any meals during their inpatient stays. Reimbursement for transportation home after each of the 2 inpatient visits will be reimbursed up to $25 each with receipts (total up to $50). Valet parking vouchers will be provided for outpatient visits.

Subjects in the pilot study will be compensated $50 for the screening visit and $250 for each night they stay in the unit (maximum compensation = $800).

CONSENT PROCEDURES

Explain in detail how, when, where, and by whom consent is obtained, and the timing of consent (i.e., how long subjects will be given to consider participation). For most studies involving more than minimal risk and all studies involving investigational drugs/devices, a licensed physician investigator must obtain informed consent. When subjects are to be enrolled from among the investigators' own patients, describe how the potential for coercion will be avoided.

Subjects will not undergo any study procedures until it is clear that they understand the risks and benefits and consent to participate. At the remote screening visit (V1a), a research physician will review the consent form with prospective subjects. Women enrolling in the study must be capable of understanding the nature of the study as well as the discomforts and potential benefits. Any questions or areas of concern regarding the protocol or alternative treatment options will be addressed by one of the physician investigators. If a woman feels comfortable with the study procedures, she will sign the consent form along with the physician investigator using the electronic REDCap e-consent template. The consent form will include information about the risks and benefits of study participation and contact information for the study physicians and research staff. The study protocol, consent forms, telephone screens, and all other study procedures will be approved by the IRB before the study is initiated.
Enrolled subjects will be given a copy of the completed consent form before any study procedures are completed, and a copy of the signed consent form will also be placed in the subject's research file. If subjects have reservations about study participation at the first visit, they have the option to take the consent form home to review the decision to participate in the study again with research physicians before signing the consent form and initiating study procedures.

Additional considerations: Because of the nature of drug administration to healthy volunteers, the

informed consent process will include a discussion about the use of leuprolide for FDA- approved indications in premenopausal women (e.g., endometriosis, fibroids). It will be made clear that study participants will have no direct health benefit from the treatment because they have no medical reason to receive the treatment. Participants will be informed that the motivation to administer a GnRH agonist to healthy volunteers is to understand metabolic consequences of sleep fragmentation associated with hot flashes induced by leuprolide, as well as that of hot flashes and estradiol withdrawal. It will be explained that a research participant retains the right to withdraw from a study at any point, but that the effects of the GnRH agonist on gonadal hormones cannot be reversed if she chooses to withdraw after receiving leuprolide because leuprolide is being administered in a depot form. Participants will also be informed that the study procedures are being conducted with the approval and monitoring of the IRB and FDA. They will also be told that if any abnormalities are detected as part of study procedures (e.g., on the DXA scan), results of these tests will be relayed to the subject and her physician (with her permission) so that follow up assessments and treatment can be undertaken if necessary.

DATA AND SAFETY MONITORING

Describe the plan for monitoring the data to ensure the safety of subjects. The plan should include a brief description of (1) the safety and/or efficacy data that will be reviewed; (2) the planned frequency of review; and (3) who will be responsible for this review and for determining whether the research should be altered or stopped. Include a brief description of any stopping rules for the study, when appropriate. Depending upon the risk, size and complexity of the study, the investigator, an expert group, an independent Data and Safety Monitoring Board (DSMB) or others might be assigned primary responsibility for this monitoring activity. NOTE: Regardless of data and safety monitoring plans by the sponsor or others, the principal investigator is ultimately responsible for protecting the rights, safety, and welfare of subjects under his/her care.

We will continue to use the Safety Monitoring Group (SMG) that we set up for prior studies using leuprolide in healthy volunteers, consistent with regulatory guidelines. This Group will continue to serve as the SMG for the proposed project as well, meeting approximately every 6 months to review the progress of the study. The Group comprises two research physicians at Massachusetts General Hospital, Harvard Medical School, who are familiar with clinical research and the study medications and their side effects, but they are not collaborators or close colleagues of the PI or co-investigators. The two physicians are a neuro-endocrinologist ([redacted]) and a psychiatrist/ neurologist ([redacted]). Drs. [redacted] will review all adverse events and unanticipated problems that arise during the study. Adverse events, including serious adverse events, and unanticipated problems will be managed and reported to our local Institutional Review Board consistent with their guidelines. We have also amended Dr. Joffe's IND (#73144) to link it with this study and will continue to submit annual reports, including IRB updates and SMG minutes, to the FDA as part of the safety monitoring of this IND.

Describe the plan to be followed by the Principal Investigator/study staff for review of adverse events experienced by subjects under his/her care, and when applicable, for review of sponsor safety reports and DSMB reports. Describe the plan for reporting adverse events to the sponsor and the Partners' IRB and, when applicable, for submitting sponsor safety reports and DSMB reports to the

Partners' IRBs. When the investigator is also the sponsor of the IND/IDE, include the plan for reporting of adverse events to the FDA and, when applicable, to investigators at other sites.

