COMIRB Protocol

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Protocol #: 16-1479
Project Title: The impact of estrogen status on the biological function of brown adipose tissue in women measured using quantitative PET/CT
Principal Investigator: Edward Melanson, Ph.D.
Version Date: 2/7/2022

I. **Hypotheses and Specific Aims**: The physiological relevance of brown adipose tissue (BAT) in humans is largely unknown. We have shown that suppressing ovarian function in premenopausal women reduces resting energy expenditure (REE), and this is prevented by adding back estradiol (E_2). Our preliminary data suggest that this may be due, in part, to reduced brown adipose tissue (BAT) activity. Our overarching hypothesis is that BAT activity in humans is modulated by E_2. To determine if natural declines in endogenous E_2 contribute to changes in BAT activity, we will compare BAT activity in pre-and post-menopausal women. We will also explore whether suppression of ovarian hormones in pre-menopausal women impairs BAT activity. BAT activity will be quantified using dynamic positron emission topography/computed tomography (PET/CT) imaging combined with ^{11}C-acetate tracers. We will assess the thermogenic response of BAT by measuring cold-induced changes in REE, shivering, and skin and core temperature.

SA1: To determine if menopausal state influences constitutive BAT oxidative metabolism and activity volume under thermoneutral conditions (room temperature).

$H1_a$: BAT oxidative metabolism and activity volume will be greater in pre- than postmenopausal women
$H1_b$: REE will be associated with BAT oxidative metabolism and activity volume.

SA2: **To determine if menopausal state influences stimulated BAT oxidative metabolism and activity volume in response to very mild cold exposure**.

$H2_a$: In response to a mild cold exposure, BAT oxidative metabolism and activity volume will be greater in pre- than postmenopausal women

SA3 (exploratory): To determine whether 6 mo. $GnRH_{AG}$ in premenopausal women attenuates basal and stimulated BAT oxidative metabolism and activity volume.

$H3_a$: Basal and cold-stimulated BAT oxidative metabolism and activity volume will be reduced after $GnRH_{AG}$.
$H3_b$: The decrease in REE after $GnRH_{AG}$ will be associated with the decrease in BAT oxidative metabolism and activity volume.

II. **Background and Significance**: In rodents, the loss of ovarian function disrupts energy balance resulting in excess fat gain, and that this is prevented or reversed by estradiol (E_2).[71,75,89] In premenopausal women, we have shown that suppressing ovarian function decreases in REE (~50 kcal/d),[22,49] independent of changes in body composition,[22] which may contribute to the risk for weight gain and obesity-related diseases that occurs with menopause.[35,45,88] We have also shown that replacing E_2 prevents this decrease in REE.[49] ***However, the mechanisms by which the loss of E_2 suppresses REE in women remain unknown.***

In humans, assessing the metabolic activity of BAT *in vivo* is challenging. Most studies have assessed ^{18}F fluoro-2-deoxyglucose (^{18}FDG) uptake using PET/CT.[4,38,39] This approach provides an indication of the volume of active BAT, but is not a quantitative marker of BAT metabolic activity.[4] In contrast, dynamic PET imaging (i.e., series of frames over contiguous time intervals) combined with metabolic tracers (e.g., ^{11}C-acetate) provides a quantitative measure of *in vivo* BAT metabolic activity. Using this approach, we have confirmed that BAT metabolic activity (clearance rate of ^{11}C in BAT after ^{11}C-acetate administration) increases in men exposed to a mild cold stimulus.[6,58] We have also demonstrated that BAT is

metabolically active in humans under thermoneutral conditions,[6] but have not yet quantified the contribution of this activity to REE.

Our overarching hypothesis is that BAT activity in humans is modulated by E_2. Specifically, we will determine if BAT metabolic activity contributes to resting energy expenditure (REE), and whether BAT metabolic activity under basal and stimulated conditions is modulated by estrogen status. We will assess the contribution of BAT metabolic activity to REE under both basal (thermoneutral) and stimulated (cold exposure) conditions. We will compare BAT oxidative metabolism (^{11}C clearance rate in BAT) and BAT activity volume (volume of ^{18}FDG uptake) in pre- and postmenopausal women, and in a subset of premenopausal women after 6 mo. of ovarian hormone suppression using gonadotropin releasing hormone agonist ($GnRH_{AG}$) treatment.

III. **Preliminary Studies/Progress Report:**

Estrogen and fat gain: Basic and preclinical studies provide evidence that the loss of E_2 or E_2 signaling causes excess fat gain[32,37,72,89,90] which is prevented by E_2 treatment.[50] In humans, suppression of gonadal function by gonadotropin releasing hormone agonist ($GnRH_{AG}$) therapy causes excess fat gain[24-26,65,90] and abdominal fat accrual.[75] In both women[75] and men,[26] evidence suggests that such changes are specifically related to E_2 deficiency. A system-level mechanism that contributes to excess fat gain in response to E_2 suppression is a reduction in total energy expenditure (TEE).[13,90] We have demonstrated that, like OVX and disruption of E_2 signaling in animals, suppression of ovarian function in women causes a decrease in REE and TEE. We have used a pharmacological approach (gonadotropin releasing hormone agonist, $GnRH_{AG}$) to suppress ovarian function to explore the role of E_2 in modulating REE. In our first study in premenopausal women (N=14), REE was significantly lower after 6 days of sex hormone suppression (1334±36 kcal/d, mean±SE) when compared with the mid-luteal phase (1405±42 kcal/d) or the early follicular phase (1376±43 kcal/d).[22] This also reduced SNS support of REE.[21] In our recently completed study,[49,75] 70 premenopausal women were randomized to 5 months of $GnRH_{AG}$ with ($GnRH_{AG}$+E_2) or without ($GnRH_{AG}$+PL) E_2 add back. Our major findings were that $GnRH_{AG}$+PL caused a decrease in REE (-57±105 kcal/d), which was prevented by E_2 (-4±75 kcal/d). Total daily energy expenditure (TDEE) was also decreased in response to $GnRH_{AG}$+PL (-128±42 kcal/d), but this was not fully prevented by $GnRH_{AG}$+E_2 (-96±30 kcal/d) (Figure 1).

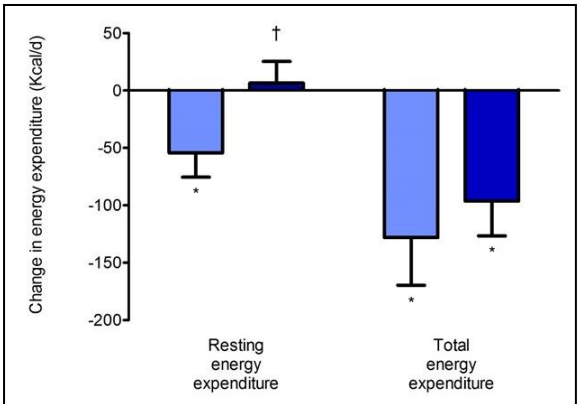

Fig 1. Change in resting (REE) and total EE (TEE) in pre-menopausal women after 5 mo. $GnRH_{AG}$ treatment with ($GnRH_{AG}$+E_2 dark bars) or without ($GnRH_{AG}$+PL, light bars) E_2 add back. * P<0.05 vs. baseline; † P<0.05 vs. $GnRH_{AG}$+PL

