Title: Bioenergetic and Metabolic Consequences of the Loss of Ovarian Function in Women

Working title: Females, Aging, Metabolism, and Exercise (FAME)

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1. Specific aims

Gonadal aging plays a distinct role in mediating some of the biological changes ascribed to the aging process. The most well-researched example of this is the accelerated decline in bone mineral density (BMD) due to the decline in ovarian estrogens at the menopause. Restoring normal estrogen levels can effectively mitigate menopause-related skeletal changes, but eventually other non-gonadal aging factors trigger a decline in BMD.[1] Because it is difficult to isolate the consequences of gonadal aging from chronologic aging, it is not clear to what extent the loss of gonadal function increases risk for age-related diseases other than osteoporosis. However, if gonadal aging increases chronic disease risk, this would be expected to have a greater adverse effect in women than in men, because the loss of gonadal function occurs at an earlier age in women.

The effect of the loss of gonadal function on physical activity in a number of animal species is startling. Ovariectomy (OVX) causes a **30% to 80% decrease** in physical activity (Fig 1)[2-7] and, although evidence is less abundant, orchiectomy has a similarly dramatic effect **(65% decrease**).[8] Physical activity levels do not return to normal spontaneously in gonadectomized animals, but full rescue occurs with estrogen treatment in females (Fig 2) and testosterone treatment in males. The loss of gonadal function in humans may also trigger a decline in physical activity. Gonadotropin releasing hormone agonist ($GnRH_{AG}$) therapy to suppress gonadal function causes an increase in fat mass;[9-11] these studies did not evaluate whether the disruption of energy balance was due to a decline in physical activity. Observational studies of women through the menopausal transition indicate that fat gain accelerates 3 to 4 years before ovarian failure. To our knowledge, only one prospective study included an objective measure of physical activity and found a **55% decrease** over the final 3 peri-menopausal years (Fig 6).[12] Based on the evidence report for the 2008 Physical Activity Guidelines for Americans,[13] a gonadal aging-related decline in physical activity could have multiple adverse effects on health. There is **strong evidence** that low physical activity is associated with all-cause mortality, coronary heart disease, high blood pressure, stroke, type 2 diabetes, metabolic syndrome, colon cancer, breast cancer, depression, low cardiorespiratory and muscular fitness, and increased body mass.[13]

The primary goal of the proposed research is to use a controlled intervention strategy to determine whether the suppression of ovarian function in women approaching the menopause causes a marked decline in physical activity. Additional goals are to assess changes in other components of energy balance, determine whether the disruption of energy balance is associated with changes in biomarkers of chronic disease risk, and determine whether programmed exercise can prevent these changes. To achieve these aims, women aged 42 to 52 years with normal menstrual cycle function will be randomized to receive 24 weeks of placebo, $GnRH_{AG}$, or $GnRH_{AG}$+exercise intervention. Specific aims (SA) and hypotheses (H) are:

Primary SA1: Determine whether 24 weeks of $GnRH_{AG}$ therapy causes a decline in physical activity when compared with placebo. Change in physical activity energy expenditure (PAEE) from baseline will be measured after 12 (secondary outcome) and 24 wk (primary outcome) of $GnRH_{AG}$ vs placebo therapy. PAEE will be the difference between total daily EE (TEE) measured by the doubly-labeled water technique (DLW) and sleeping EE (SEE) measured by indirect calorimetry, adjusted for the thermic effect of feeding (TEF).[14]

H1: PAEE will decrease significantly after 12 and 24 weeks of $GnRH_{AG}$ therapy when compared with placebo.

Secondary SA2: Determine the effects of gonadal function on multiple components of energy expenditure under free-living (DLW) and controlled (metabolic chamber) conditions, including 1) 24-h TEE measured by DLW, and 2) resting EE (REE), SEE, and TEF measured by indirect calorimetry in a metabolic chamber.

H2: When compared with placebo, $GnRH_{AG}$ therapy will cause a decrease in TEE. The relative contributions of REE, SEE, TEF, and PAEE to the decline in TEE will be evaluated.

Secondary SA3: Determine whether the disruption of energy balance in response to $GnRH_{AG}$ therapy leads to unfavorable changes in biomarkers of disease risk, including adiposity, insulin resistance, and inflammation. This will include the measurement of biomarkers in adipose tissue samples.

H3: When compared with placebo, $GnRH_{AG}$ therapy will cause increases in adiposity, insulin resistance, and fasted serum inflammatory cytokines.

Secondary SA4: Because programmed exercise mitigates metabolic consequences of gonadectomy in animals, we will determine whether the bioenergetic and metabolic consequences of $GnRH_{AG}$ in women can be

mitigated through a programmed, supervised exercise intervention ($GnRH_{AG}$ vs $GnRH_{AG}$+exercise).