NOTE: In addition to the adverse event reporting requirements of the sponsor, the principal investigator must follow the Partners Human Research Committee guidelines for Adverse Event Reporting

Subjects will be closely monitored for adverse events at every visit. Research staff will record any serious or non-serious adverse events using standard spontaneous IRB-approved reporting procedures. Participants will be informed that they can contact the investigator between visits if they have concerns about potential side effects. Routine adverse events and serious adverse events will be reported to the FDA and Human Research Committee in accordance with the FDA and Human Research Committee reporting guidelines, following the timeframes specified by the Partners Investigator's Guidelines. In addition, the DSMG will review all adverse events and investigate those considered serious and unexpected consistent with standard ISM procedures. In case of a serious adverse event, study procedures will be discontinued and appropriate diagnostic and therapeutic measures will be taken, as decided by the investigator. All adverse event reports will be reviewed by the principal investigator.

MONITORING AND QUALITY ASSURANCE

Describe the plan to be followed by the principal investigator/study staff to monitor and assure the validity and integrity of the data and adherence to the IRB-approved protocol. Specify who will be responsible for monitoring, and the planned frequency of monitoring. For example, specify who will review the accuracy and completeness of case report form entries, source documents, and informed consent.

NOTE: Regardless of monitoring plans by the sponsor or others, the principal investigator is ultimately responsible for ensuring that the study is conducted at his/her investigative site in accordance with the IRB-approved protocol, and applicable regulations and requirements of the IRB.

The PI will meet with the study coordinator on a weekly basis to review and monitor the validity and integrity of all data in study binders and adherence to the IRB-approved protocol. A checklist will also be included in the binders for each subject at each study visit. This checklist will be completed by the research coordinator and reviewed by the PI to confirm that all data are collected. In addition, all study personnel will meet on a monthly basis to review study data and discuss QA issues related to protocol adherence. Forms available on the IRB website will be used to keep track of adverse events and minor protocol violations.

PRIVACY AND CONFIDENTIALITY

Describe methods used to protect the privacy of subjects and maintain confidentiality of data collected. This typically includes such practices as substituting codes for names and/or medical record numbers; removing face sheets or other identifiers from completed surveys/questionnaires; proper disposal of printed computer data; limited access to study data; use of password-protected computer databases; training for research staff on the importance of confidentiality of data, and storing research records in a secure location.

NOTE: Additional measures, such as obtaining a Certificate of Confidentiality, should be considered and are strongly encouraged when the research involves the collection of sensitive data, such as sexual, criminal or illegal behaviors.

Confidentiality will be maintained by storing data with a subject identification number only. Only the PI, co-investigators, and study staff will have access to the database and the study binders. Data will be entered into a secure, HIPAA-compliant Research Electronic Data Capture (REDCap) database hosted by Partners HealthCare and analyzed using STATA 14.0 (College Station, Texas). All exported data will be de-identified using only the subject's identification number. DXA data will be reviewed by experts in bone densitometry and a radiologist, respectively. Analysis of all study data will be conducted by co-investigators/project leaders (MD/PhD level) under the purview of Dr. Joffe in consultation with [redacted] the statistical consultant on this project.

SENDING SPECIMENS/DATA TO RESEARCH COLLABORATORS OUTSIDE PARTNERS

Specimens or data collected by Partners investigators will be sent to research collaborators outside Partners, indicate to whom specimens/data will be sent, what information will be sent, and whether the specimens/data will contain identifiers that could be used by the outside collaborators to link the specimens/data to individual subjects.

De-identified data will be sent to our statistical consultant working with us at University of Massachusetts Medical School[redacted] Deidentified lab specimens will be sent to[redacted] at Medical College of Wisconsin and the NSF International laboratory for assays. NSF International is not considered a collaborator; rather, they are fee-for-service. All study materials will be labeled with a de-identified study ID only. No information that could be used to link the data to an individual subject will be sent to[redacted] or NSF.

Specifically address whether specimens/data will be stored at collaborating sites outside Partners for future use not described in the protocol. Include whether subjects can withdraw their specimens/data, and how they would do so. When appropriate, submit documentation of IRB approval from the recipient institution.

No data or specimens will be stored at collaborating sites outside partners. Participants will be asked for permission to store serum samples and health information for future research related to menopause and sleep disturbance at Brigham and Women's Hospital. Participants may withdraw their consent to store samples at any time.

RECEIVING SPECIMENS/DATA FROM RESEARCH COLLABORATORS OUTSIDE PARTNERS

When specimens or data collected by research collaborators outside Partners will be sent to Partners investigators, indicate from where the specimens/data will be obtained and whether the specimens/data will contain identifiers that could be used by Partners investigators to link the specimens/data to individual subjects. When appropriate, submit documentation of IRB approval and a copy of the IRB-approved consent form from the institution where the specimens/data were collected.

We will not be receiving specimens or data collected by research collaborators outside Partners.