Evidence for BAT in adult humans: The primary function of BAT is to produce heat. BAT is highly vascularized and richly innervated by the SNS.[66] When activated, BAT generates heat through mitochondrial uncoupling of oxidation and phosphorylation, mediated by UCP1 that is uniquely and abundantly expressed in BAT mitochondria. BAT activity is stimulated by factors that increase SNS activity, such as acute cold exposure.[91,92] Even exposure to room temperature (~22 °C) may be sufficient to stimulate BAT activity.[5,6,15,16,19,58,83,86,91,92] BAT is present in rodents and other mammals throughout the lifecycle.[14] Until recently, BAT in humans was thought to be present in significant volume and activity only in infants. This was despite the existence of necropsy studies demonstrating that BAT persisted in adults, albeit in fewer sites and smaller volumes.[31] This view began to shift in the early 2000's, as radiological reports documented non-tumor related ^{18}FDG uptake in tissues with low radiodensity (indicative of an adipose tissue).[57] In these studies, bilateral, symmetrical ^{18}FDG uptake was often observed in the cervical, clavicular, and paraspinal regions. Because these areas were within fat depots (based on CT Hounsfield units), and were more pronounced when patients were not kept warm, many nuclear imaging departments began referring to these as BAT. Subsequent studies established that ^{18}FDG uptake in these areas was stimulated by cold exposure,[73,83] and biopsy studies confirmed these areas as BAT via morphological assessment and identification of UCP1.[20,86] The reported prevalence of adults with detectable BAT varies markedly. Retrospective analysis of large cohorts who had undergone clinical

PET/CT scanning suggests that the prevalence of spontaneously detectable BAT is 5-10%, and identified a number of factors that increase the prevalence of spontaneously detectable BAT including colder outdoor temperature, female sex, younger age, and lower BMI and body fat content.[1] Studies of cold exposed individuals have since shown that the prevalence of metabolically active BAT using ^{18}FDG is much higher, ranging from 30 to 100%.[5-7,58] Cold-induced BAT activity decreases with age,[47,71,93] but has been observed even in humans up to the age of 64 yrs.[5] Cold-induced BAT activity is inversely associated with body fatness,[47,83,85] suggesting that BAT plays a role in body weight regulation, but whether a decline BAT activity contributes to the development of human obesity is unclear.[2]

Most BAT studies in humans have used static PET/CT acquisitions to measure whole-body ^{18}FDG uptake, expressed as the standardized uptake value (SUV).[4,38] SUV is calculated as the average radioactivity concentration in a region of interest, relative to the injected dose and normalized to body weight. Traditionally, total volume of activity of ^{18}FDG uptake is used to provide estimates of BAT volume. However, there are several limitations to this approach: **1)** SUV is subject to many sources of variability including, but not limited to body composition, length of uptake period, and endogenous plasma glucose levels; **2)** ^{18}FDG uptake is influenced by the energy status of BAT (e.g. when BAT triglyceride (TG) levels are high, ^{18}FDG uptake is reduced); and **3)** BAT glucose uptake is associated with BAT blood flow but not thermogenesis.[4,36,40] Thus, static ^{18}FDG PET/CT acquisition helps determine the biodistribution of glucose to different organs under a given physiological condition (e.g. cold-exposure), but SUV is a semi-quantitative unit that does not characterize physiological functions. In contrast, quantitative dynamic PET/CT imaging combined with metabolic tracers involves the collection of a series of frames over contiguous time intervals, which are used to model physiological parameters such as rate of tracer uptake or clearance in different tissues.[55] For example, ^{18}FDG is used to quantify facilitated transport and hexokinase-mediated phosphorylation of glucose, whereas the rapid conversion of ^{11}C-acetate to acetyl-CoA and ultimate entry into the TCA cycle can provide a measure of tissue oxidative metabolism.[41] Combining static and dynamic PET/CT methods is essential to provide accurate and valid assessments of changes in BAT metabolic rate and glucose uptake.

The contribution of BAT to EE in humans: Our collaborators, Drs. Haman and Blondin, have shown that BAT oxidative metabolism contributes to the increased EE during acute cold exposure in humans. In one study, healthy young men (N=12, 23-42 yrs, BMI=24-31 kg·m^2) were studied during 3 hr. of mild cold exposure (18º C). Significant uptake of ^{18}FDG was noted in areas identified as BAT as well as in deep muscles of the thoracic region. Conversely, BAT oxidative metabolism (quantified based on the fast monoexponenital decay of ^{11}C-acetate in BAT, k_{mono}) increased significantly (two-fold) in BAT during cold-exposure, but not in adjacent skeletal muscles or subcutaneous adipose tissue (Figure 2).[6] This indicates a cold-induced activation of oxidative metabolism in BAT, but not skeletal muscle or subcutaneous adipose tissue. **Interestingly, an increased ^{11}C clearance rate was also observed in BAT during measurements obtained at room temperature, suggesting that BAT oxidative metabolism was active even under ambient conditions (Figure 3)**. There was an inverse association between BAT activity volume and shivering, suggesting those with greater BAT volumes produced more heat via non-shivering thermogenesis (NST). In another study, 25 men (22-64 yrs, BMI=23-35 kg·m^2) were studied using a similar protocol.[5] Even in older, overweight men with type 2 diabetes (T2DM) who demonstrate a lower ^{18}FDG uptake in BAT relative to young, healthy men, BAT oxidative metabolism was increased during cold exposure to similar levels as their young, healthy counterparts.

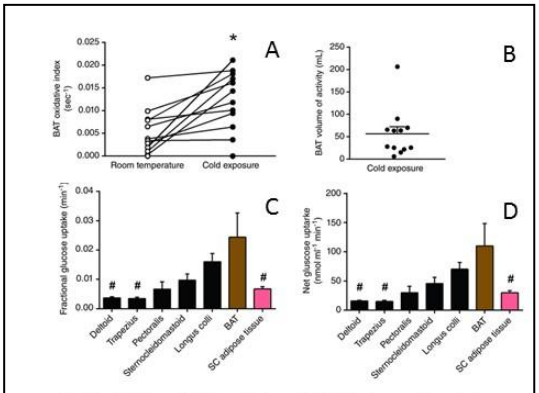

Fig 2. A) BAT oxidative index; B) BAT volume of activity; and C) fractional and D) net glucose uptake in cervicothoracic tissues during acute cold exposure (18 ºC). * vs. room Temperature (P<0.01); # vs. BAT (P<0.05). Blondin et al., J Physiol, 2015

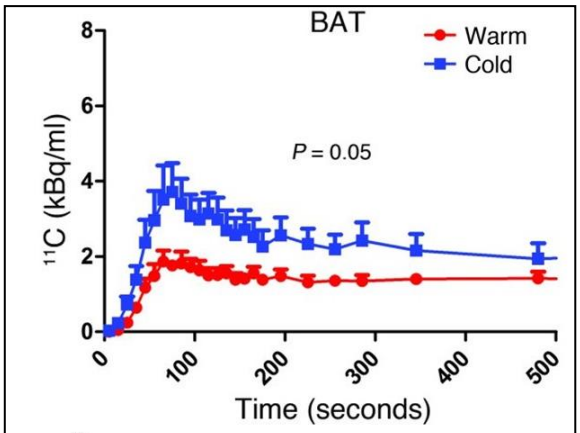

Fig 3. ^{11}C-acetate uptake during warm and cold-stimulated conditions. Ouellette et al., J Clin Invest, 2012