H4: Programmed exercise will attenuate the decrease in TEE and the increases in adiposity, insulin resistance, and inflammatory cytokines.

Exploratory Aim: To evaluate the effects of chronic sex hormone suppression and exercise on arterial stiffness and endothelial function. This preliminary data will be used for a new grant application examining the effects of exercise on modulating vascular function in E2-deficient women.

2. Research Strategy

2.a. Significance

Physical activity and disease risk. The evidence report for the 2008 Physical Activity Guidelines for Americans[13] found **strong evidence** that low levels of physical activity are associated with increased all-cause mortality, coronary heart disease, high blood pressure, stroke, type 2 diabetes, metabolic syndrome, colon cancer, breast cancer, depression, low cardiorespiratory and muscular fitness, increased body mass, and a biomarker profile that is not favorable for the [illegible] osteoporosis. Leisure-time physical activity levels decline with age and, at any adult age, women are less likely to be highly active and more likely to be insufficiently active or inactive than men.[15,16] **These observations suggest that older adults, and specifically older women, may be at increased chronic disease risk because of inadequate physical activity.** The reasons for sex differences and age-related changes in physical activity are not clear. There are well-described sex- and age-related differences in such physiological factors as muscle strength and aerobic power that would explain why the absolute intensity or volume (e.g., kcal/d) of physical activity would be lower in women than men and decline with aging.[17] However, the trend and prevalence estimates discussed above[15,16] were based on the **time spent participating in physical activity**, and there is no obvious biological reason why this should be different between women and men or decline with age. Because leisure-time physical activity is typically thought to be voluntary, it is possible that women simply choose to be less active than men and that adults choose to become less active as they age. However, it is also possible that there are biological regulators of physical activity.[18]

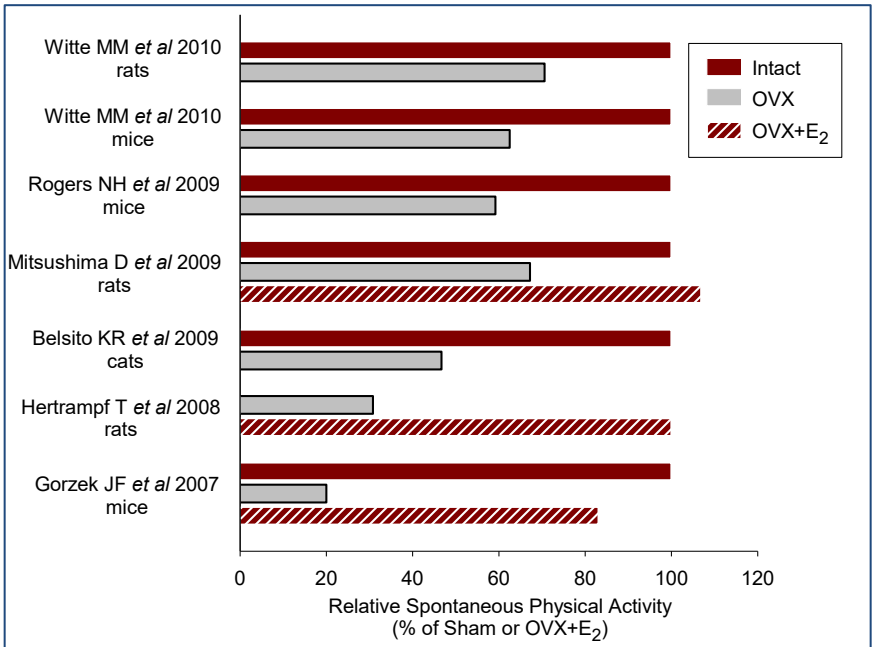


Figure 1. Recent preclinical studies demonstrating a reduction in spontaneous physical activity in response to ovariectomy (OVX) and the rescue by estradiol (E_2).

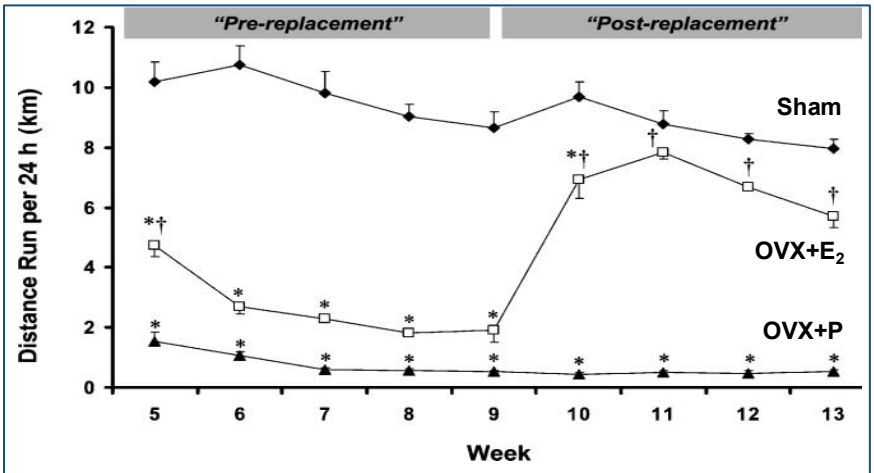


Figure 2. Effects of ovariectomy (OVX) vs sham surgery on voluntary wheel-running activity in mice and the rescue of normal activity by estradiol (E2) but not progesterone (P). From: Gorzek JF et al. *Med Sci Sports Exerc* 39:248, 2007

Gonadal regulation of physical activity – preclinical evidence. There is compelling evidence from studies of laboratory animals that spontaneous physical activity is regulated by gonadal function,

and specifically by sex hormones. Figure 1 summarizes the results of several recent preclinical studies of the effects of OVX on physical activity. When compared with animals with normal ovarian function (intact; solid dark red bars), **OVX (solid light gray bars) resulted in a dramatic decline in physical activity of 30% to 80%** that was not attributable to the effects of surgery. Further, physical activity was rescued in OVX animals treated with estradiol (E_2; hatched bars).[2-7] The regulation of physical activity by E_2, specifically, in female animals is further supported by the observation that E_2, but not progesterone, restored normal activity in OVX animals (Fig 2).[7] Although the gonadal regulation of physical activity has received less attention in male animals than females, orchiectomy (ORX) was reported to reduce voluntary wheel-running activity as dramatically as OVX (~65%) and normal levels were restored by testosterone treatment.[8]

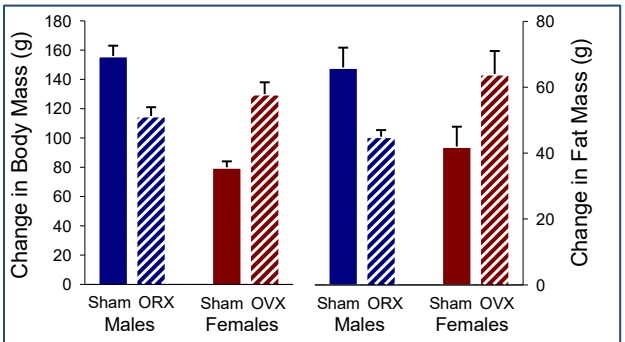


Figure 3. Changes in body mass and fat mass in intact (sham) and gonadectomized animals. Adapted from: Richard D et al *Int J Obesity* 26:344, 2002

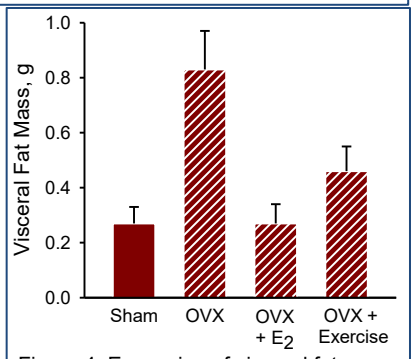


Figure 4. Expansion of visceral fat mass after OVX and protection by estradiol (E_2) and exercise. Adapted from: Wohlers LM

Sex differences in bioenergetic consequences of the loss of gonadal function – preclinical evidence. One consequence of **OVX** in laboratory animals is excess weight gain.[2-7] For example, the mean body weight of OVX mice was approximately 20% higher than sham controls 12 wk after surgery.[3] The disruption in energy balance following OVX may be mediated by not only the decrease in physical activity (Fig 1),[2-7] but also a decrease in metabolic rate that is independent of the decline in activity[3] and, in some species, an increase in energy intake (EI).[2] In contrast, **ORX** does not result in excess weight gain in male animals[8,19-23] and has, in fact, been found to significantly reduce body weight.[23] Even a 65% decrease in wheel-running activity in response to ORX did not result in an increase in body weight.[8] The sex dimorphism in the effects of gonadectomy on fat mass mirrors the effects on body mass (Fig 3).[23] There is also evidence that the abdominal visceral fat depot expands markedly in response to gonadectomy in females (Fig 4),[24] but this does not occur in males.[19] These findings indicate that **the bioenergetic consequences of the loss of gonadal function are markedly different in female rodents than in males.** The loss of E_2 appears to be the mechanistic trigger in females, because E_2 therapy mitigates the decline in physical activity (Fig 1) and the increase in visceral fat mass (Fig 4). Exercise has also been found to attenuate the gain in visceral fat in OVX animals (Fig 4). **Because of the apparent sex differences in the bioenergetic response to gonadectomy and the constrained resources of the UCAMC SCOR, the clinical project will focus on women only**. Based on the preclinical evidence discussed above, we postulate that the loss of ovarian function will lead to a marked decline in physical activity and an increase in total and abdominal adiposity.