Sex differences in BAT: In rodents, BAT activity is typically higher in females than males.[69,81] In humans, retrospective studies have reported that the prevalence of BAT is greater in women than men but this sex difference diminishes with age, suggesting a potential role of female sex hormones.[1,18,19,59,63,64] Conversely, acute cold exposure studies in humans have suggested that the volume of metabolically active BAT and BAT glucose uptake are similar in men and women.[16] However, this may be attributed to limitations in the cold exposure protocols used. For example, one particular study recently reported that glucose uptake in BAT during a mild cold exposure was 50% lower in women compared to men. However, cold-induced thermogenesis (CIT, the increase in energy expenditure upon cold exposure) was also lower in women compared to men (0.3 vs. 0.9 kJ/min), suggesting the cold stimulus was not adequate to stimulate BAT in the women.[30]

Evidence for regulation of BAT by estrogens: Several lines of evidence suggest that sex hormones regulate BAT activity.[67,68,84,87] Androgens and estrogens exert opposing effects in brown adipocytes.[51,70] Testosterone reduces UCP1 expression and thermogenic activity, whereas E_2 has the opposite effects.[67,68,70] BAT function is compromised when ERα signaling is disrupted.[13,90] Additionally, OVX rats have decreased BAT thermogenic activity and UCP1 expression in brown adipocytes,[32,56,62,94] but these effects are reversed by E_2 treatment.[3,27,42,46,87]

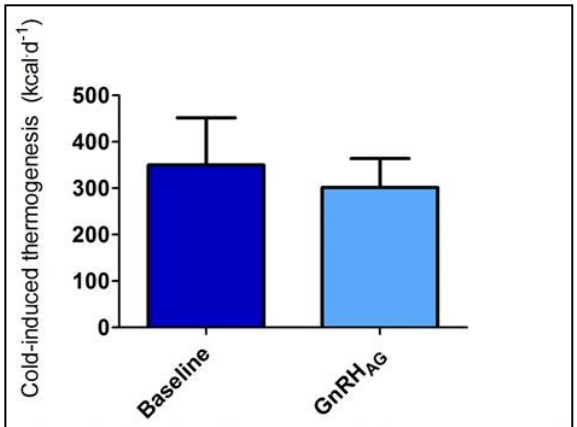

Fig 4. Cold-induced thermogenesis in pre-menopausal women before and after 3 mo. GnRHAG treatment.

CIT varies with E_2: We recently performed a pilot study in healthy, premenopausal women (n=5; 31±6 yr.; BMI 24.3±4.5 kg/m^2) to determine the effects of ovarian hormone suppression on REE, CIT, and BAT activity. Before and after 3 mo of GnRH$_{AG}$, participants underwent a 2-hr cooling protocol using a surgical cooling vest filled with water (~16-18º C). Following GnRH$_{AG}$, REE decreased from 1382±218 kcal/d to 1341±209 kcal/d; the decrease (-41±160 kcal/d) was similar in magnitude to that observed in response to 5 mo of GnRH$_{AG}$+PL in the study described above (-57±105 kcal/d). CIT was also attenuated (350±228 vs. 302±139 kcal/d, Figure 4). We also performed ^{18}FDG PET/CT measurements to demonstrate the feasibility of measuring BAT activity with this protocol. After GnRH$_{AG}$, no significant differences in ^{18}FDG uptake were observed, but ^{18}FDG uptake in BAT decreased in 3 of the 5 individuals studied (Figure 5). There are two limitations to consider in this pilot study. Firstly, the period of hormone suppression may have been too short to invoke substantial changes in BAT activity and CIT, because a single injection of GnRH$_{AG}$ produces an initial sex hormone flare (for up to 3 wk) followed by continuous suppression with additional doses of GnRH$_{AG}$. **In the proposed study, we will use a longer period of ovarian suppression (6 mo) (SA3, exploratory)**. Secondly, the use of ^{18}FDG PET/CT to study BAT metabolism is influenced by the energy status of the BAT. Because FM increased following GnRH$_{AG}$, BAT TG content may have also increased, which will reduce ^{18}FDG uptake independent of any changes in BAT metabolism.[4] **In the proposed study, in addition to ^{18}FDG, we will use PET to measure clearance of the tracer ^{11}C-acetate in BAT, which is not dependent on the energy status of the tissue and provides a direct measure of BAT thermogenesis.** We expect to see a more clear suppression of BAT thermogenesis with a more prolonged period of ovarian suppression (pre- vs. postmenopausal women, **SA1 and SA2**; 6 mo.

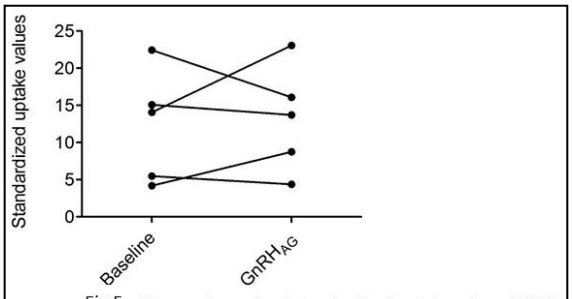

Fig 5. Change in maximal standardized uptake values (SUV) of ^{18}FDG in BAT in pre-menopausal women N=5) before and after 3 mo. GnRHAG treament.

GnRHAG, **SA3**) in combination with the use of ^{11}C-acetate PET methodologies.

IV. Research Methods

A. Outcome Measure(s): *The primary study outcome* is BAT oxidative metabolism (^{11}C-acetate clearance rate) during basal and cold-stimulated conditions. *Secondary outcomes* are BAT activity volume (^{18}FDG uptake) during basal and cold-stimulated conditions; CIT; ^{18}FDG update into muscle; shivering (sEMG & ^{18}FDG uptake in deep skeletal muscles); and changes in skin and core temperature during cold exposure. Serum concentrations of hormones and metabolites will be measured primarily as descriptive variables, but we will also explore the association of these measures with the primary study outcomes.

B. Description of Population to be Enrolled: Premenopausal volunteers will be healthy, eumenorrheic women, aged 18-50 yrs. Eumenorrheic status will be verified by regular menses (no missed cycles in previous year; cycle length 25-35 d). Postmenopausal volunteers will be healthy women who have no menses for at least 12 months; postmenopausal status will be confirmed based on FSH (≥50 mIU/ml). The primary **exclusion** criteria are:

- Pre- and Postmenopausal women
  - BMI>40 kg/m^2; we have purposely used a wide range of BMI to facilitate recruitment.
  - Thyroid dysfunction, defined as an ultrasensitive thyroid stimulating hormone (TSH) <0.5 or >5.0 mU/L (pre- and postmenopausal women); volunteers with abnormal TSH values will be re-considered for participation in the study after follow-up evaluation by the PCP with initiation or adjustment of thyroid hormone replacement.
  - Uncontrolled hypertension defined as resting systolic BP >150 mmHg or diastolic BP>90 mmHg; participants who do not meet these criteria at first screening will be re-evaluated, including after follow-up evaluation by the PCP with initiation or adjustment of anti-hypertensive medications
  - Self-reported diabetes or fasting glucose >126 mg/dL
  - Use of tobacco products (last 6 months)
  - Selective serotonin reuptake inhibitors (SSRIs) or any other medication or condition that may affect brown fat activity. The decision will be based on the judgement of the study physician and primary investigator.
  - Serious illness within the last 6 months
  - Confirmed positive COVID test within the last 6 months

- Additional exclusion criteria for postmenopausal women
  - Current use of hormonal replacement therapy or use <10 yrs ago.
  - Women who have undergone surgical menopause.

- Additional exclusion criteria for premenopausal women
  - Current hormonal contraceptive use (past 6 mo).
  - Pregnant, lactating or intention to become pregnant during the period of study
  - FSH and estrogen levels that suggest an individual may be approaching perimenopause (reviewed by PI and study MD)
  - Because of the risks associated with $GnRH_{AG}$ treatment, women who meet any of the following criteria will be excluded from the $GnRH_{AG}$ intervention study, but will be permitted to participate in the cross sectional study
    - History of fragility fracture
    - Low BMD (i.e., proximal femur or lumbar spine z scores < -2.0)
    - Abnormal vaginal bleeding
    - History of breast cancer or other estrogen-dependent neoplasms
    - History of venous thromboembolic events
    - Hypersensitivity to leuprolide acetate or benzyl alcohol (the vehicle for injection of leuprolide acetate)
    - Evidence for depressive symptoms (Score > 16 on the Center for Epidemiologic Studies Depression Scale, CESD)
    - Moderate or severe renal impairment defined as a calculated creatinine clearance <50 mL/min based on the equation of Cockcroft and Gault[91]

- Chronic hepatobiliary disease, conservatively defined as liver function tests (AST, ALT, alkaline phosphatase, Total Bilirubin) >1.5 times the upper limit of normal (if such values are obtained on initial screening and thought to be transient in nature, repeated testing will be allowed)

C. Study Design and Research Methods

Our hypothesis is that in humans, BAT activity is modulated by E_2. To test this hypothesis, we will compare BAT oxidative metabolism (rate of ^{11}C clearance in BAT) and BAT activity volume (volume of ^{18}FDG uptake) in healthy pre- and postmenopausal women (**SA1 and SA2)** under basal (thermoneutral) and stimulated (cold exposure) conditions. After providing informed consent, volunteers will undergo screening procedures. Volunteers who meet the inclusion/exclusion criteria (Section 2.3.5) will be invited to participate in the cross-sectional study (Table 1) to measure basal and cold-stimulated BAT activity (PET/CT). A subset of premenopausal women will also be studied after 6 mo. of $GnRH_{AG}$ treatment (**SA3, exploratory**).

Table 1. Schedule of Procedures.

	Cross Sectional Study (SA1-2) Pre- (N=32) vs. Post-menopausal (N=32)			**Intervention Study (SA3)** Pre-menopausal (N=15)			
Measurement	Screening (~2 hrs)	Visit #1 Thermoneutral (~5 hrs)	Vist #2 Cold (~6 hrs)	Visits (#3-8) (~1hr)	Visit #8a (~1 hr)	Visit #9 Themoneutral (~5 hrs)	Visit #10 Cold (~6 hrs)
Health history and physical exam	X						
Screening labs	X						
Urine Pregnancy Test	X[1]	X[1]	X[1]	X	X[1]	X[1]	X[1]
Blood chemistries	X*[5]		X[4]		X[4]		X[4]
DXA (body composition, bone mineral density) and waist circumference	X				X		
PET/CT studies		X[2]	X[3]			X[2]	X[3]
Monthly $GnRH_{AG}$ injections				X			

[1] – Performed by the PRA

[2] - A single dose of ^{11}C-acetate (185 MBq) will be administered, followed by a regional CT and dynamic PET scan (nose to aortic arch)

[3] – A dose of ^{11}C-acetate (185 MBq) will be administered, followed by a regional CT and dynamic PET scan (nose to aortic arch). A dose of ^{18}F-FDG (185 MBq) will then be administered, followed by another dynamic PET scan, and then whole body static PET and whole body CT scan (eye level to knee caps)

[4] – blood samples will be obtained before and after the cold exposure study

[*5] – FMD/CIMT/blood draw procedures will be obtained at the screening visit or Visit #1

[+] – Waist circumference obtained)

D. Description, Risks and Justification of Procedures and Data Collection Tools:

Pre-enrollment screening - The initial interaction is initiated by individuals who read or hear an advertisement and contact staff to learn more about the study. A brief phone screen will be conducted. Callers who meet general study criteria and are interested in learning more about the study will be scheduled for an orientation session. Volunteers will be sent a copy of the consent form in advance. Individuals may also be recruited through the VA database. An initial letter and informational flyer may be sent by U.S. mail inviting individuals to participate.

Orientation and informed consent – Interested volunteers will meet with a member of the research team to **a)** have the study and what is expected of them explained in detail; **b)** discuss any practical problems (e.g., scheduling conflicts, vacations) that could interfere with participation; **c)** have their questions

answered; and **d)** demonstrate their ability to provide informed consent by describing their understanding of the major study goals and what is expected of them if they choose to participate. Written, signed informed consent and HIPAA authorization will be obtained from volunteers willing to participate in the study.

Screening - After providing informed consent, volunteers will undergo screening procedures, including a review of medical history (including menstrual cycle history), and a clinical laboratory evaluation including fasting glucose, creatinine, liver function tests, thyroid function tests, and a complete blood count. TSH will be measured in the Clinical and Translational Research Center (CTRC) core lab; other chemistries (metabolic panel, CBC) will be performed by the University of Colorado Hospital laboratory. This will be followed by measurement of bone mineral density (BMD). Premenopausal volunteers with a history of fragility fracture or low BMD (lumbar spine or proximal femur z-scores ≤ –2.0) will not be enrolled in the intervention study because of the possibility that BMD will decrease during $GnRH_{AG.}$

Serum hormones and metabolites - At study entry and at the end of the intervention (on the day of the cold exposure study), fasted blood samples will be obtained for the measurement of estrone (E_1), E_2, progesterone, testosterone, sex hormone binding globulin (SHBG), follicular stimulating hormone (FSH), leptin, cortisol, free fatty acids, glycerol, and thyroid hormones (T3, T4). An additional sample will be obtained at the end of the cooling protocol (at minute 120, before the 11C acetate infusion) for measurement of T3, T4, TSH, free fatty acids and glycerol. Changes in these parameters will be evaluated for correlation with the changes in the primary outcomes. In rodents, BAT activation improves glucose tolerance.[76,82] In humans, BAT activity is inversely associated with fasting blood glucose and hemoglobin A1c (HbA1c),[47] and cold acclimation induces improvements in insulin sensitivity.[43] Thus, samples will also be obtained for the measurement of glucose and insulin for the assessment of insulin resistance by the homeostasis model.[48] Assays will be performed in the Clinical and Translational Research Center (CTRC) core lab. Samples will be stored at -80º C.

Dual-energy X-ray Absorptiometry (DXA) – Body composition and BMD will be measured using a Hologic Discovery DXA instrument (Waltham, MA).

Waist Circumference (WC) – will be measured using a spring-loaded tape measure. Circumference will be measured at baseline, Visit 3-8, and after 6 months of suppression.