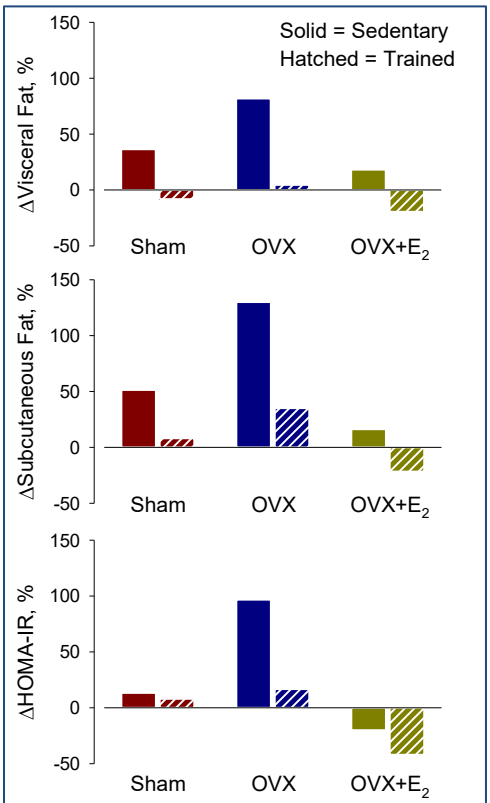


Figure 5. Relative changes over 6 wk in visceral fat, subcutaneous fat, and insulin resistance in sham-, OVX-, and OVX+E_2-treated rats that were sedentary (solid bars) or exercise trained (hatched bars). Adapted from: Pighon A et al *Climacteric* 13:238, 2010

Benefits of programmed exercise. The effects of OVX to decrease physical activity and increase adiposity, leading to metabolic dysfunction, can be largely counteracted with programmed exercise (e.g., treadmill exercise).[25-29] For example, the exaggerated increases in visceral fat, subcutaneous fat, and insulin resistance that occur in OVX (Fig 5, middle solid bars) compared with sham rats (left solid bars) are largely prevented in OVX rats that perform treadmill exercise (middle hatched bars).[27] The greatest benefits occur in OVX animals treated with both E_2 and exercise (right hatched bars). The PI has also demonstrated that exercise training and estrogen therapy have

independent and additive benefits on insulin action in women.[30]

Is there evidence for gonadal regulation of physical activity in women? The European Society of Human Reproduction and Embryology (ESHRE) workshop in 2010 on perimenopausal risk factors and future health[31] reported that "body weight increases because the decline in physical activity during the perimenopause is greater than the concomitant decline in energy intake." However, based on the information in the report and to our knowledge, **only one prospective cohort study** has reported changes in physical activity and energy intake (along with body composition, fat distribution, and energy balance) over the menopausal transition.[12] Lovejoy and colleagues conducted annual study visits over 4 years in 129 women aged 43 years or older with normal menstrual cycle function. By year 4, 51 women were postmenopausal, 44 were perimenopausal, and 34 remained premenopausal. Physical activity was assessed objectively at each annual visit by triaxial accelerometry over 4 consecutive days; food records were obtained simultaneously for the estimation of energy intake. Over the 4 years before women became menopausal, the average **physical activity level decreased by more than 50%** (Fig 6, bottom left). **Energy intake also decreased ~30%** (bottom right), but not to a sufficient extent to prevent a gain in body weight (upper left) and an increase in body fat (~5% of body mass; not shown). There was also a striking increase in abdominal visceral fat area over the 4 years leading up to menopause (Fig 6, upper right). In a subset of women, TEE and SEE measured in a room calorimeter tended to decrease more in women who became menopausal than in those who remained premenopausal. **These findings suggest that relatively large reductions in energy intake during the menopausal transition do not sufficiently counter the decrease in physical activity to prevent excess abdominal fat gain.** The proposed study will determine whether programmed exercise is an effective strategy for maintaining energy balance.

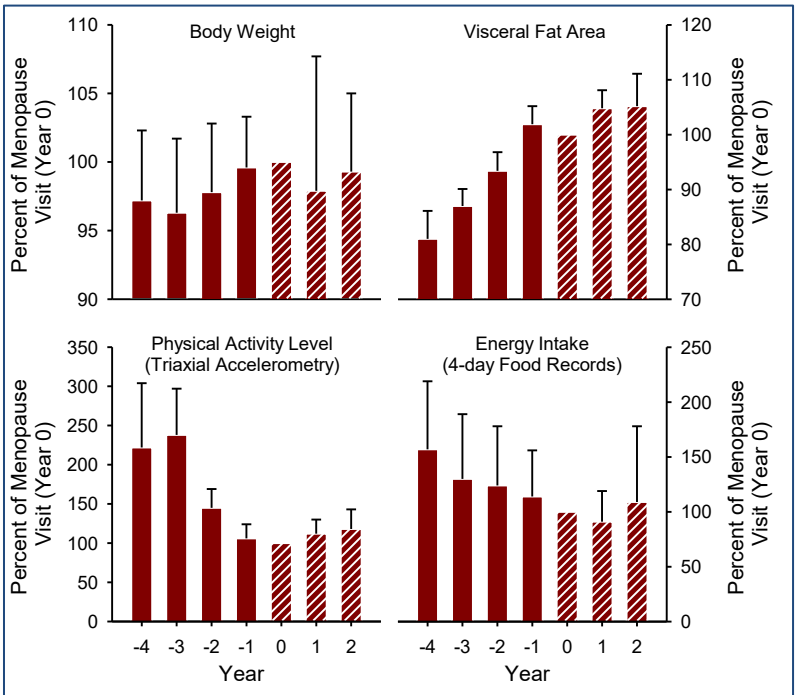


Figure 6. Relative prospective changes in body weight, visceral fat area, physical activity, and energy intake in women followed through the perimenopause-to-menopause transition (year 0 = menopause). Adapted from: Lovejoy JC et al *Int J Obesity* 32:949, 2008

Additional evidence for gonadal regulation of bioenergetics in women. There is considerable evidence, as recently reviewed,[32,33] that energy balance is disrupted in postmenopausal women in a manner that increases the propensity for weight gain and fat gain, particularly in the abdominal region. Evidence that this is mediated specifically by estrogen deficiency, as in rodents, comes from the Postmenopausal Estrogen Progestin Intervention (PEPI) trial.[34] Women randomized to menopausal hormone therapy (HT) in the PEPI trial gained less weight over 3 years and had less of an increase in waist girth than placebo-treated women. Although there were no significant differences between estrogen-only and estrogen+progestin HT, women on estrogen-only HT had the smallest gains in weight and waist size. Meta-analyses of randomized controlled trials of HT vs placebo indicate that HT has favorable effects on abdominal adiposity and insulin resistance.[35] Additional evidence that the loss of ovarian function disrupts energy balance is that the clinical use of $GnRH_{AG}$ therapy for endometriosis or uterine fibroids results in a significant gain in fat mass.[9,10,36] We have also found that acute, short-term suppression of ovarian function results in a significant decline in REE (Preliminary Data, Fig 8).[37] However, if body weight increases with more prolonged suppression of ovarian function, REE could potentially increase.

Significance – summary. The NIH defines the significance of a project by such factors as whether it addresses an important problem and whether the successful achievement of the aims will expand scientific knowledge or change the concepts, methods, technologies, treatments, services, or preventative interventions that drive the field. The proposed project ranks high in these areas of significance. If we find that the suppression of ovarian

function in women does, indeed, increase the *biological drive* toward sedentary behavior, as it does in laboratory animals, this will generate knowledge regarding a novel pathway by which the menopause increases risk for metabolic dysfunction and multiple diseases. It is expected that such a finding would catalyze research on neuroendocrine and tissue-specific mechanisms by which gonadal function regulates physical activity. Such a finding would also indicate a need for behavioral or pharmacologic strategies to compensate for or counteract the menopause-triggered decline in physical activity. The proposed study will provide insight as to whether a biological drive to become more sedentary can be overridden through programmed exercise.

2.b. Innovation

The NIH-defined determinants of innovation include whether the application 1) will shift current research or clinical practice paradigms by utilizing novel theoretical concepts, approaches, instrumentation, or interventions; 2) advances concepts, approaches, instrumentation, or interventions that are novel; or 3) involves a refinement, improvement, or new application of theoretical concepts, approaches, instrumentation, or interventions. The primary innovation of the current application is that it will be the **first study to evaluate the gonadal regulation of physical activity in women using a randomized controlled intervention approach**. Although there is solid *preclinical* evidence for the regulation of spontaneous physical activity by estradiol, this is a novel theoretical concept in the clinical research community. The proposed study will provide proof-of-concept evidence for the gonadal regulation of physical activity in women. If the hypotheses are upheld, this is expected to stimulate novel research on the 1) mechanisms by which physical activity is regulated, 2) the health consequences of a menopause-related decline in physical activity, and 3) strategies (i.e., behavioral and pharmacologic) to prevent the bioenergetic and metabolic consequences of gonadal aging. Another innovative aspect of the proposal is that it will utilize state-of-the-science technologies to measure the effects of $GnRH_{AG}$ therapy on energy balance under both free-living conditions (i.e., over 10 days by DLW) and well-controlled conditions (i.e., over 24 hours by indirect calorimetry). The measurement of the components of TEE under well-controlled conditions will provide insight into whether there are decreases in EE in response to a controlled glucose challenge (TEF), a controlled exercise challenge (exercise EE), while at rest (REE), and during sleep (SEE).