Experimental procedures – PET/CT imaging studies will be performed on two separate days. These studies will be performed as close as possible to each other (e.g., within 1-2 weeks) and within the luteal phase for pre-menopausal women). Studies will be performed in the morning (~7 am). Participants will be asked to fast overnight (12 h) and refrain from strenuous physical activity for 48 h prior to the study day. A urine pregnancy test will be performed (premenopausal women) prior to initiating study procedures. Clothing will be standardized, with participants clad only in slippers socks, shorts, and a short sleeve t-shirt. During Visit #1, BAT activity under thermoneutral (room temperature) conditions will be determined by administering a single dose of ^{11}C-acetate and quantifying the decay using dynamic PET. A regional CT scan will also be performed to define the regions of interest. During Visit #2, BAT activity following cold-exposure will be determined by administering a single dose of ^{11}C-acetate and quantifying the decay using dynamic PET. BAT volume and activity will then be determined by administering a single dose of ^{18}F-FDG followed by another dynamic PET scan, and then whole body static PET and whole body CT scan (eye level to knee caps)

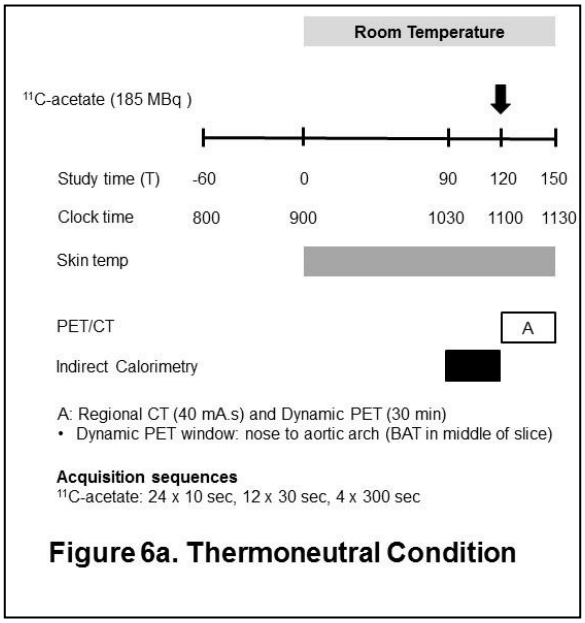

Figure 6a. Thermoneutral Condition

Visit #1 (Thermoneutral conditions, Figure 6a) – Participants will report to the Colorado Research Imaging Center at 8:00 AM. Upon arrival, a urine pregnancy test will be performed in pre-menopausal

volunteers. A fasted blood sample will be obtained for the measurement respiration and protein measures of mitochondrial markers, then FMD and C-IMT will be performed.

Participants will be escorted to the CTRIC imaging center and be instrumented with wireless skin temperature sensors (Thermochron iButton model DS1922H; Maxim integrated, San Jose, CA, USA) and sEMG electrodes (Delsys, EMG System, Natick, MA, USA). Indwelling catheters will then be placed in an antecubital vein for tracer infusions. Participants rest in a supine position under thermoneutral conditions (~25°C) for 120 min. After 90 min, REE will be measured using indirect calorimetry with a ventilated hood. At 120 min, BAT oxidative metabolism will be determined by administering a ~185 MBq bolus of ^{11}C-acetate followed by a 30 min list-mode dynamic PET acquisition and a regional CT scan (40 mA.s) centered at the cervico-thoracic junction to correct for attenuation and to define PET regions of interest (ROI).

Visit #2 (Cold exposure, Figure 6b) – Participants will report to the Colorado Research Imaging Center at 8:00 AM. Upon arrival, a urine pregnancy test will be performed in premenopausal women. A fasted blood sample will then be obtained for the measurement of estrone (E_1), E_2, progesterone, testosterone, sex hormone binding globulin (SHBG), FSH, leptin, cortisol, free fatty acids, glycerol, and thyroid hormones (total T3, free T4). Changes in these parameters will be evaluated for correlation with the changes in the primary outcomes. In rodents, BAT activation improves glucose tolerance.[76,82] In humans, BAT activity is inversely associated with fasting blood glucose and hemoglobin A1c (HbA1c),[47] and cold acclimation induces improvements in insulin sensitivity.[43] Thus, samples will also be obtained for the measurement of glucose and insulin for the assessment of insulin resistance by the homeostasis model.[48] Assays will be performed in the CTRC core lab. Samples will be stored at -80º C. Participants will be escorted to the CTRIC imaging center and then be instrumented with wireless temperature sensors (Thermochron iButton model DS1922H; Maxim integrated, San Jose, CA, USA) and sEMG electrodes (Delsys, EMG System, Natick, MA, USA). Temperature sensors will be placed on the forehead, chest, forearm, back of the hand, lower back and quadriceps to measure mean skin temperature.[60] sEMG electrodes will be placed on the belly of 8 large muscles known to contribute significantly to shivering during cold exposure (*m. trapezius, m. deltoideus, m. sternoclastomastoid, m. pectoralis major, m. rectus abdominis, m. vastus lateralis, m. vastus medialis, m. rectus femoris*).[29] Participants will then be fitted with the liquid-conditioned suit, swallow a telemetric thermometry capsule to measure core temperature (Vital Sense monitor and Jonah temperature capsule, Mini Mitter Co., Inc., Bend, OR, USA) and will perform a series of maximal voluntary contractions (MVCs) of each of the eight muscles being recorded by sEMG for normalization of the shivering measures. Indwelling catheters will then be placed in an antecubital vein for tracer infusions.

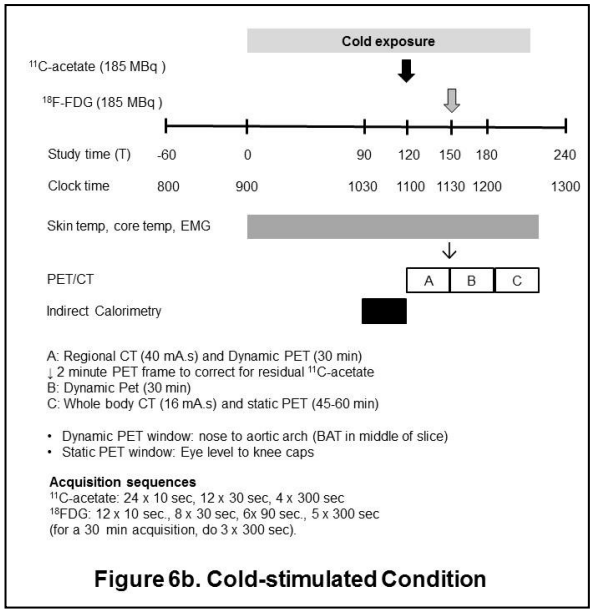

Figure 6b. Cold-stimulated Condition

The liquid-conditioned suit will be perfused with 18° C water for 210 min (min 0-210). This is expected to induce a thermogenic response (~75% increase in REE) but minimize shivering. In our previous study using the same cold stimulus, mean skin temperature was 33.1±0.3º C at room temperature, and decreased to 28.6±0.3 ºC during cold exposure; shivering, measured using sEMG was minimal (1.6% of MVC); and REE increased from 6.7±0.3 to 12.2±0.8 kj·min^{-1} (~2300 to 4170 kcal·d^{-1}).[6] This suggests that the contribution of NST to CIT was substantial and at its highest level.[9] Participants will be monitored continuously for shivering using sEMG collected using a digital recorder (Trigno Wireless EMG System, Delsys, Inc., Boston, MA). REE will then be measured at minute 90 to determine CIT (cold-simulated REE – baseline REE). At time 120 min, a fasted blood sample will be obtained for the measurement of thyroid hormones (TSH, total T3, free T4) and markers of lyploysis (free fatty acids and glycerol). Following the blood sample, a ~185 MBq bolus of ^{11}C-acetate will be injected intravenously, followed by a 30 min list-mode dynamic PET acquisition. This will be immediately followed by a regional CT scan