2.c. Preliminary data

This section will a) highlight the experience of the investigators and their long-standing interest in estrogens as a mediator of energy balance and metabolism in women; b) present preliminary evidence for the potential regulation of energy balance by estrogens; c) document the precision of measurement of EE; and d) demonstrate the ability of the investigators to successfully carry out an intensive intervention trial involving pharmacologic and behavioral interventions and a relatively burdensome testing schedule.

Experience of the investigators. The PI, Dr. Kohrt, has been conducting trials involving exercise, hormone, and/or nutrition interventions in young and older adults for more than 20 years.[30,38-45] Drs. Kohrt and Van Pelt have been working together since 1998 on studies of fat distribution as a determinant of disease risk in postmenopausal women[46-49] and of the role of estrogens in the regulation of the glycolytic and anti-lypolytic actions of insulin.[30,45,50-52] Drs. Kohrt and Melanson have been working together for 10 years through their roles as director and associate director, respectively, of the Energy Balance Core Laboratory (EBL), which is a component of the Nutrition and Obesity Research Center (NORC). Dr. Melanson maintains the metabolic chamber, a resource supported by the EBL, which utilizes state-of-the-science technology to measure 24-h energy expenditure under controlled conditions in humans.[53] Dr. Melanson is an expert in the assessment of physical activity and energy balance.[53-57] Dr. Shea is currently an instructor in the Division of Geriatric Medicine working with Dr. Kohrt. Her primary research interest is in the effects of weight loss on bone metabolism in older women, which is tangentially aligned with the proposed project.[58] Through her role as a co-investigator, she will gain valuable experience in carrying out an intensive clinical intervention study that will benefit her career advancement. She will also have the opportunity to develop an ancillary study to the parent project focused on skeletal outcomes of the interventions.

Drs. Kohrt, Melanson, and Van Pelt have documented experience with all of the proposed procedures with the exception of the measurement of TEE by DLW (Dr. Melanson is currently using DLW in an R01-supported study). Because the DLW technique will be used to generate the primary outcome of the study (PAEE), we have enlisted Dr. Dale Schoeller from the University of Wisconsin as a consultant for the project. Dr. Schoeller developed the DLW approach to measure TEE in humans nearly 20 years ago[59] and is internationally

renowned for his work in this area. He will provide advice on the DLW sample analysis and data reduction.

Regulation of energy balance by sex hormones. Previous work of the PI supports a role of sex hormones in the regulation of energy balance. Her original interest in this area was generated by a serendipitous observation in a study of the interactive effects of exercise and HT on bone mineral density (BMD).[39] In that study, postmenopausal women who were assigned to HT (n=16) or no therapy (n=18) had similar reductions in fat mass (Fig 7, left panel, solid bars) and waist girth (right panel, solid bars) in response to a supervised exercise intervention. Women continued the hormone intervention for 6 months after the cessation of the exercise program. During that 6-month interval (Fig 7, hatched bars), women not on HT gained back most of the fat mass and waist girth they had lost. In contrast, reductions in fat mass and waist girth were largely preserved in women on HT. Physical activity was not assessed, but the observed changes are consistent with a favorable effect of HT to maintain energy balance.

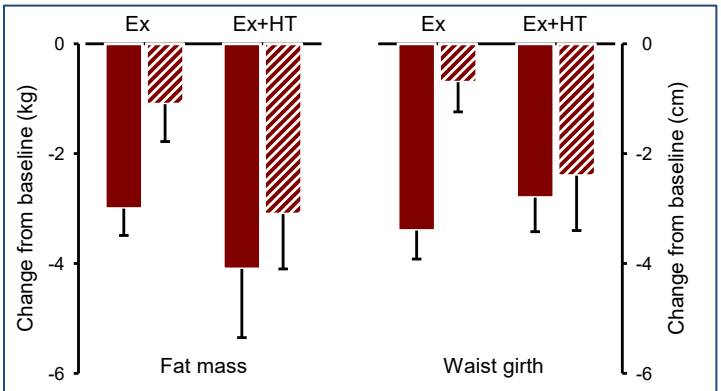


Figure 7. Changes in fat mass (left) and waist girth (right) in response to exercise training (▬) and 6 months after the cessation of the supervised exercise program (▨). Participants were postmenopausal women on estrogen-based hormone therapy (HT) or no HT. Adapted from: Kohrt WM et al. *J Appl Physiol* 84:1506 1998

The PI also has evidence that the suppression of ovarian function in premenopausal women may disrupt energy balance by decreasing REE.[37] Ovarian function was suppressed acutely for 6 days in premenopausal women (n=14) with a GnRH antagonist. REE was measured in the early follicular and mid-luteal phases of the menstrual cycle, and after 6 days of $GnRH_{ANT}$. Suppression of ovarian function caused a decrease in REE when compared with both the follicular (-42 kcal/d) and luteal phases (-71 kcal/d) (Fig 8).

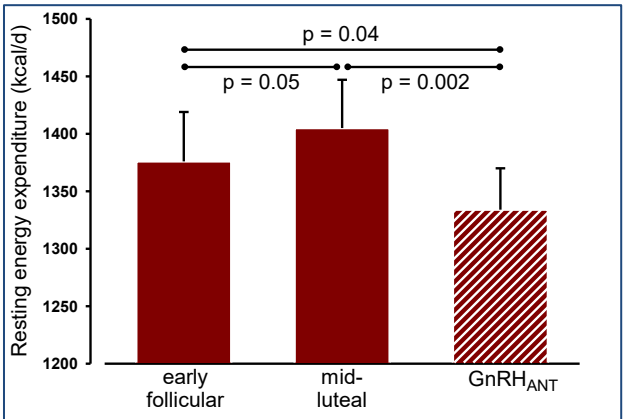


Figure 8. REE (kcal/d) in premenopausal women during the early follicular and mid-luteal phases of the menstrual cycle and in response to 6 days of $GnRH_{ANT}$ therapy. Adapted from: Day DS et al. *J Clin Endocrinol Metab* 90:3312, 2005

Precision of measurement of EE. Dr. Schoeller validated the measurement of EE by DLW against indirect calorimetry in adults and found it to be accurate within 1-2%, with a standard deviation of 6-8%.[59-61] He has also performed reproducibility studies to assess the technical and biological variability in repeated measurements of TEE by DLW and found the intraclass correlation coefficients to be very high (0.98 – 0.99).[62,63] Dr. Melanson has performed repeated measurements of TEE (N=15) in the metabolic chamber and also found the intraclass correlation coefficient to be high (r=0.964). The difference between repeated measurements was 38±76 kcal/day.

Likelihood that the proposed study can be carried out successfully. A major determinant of the success of the proposed study will be the ability to recruit women into a project that involves the manipulation of sex hormone levels, an exercise intervention, and a burdensome testing schedule. The PI is currently conducting a study (R01 AG018198) in premenopausal women that involves a similar intervention (all women undergo $GnRH_{AG}$ with add-back of placebo or E_2 for 5 months) and a similarly burdensome testing schedule. The primary aims of that study are to determine 1) whether the suppression of ovarian function with placebo add-back causes a decrease in REE (as a mechanism for fat gain) and an exaggerated serum cortisol response to physiologic and pharmacologic stressors (as a mechanism for abdominal fat gain); and 2) whether E_2 add-back mitigates these responses. Despite early delays caused by pharmaceutical company takeovers, this study is on track to reach completion in early 2013. As of Sept 2011, 121 women had provided informed consent for the project; of these, 13 did not qualify and 31 withdrew before randomization (primary reasons: 11 did not return calls, 6 had time concerns, 3 wanted to start hormonal contraception). Of the remaining 77, 13 were undergoing pre-randomization testing and 64 were randomized to the intervention. Of the 64 randomized, only 7 were lost to follow-up before the post-intervention evaluations (1, side effects of $GnRH_{AG}$; 2, lack of time; 4, family

problems). The investigators will remain blinded to treatment condition until the end of the trial, so preliminary data cannot be presented in a treatment-specific manner. However, for the 41 women who have had measurements of REE before and after the intervention, the average change is -33±113 kcal/d. Our hypothesis is that REE will decrease by 40 to 50 kcal/d in women on $GnRH_{AG}$+placebo and be maintained in women on $GnRH_{AG}$+E_2; the pooled mean data are consistent with the expected pattern of change.

Summary. The investigators are experienced in the methodologies to be employed and in carrying out intervention trials of similar complexity. They are well-qualified and well-prepared to carry out the proposed study. Preliminary data support the concept that sex hormones influence energy balance. The SCOR will facilitate the expansion of this research to determine how the suppression of ovarian function influences PAEE and other determinants of energy balance (TEE, SEE).