(40 mA.s) centered at the cervico-thoracic junction to correct for attenuation and to define PET ROI. At time 150 minute, a 2 minute PET frame will be performed to account for any residual ^{11}C-acetate. A ~185 MBq i.v. bolus of ^{18}FDG will then be administered followed by a 30 minute dynamic mode PET scan. At time 180 min, participants will be instructed to void to minimize radiation exposure (30 min. after ^{18}FDG administration). A whole body CT scan (16 mA.s) and whole body static PET acquisition will then be performed to determine whole body ^{18}FDG organ distribution and tissue SUV. To minimize radiation exposure, participants will be instructed to void a second time as soon as the study is completed (2 hrs. after ^{18}FDG administration).

Pre-menopausal women participating in the intervention study will repeat the PET/CT studies after 6 month of ovarian hormone suppression (Visits #9 and 10).

PET/CT image analysis - Dr. Scherzinger, a co-I in the Department of Radiology, will oversee data acquisition of all PET images and analysis of whole-body FDG PET acquisitions, with the guidance of our collaborator Dr. Denis Blondin. Drs. Blondin and Dr. Carpentier will provide additional guidance in the analysis and interpretation of the ^{11}C-acetate data and the ^{18}FDG dynamic PET data.

The volume of supraclavicular BAT will be determined according to the following criteria: tissue radio density between −30 and −150 Hounsfield units and ^{18}FDG uptake during cold exposure of more than 1.5 SUV units.[7,44] BAT volume of ^{18}FDG will be quantified by from the voxel size of the image (i.e., in-plane length x width of the pixel x slice thickness), and BAT mass and activity will be quantified from volume using the assumption of a density of fat of 0.90 g/mL.[58,59] The maximal and mean SUV, defined as the radioactivity per mm within the regions of interest, divided by the injected dose in MBq/kg BW, will be determined. Tissue-specific BAT oxidative metabolism index (the rapid fractional tissue clearance of ^{11}C-acetate, k, in s^{-1}) will be estimated from BAT ^{11}C activity over time using a monoexponential fit from the time of peak tissue activity.[11] Total BAT oxidative metabolism index will be calculated as the product of the total volume of supraclavicular BAT and BAT oxidative metabolism index.[7] BAT fractional glucose uptake (K_i) will be determined by Patlak linearization analysis of the ^{18}FDG data.[61] BAT glucose uptake (K_m) will be calculated by multiplying K_i glucose by plasma glucose levels, which assumes a lump constant value of 1.[77] Drs. Blondin and Carpentier will oversee data analysis of ^{11}C-acetate dynamic PET acquisitions.

sEMG - Raw EMG signals (collected at 1000 Hz) will be filtered to remove spectral components <20 Hz and >500 Hz, as well as 60 Hz contamination and related harmonics, and analyzed using custom-designed MATLAB algorithms (Mathworks, Natick, MA, USA). Shivering intensity of individual muscles will be determined from root-mean-square (RMS) values. Baseline RMS values ($RMS_{baseline}$: 5 min RMS average measured prior to cold exposure) will be subtracted from shivering RMS (RMS_{shiv}) values and RMS values obtained from the maximal voluntary contractions of individual muscles (RMS_{mvc}). Shivering intensity will be normalized to RMS_{mvc}, as previously described.[5] sEMG data will be analyzed in Dr. Haman's laboratory.

$GnRH_{AG}$ therapy – Fifteen premenopausal women will be recruited from the cross-sectional study to participate in the longitudinal study. Following the next menses after baseline testing, participants will initiate 6 mo of $GnRH_{AG}$ therapy (intramuscular injection of leuprolide acetate 3.75 mg for depot suspension; Lupron; TAP Pharmaceutical Products, Inc.; Lake Forest, IL) to chronically suppress ovarian hormones. A single injection of leuprolide acetate produces an initial stimulation (for up to 3 wk) followed by a prolonged suppression of pituitary gonadotropins and ovarian hormones. Repeated monthly dosing suppresses ovarian hormone secretion. $GnRH_{AG}$ therapy has been used clinically for up to 1 year without serious adverse events, although there is a decrease in BMD that recovers following cessation of treatment.[34,79] The drug is used for the management of endometriosis and for pre-operative management of anemia caused by uterine fibroids. Side effects are those typical of hypoestrogenemia. In studies of women with uterine fibroids, $GnRH_{AG}$ 3.75 mg/mo. induced amenorrhea in 61%, 86%, and 90% of women after 1, 2, and 3 mo of treatment, respectively.[28,74] Return of menses typically occurs within 1 to 3 mo of cessation of therapy.

Absence of pregnancy (urine test) will be confirmed before each dosing. Participants will be observed for 30 min after drug injection to monitor for hypersensitivity reactions. Women who do not tolerate $GnRH_{AG}$ therapy in the proposed study can refuse further treatment after each monthly dosing. Among the first 94 women treated with $GnRH_{AG}$ in our previous and ongoing studies (R01 AG018198, P50 HD073063), this

occurred only once. Participants will also meet with the study research nurse practitioner, providing a regular mechanism by which adverse events will be queried. A health status questionnaire will be used to track changes in use of medications (prescription and over-the-counter) or health (e.g., doctor visits, hospitalizations), as well as any study-related problems/concerns over the past 4 weeks. Participants will also complete a Menopausal Symptom List (MSL) to monitor the frequency and severity of menopause-related symptoms (e.g., hot flashes, depressed feelings); the Eating Behavior Questionnaire (EBQ) to monitor dietary restraint, disinhibition, and hunger;[78] and the Pittsburgh Sleep Quality Index (PSQI) to monitor sleeping behavior.[12]

Potential risks

- *Leuprolide acetate* – A subset of premenopausal women will receive $GnRH_{AG}$ (leuprolide acetate) 3.75 mg/mo by intramuscular injection every 4 weeks for 24 weeks (6 doses).
    - Contraindications for use include: hypersensitivity to GnRH, GnRH agonist analogs or any of the excipients in Lupron Depot; undiagnosed vaginal bleeding; known or suspected pregnancy; lactation.
    - In women who are pregnant, use of leuprolide acetate may cause fetal abnormalities.
    - Leuprolide acetate may cause an anaphylactic reaction in volunteers with hypersensitivity to GnRH.
    - Regular menstruation should stop during leuprolide acetate therapy but spotting or breakthrough bleeding may occur. Cessation of menses does not ensure that pregnancy will not occur. Normal menstrual function is usually restored within 2-3 months after therapy is discontinued.
    - Leuprolide acetate may cause symptoms related to hypoestrogenism including hot flashes, headaches, nausea, emotional lability, decreased libido, acne, myalgia, sleep disorders, reduction in breast size, hypertension, and vaginal dryness. Bone loss may occur, although the amount lost over the 24-week intervention should be recovered slowly after discontinuation of leuprolide acetate treatment. There may be a development or worsening of depression and the occurrence of memory disorders
    - In rare cases, leuprolide acetate may cause seizures (post-marketing concern).
    - Leuprolide acetate therapy for 24 weeks may cause an increase in FM (~1.0-1.5 kg), a decrease in fat-free mass (1-1.5 kg), an increase in total, LDL, and HDL cholesterol (5-10%), and an increase in triglycerides (15-20%).
    - Local injection site reactions including induration and abscess may occur.
- *Risk of radiation exposure*: Participants will be exposed to radiation during the PET/CT and DXA scans. During the PET/CT scans, participants are exposed to radiation from the CT and from the radiopharmaceuticals. The primary risk of this level of radiation exposure is to the unborn fetus of pregnant women. All participants will perform one DXA and PET/CT study, and a subset of premenopausal women will perform these tests twice. The estimated radiation exposure during each study visit is listed in the table below

Table 2. Estimated radiation exposure (mSv)

Visit #	C-11	F-18	CT	DXA
0				0.015
1	0.91		0.96	
2	0.91	3.5	0.96	
			2.55	
8				0.015
8	0.91		0.96	
9	0.91	3.5	0.96	
			2.55	

    - During the DXA scans, the radiation exposure is approximately 0.015 mSv per total body

scan.
  - During study visit #1 (room temperature), participants will be exposed to approximately 1.87 mSv of radiation.
  - During study visit #2 (cold exposure), participants will be exposed to approximately 7.92 mSv of radiation.
  - After allowing for 20% extra exposure from the procedures, women who complete study visits #1 and #2 will be exposed to a total of up to 11 mSv or radiation (~3.6 cross-country flights). Pre-menopausal women who complete study visits #8 and 9 will be exposed to a total of up to 22 mSv of radiation (7.3 cross-country flights). This is below the annual amount of exposure for radiation workers (50 mSv).

FDG will accumulate in the bladder. The estimated whole body radiation exposure following the FDG infusion depends on when subjects void. Participants will be instructed to void 1 hour after the FDG infusion. Please note that the cold exposure will induce diuresis, and in our experience, subjects void immediately after the procedures. The void volume will also be larger than what might be seen at room temperature, and this increases elimination of the radiopharmaceuticals

- *PET/CT:* Aside from the radiation exposure, the risks associated with the PET/CT scan are the chance of incidental findings. PET/CT is used to diagnose a few diseases like some cancers and diseases of the brain. There is a very small risk that diseases like this may be found. Some participants may also experience claustrophobia in the scanner. Some participants may experience a burning sensation in the arm during the ^{11}C-acetate infusion. The infusion only lasts for a few seconds and the burning sensation quickly diminishes after the infusion is complete.
- *sEMG and skin temperature sensors*: The sEMG and temperature sensors may cause some minor discomfort and/or skin irritation due to the paste used to attach the sensors.
- *Core temperature pills*: The temperature pill moves through the intestinal tract like food does, but on rare occasions, can become stuck in the intestines.
- *Venipuncture and IV catheters*: Venipuncture will be performed to obtain blood samples for laboratory analyses, and an IV catheter will be used during the ^{11}C-acetate and ^{18}FDG infusion. There is a small risk of syncope, local hematoma, infection, and thrombosis associated with intravenous blood sampling.
- *Confidentiality and privacy*: The use of questionnaires, interviews, and collection of personal medical information poses a risk to confidentiality and privacy and may cause embarrassment.

Plans to minimize risks

Leuprolide acetate: The risks associated with use of the study drugs will be minimized by enrolling participants who do not have contraindications for their use. Volunteers will undergo a medical history and physical examination by a study clinician. Absence of pregnancy (urine test) will be confirmed before $GnRH_{AG}$ injections are administered. Participants will be observed for 30 min after drug injection to monitor for new hypersensitivity reactions. Women will be instructed that they should not become pregnant while taking study drugs because of risks to the fetus. This physician will discuss non-hormonal based contraceptive methods during the health and physical examination. They will be instructed that cessation of menses may or may not occur during the study and that cessation of menses does not provide protection against pregnancy; contraception (not hormonal) must be used.

Women who do not tolerate $GnRH_{AG}$ therapy in the proposed study can refuse further treatment after each monthly dosing. Among the first 94 women treated with $GnRH_{AG}$ in our previous and ongoing studies (R01 AG018198, P50 HD073063), this occurred only once. Participants will also meet with the study research nurse practitioner, providing a regular mechanism by which adverse events will be queried. A health status questionnaire will be used to track changes in use of medications (prescription and over-the-counter) or health (e.g., doctor visits, hospitalizations), as well as any study-related problems/concerns over the past 4 weeks. Participants will also complete e Menopausal Symptom List (MSL) to monitor the frequency and severity of menopause-related symptoms (e.g., hot flashes, depressed feelings); the Eating Behavior Questionnaire (EBQ) to monitor dietary restraint, disinhibition,

and hunger;[78] and the Pittsburgh Sleep Quality Index (PSQI) to monitor sleeping behavior.[12] After completing the study, Participants will be asked to notify study team when menstrual cycles have resumed. The study team will follow up after 2 months if no contact has been made by participant.

Radiation exposure: All participants will perform one DXA and PET/CT study, and a subset of premenopausal women will perform these tests twice. Women who complete study visits #1 and #2 will be exposed to a total 9.91 mSv or radiation. Pre-menopausal women who complete study visits #8 and 9 will be exposed to 19.61 mSv of radiation.

- To minimize risk to an unborn fetus, participants must have a negative pregnancy test prior to each measurement.
- DXA: Conducting two scans before and after the intervention involves a total radiation exposure of approximately 0.43 mSv, which is <1% of the annual allowable exposure for radiation workers (50 mSv/yr over ***a 40 year career***).
- PET/CT: The FDA ***annual*** allowable exposure for radiation workers for whole body or organ specific radiation exposure is 50 mSv/yr **over a 40 year career**. Radiation exposure increases the risk of a fatal cancer by 4-5% per Sievert. Radiation exposure in this study is <50 mSv, so the increased risk is <0.001%. The highest organ radiation exposure in this study will be in the bladder. Conducting two studies before and after the intervention in the subset of premenopausal women will involve a total radiation exposure of 38 mSv in the bladder, which is ~76% of the ***annual*** allowable exposure. To minimize exposure, participants will void 30 min. after the FDG infusion, and then again as soon as the study is completed (2 hrs. after ^{18}FDG administration). In our experience the cold exposure causes all participants to void by the time the study is completed. According to the Institute for Science and International Security, the average annual dose equivalent of natural ionizing radiation in Denver is 11.8 mSv/yr. In comparison to natural background radiation, the radiation exposure in the proposed experiments is comparable to 1.6 years of natural radiation from living in Denver.

Incidental findings during the PET/CT scans: All images for this study will be de-identified and reviewed for incidental findings. Should anything significant be found, the Nuclear Medicine physician will discuss these findings with the study MD. The participant will be contacted immediately by the Nuclear Medicine physician and advised on clinical follow-up to confirm findings.

Venipuncture and IV catheter: The risks of hematoma and infection are minimized by having trained personnel perform the procedures using sterile techniques.

Core temperature pills: Participants will be advised to avoid having an MRI test for at least a week after the study. Because the capsule is a radiotransmitter, participants will also be advised to avoid travel on commercial airlines for at least a week after the study. The recording device is electrically isolated and complies with hospital standards for electrical safety.

sEMG and skin temperature sensors: Any skin irritation from the sensors is expected to be minor and temporary.

Confidentiality and privacy: These risks will be minimized by not including personal identifying information on the forms, when possible, and by conducting interviews and collection of personal information in a private setting.

Evaluation of study-related events – The subset of premenopausal women enrolled in the intervention study will meet with a study clinician every 4 weeks to review a Health Status Questionnaire that the participants will complete. The questionnaire queries about changes over the past 4 weeks in medications, health status, and any concerns with study medications. The clinician will specifically note on the form whether there are any concerns that are possibly, probably, or definitely related to the study. Participants will also be instructed to report concerns that may be study related when they occur to the research nurse practitioner, Ms. Quick, who will initiate event reports for both the programmed and spontaneous complaints.

E. Potential Scientific Problems:

We considered studying women across the menstrual cycle, rather than a cross-sectional comparison of pre- vs. postmenopausal women. The strength of the cross-sectional design is that postmenopausal

women will have been estrogen deficient for several years, which should maximize the attenuation of E_2 deficiency on BAT activity. A limitation is that we will not be able to determine whether this is also partially due to age. However, if the exploratory aim confirms that $GnRH_{AG}$ reduces BAT activity, this will support the overall hypothesis that E_2 deficiency, rather than age, is the primary factor contributing to reduced BAT in postmenopausal women.

There is no known way to distinguish the energy expenditure associated with shivering and non-shivering thermogenesis in humans. Thus, we will employ rigorous techniques to measure shivering during cold exposure using both sEMG and ^{18}FDG uptake into deep muscles. These data will be used quantify shivering and used to interpret differences and changes in CIT.

Postmenopausal women will likely have higher levels of body fat, and body fat may increase in premenopausal women after $GnRH_{AG}$. As described above, the cooling suit delivers an identical cold stimulus during each study. Our intent is not to determine the maximal activity of BAT, but rather, determine if differences in BAT activation in response to a constant stimulus is either different between pre- and postmenopausal women (**SA2**), or changes after $GnRH_{AG}$ (**SA3**). To account for differences or changes in body composition, REE will be expressed per kg of fat free mass.

We acknowledge that $GnRH_{AG}$ causes a more abrupt withdrawal of ovarian function (i.e., weeks rather than years) and does not generate the same gonadotropin response of menopause (i.e., low rather than high). However, the $GnRH_{AG}$ model is also a strength because it allows us to control the sex hormone environment in a manner that is not possible when studying the natural menopause transition.

F. Data Analysis Plan:

Sample size justification and power analyses – Sample size calculations and power analyses were performed using SAS 9.4 (SAS Institute, Inc., Cary, NC). For our estimate of effect size, we refer to our previous study that measured CIT in the follicular and luteal phases (Figure 5).[8] In that study, CIT was higher during the luteal phase (2530±524 kcal·d^{-1} vs. 2225±440 kcal·d^{-1}). Unfortunately, we did not measure BAT activity. Because there were no differences in shivering in this study, we will assume differences in CIT were due to differences in NST; we also assume that this reflects differences in BAT activity. Using a pooled SD of 484 kcal/d, 32 evaluable participants per group (64 total) will provide 80% power to detect a difference of 305 kcal/d in CIT between pre- and postmenopausal women using a 2-sided t-test and a type I error rate at 0.05. Assuming a dropout rate of 15%, 38 participants per group will need to be enrolled to yield the desired sample size of 64 participants. The primary endpoint is BAT oxidative metabolism (^{11}C clearance rate, expressed per mL of BAT per s^{-1}). In our previous study of 12 men, BAT oxidative metabolism increased during cold exposure from 0.35±0.13 to 0.74±0.19 (mean±SEM).[6] Using a pooled SD of 0.45, we estimate that we will have 80.0% power to detect a difference between groups of 0.32 during thermoneutral conditions. Using a pooled SD of 0.66, we will have 84.7% power to detect a difference between groups of 0.50 during cold exposure. In another study, BAT oxidative metabolism at room temperature was 0.35 in lean men vs. 0.05 in overweight men with T2D.[10] We have also reported that BAT oxidative metabolism increased in lean mean from 0.73 to 1.59 following a 4-week cold acclimation.[7] Thus, we consider this to be a meaningful effect size to examine differences in BAT oxidative metabolism between groups during thermoneutral conditions and during cold exposure. A secondary endpoint is BAT activity volume. In our previous study, BAT activity volume was 57±16 ml (mean±SE).[6] Using a pooled SD of 55 ml, the study will have 80.0% power to detect a difference of 39 mL between groups. In another study, we reported that BAT activity volume was 48 vs. 4 ml in young healthy men compared to older men with T2DM.[5] Thus, we consider this to be a meaningful effect size.

Analysis strategy - Statistical analyses will be performed using SAS 9.4. Analyses will begin with descriptive plots of individual variables to evaluate distributional characteristics and for quality assurance. The effect of menopausal status on each endpoint will be estimated using a linear regression model. The endpoint will be the response variable and a variable indicating menopausal status will be the explanatory variable (equivalent to a 2-sample *t*-test). If a variable that is related to the response variable is not balanced between menopausal groups, that variable will be included in the regression model to eliminate the potential of the effect of menopausal status being confounded by that variable. For all multivariable analyses, regression diagnostics will be used to ensure that the group of explanatory variables are not collinear. Secondary analyses will be performed to investigate whether REE is associated with either BAT

oxidative metabolism or BAT activity volume. Exploratory analyses will investigate the changes in BAT oxidative metabolism and BAT volume of activity associated with $GnRH_{AG}$ treatment (Aim 3) Because these aims are exploratory, results will be reported using descriptive statistics, mean change, and 95% confidence intervals (CIs) for the changes in the primary and secondary endpoints.

G. Summarize Knowledge to be Gained:

The importance of understanding mechanisms that contribute to risk for obesity cannot be overstated given the current epidemic of overweight and obesity in the U.S. and other developed countries worldwide. Although the loss of ovarian function is only one of a multitude of physiological, behavioral, and environmental factors that increase risk for fat gain, it is potentially a very important one in women. The strong evidence from multiple large RCTs and smaller physiologic trials indicates that estrogen-based HRT attenuates weight gain in postmenopausal women by an average of 40%. Further, in the absence of HRT, weight gain in postmenopausal women is accompanied by a disproportionate increase in abdominal fat. Although abdominal obesity and the related metabolic dysfunction increases risk for coronary artery disease in both women and men, the risk of mortality may be ~5-fold higher in women. Also, it has been estimated that abdominal obesity and metabolic dysfunction account for about 20% of coronary events in men, but 48% of events in women. Such statistics underscore the importance of understanding the mechanisms of abdominal fat gain in women – including both energy expenditure and energy intake regulation. In this context, the proposed study has the potential to reveal a consequence of the loss of ovarian function that has widespread health implications.

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