2.d. Research methods

2.d.1. Overview

This will be a randomized, double-blinded (to the extent possible), placebo-controlled intervention to determine whether the suppression of ovarian function with $GnRH_{AG}$ disrupts energy balance by causing a decrease in PAEE. Secondary aims are to determine the effects of the suppression of ovarian function on other components of energy expenditure and on biomarkers of disease risk and whether the consequences of $GnRH_{AG}$ can be mitigated through programmed exercise. Participants will be women who are nearing the menopausal transition based on age (42-52 y) but have normal menstrual cycle function. They will be randomized to receive monthly injections of placebo or $GnRH_{AG}$ (leuprolide acetate, 3.75 mg) for 24 weeks. Women who receive $GnRH_{AG}$ will be further randomized to no exercise or a supervised exercise program. Thus, the 3 treatment arms are: placebo, $GnRH_{AG}$, $GnRH_{AG}$+exercise. Although a 2x2 study design (placebo vs drug; no exercise vs exercise) was considered, this was not feasible given the resources available under the grant mechanism.

2.d.2. Outcome variables

The primary outcome (Aim 1) will be PAEE after 24 weeks of intervention. PAEE will be the difference between TEE measured by DLW and SEE and TEF, measured by indirect calorimetry. It will be assessed at baseline and after 12 and 24 weeks of intervention. The **rationale** for PAEE as the primary outcome is the dramatic decrease in PA in response to OVX in laboratory animals (Fig 1), which could be a primary trigger for subsequent changes in adiposity and metabolic function. This will be the first controlled intervention trial to determine whether an objective measure of PA decreases in response to the suppression of ovarian function in women. $GnRH_{AG}$ therapy for 4 months or longer increases fat mass and decrease fat-free mass (FFM).[9,10,36] One concern is that women may begin to adopt compensatory behavioral changes (e.g., decreased food intake, increased physical activity) if changes in body composition are sensed. Thus, the greatest **biologically-mediated** decrease in PAEE may occur at the intermediate testing interval (12 weeks), before measurable changes in body composition are expected to occur; this will be the primary study endpoint.

Other secondary outcomes of the study will include: 24-h TEE measured by DLW; REE, SEE, and TEF measured by indirect calorimetry in a metabolic chamber; energy intake estimated from food records; physical activity measured by accelerometry; body composition measured by dual-energy x-ray absorptiometry (DXA); glucose and insulin responses to an OGTT; serum concentrations of inflammatory cytokines (IL-6, hsCRP); and biomarkers in adipose tissue samples.

2.d.3. Study population

Volunteers will be women nearing the menopausal transition, based on age (40 to 60 y), with normal menstrual cycle function. The **rationale** for this approach was driven by the observation that a menopause-related decline in PA likely begins 3 to 4 years before menopause (Fig 6). We chose not to focus the study on women with perimenopausal symptoms for two reasons: 1) we may miss part of the perimenopause-related change in PAEE, and 2) the irregularity of menstrual cycles during the perimenopause complicates the scheduling of baseline tests around menstrual cycle phase. Specific inclusion and exclusion criteria are in Section 3.1.a.

2.d.4. Sample size justification

The expected effect of ovarian hormone suppression on PAEE was conservatively set at -25%, based on the range of decreases in physical activity observed in preclinical studies of -30% to -80% (Fig 1) and the decrease

of -50% in the prospective cohort study of women undergoing natural menopause.[12] Baseline PAEE was assumed to be 800 kcal with a SD of 330 kcal.[65] The variance of the difference between $PAEE_{pre}$ and $PAEE_{post}$ is $2(1-\rho)\sigma^2$. Using a conservative value of 0.90 for ρ (actual value, 0.98)[62] and assuming no change in variance as PAEE decreases, the SD of the change will be 148 kcal/d. Based on the analysis plan to conduct a 1-way ANOVA with two contrasts (placebo vs $GnRH_{AG}$; $GnRH_{AG}$ vs $GnRH_{AG}$+exercise), a sample size of 15 in each of 3 groups will achieve 95% power for either test to detect a 200 kcal/d difference between two groups in the change in PAEE using a *t* test at the 0.05 significance level. To account for attrition, we will enroll 22 per group.

2.d.5. Screening procedures

After providing informed consent, volunteers will undergo 2 days of screening tests. Day 1 will include an assessment of depressive symptoms (Center for Epidemiologic Studies Depression Scale (CESD), a clinical laboratory evaluation including a comprehensive metabolic profile, TSH, a complete blood count, and a medical history and physical examination by the study clinician. If volunteers are found to be depressed after administering the CESD (score of >16), the study clinician will administer the Beck Depression Inventory (BDI-II). As score of <18 on the BDI-II will be used along with clinical judgement to determine final eligibility; this approach was used in a previous study completed by Dr. Kohrt. At the second visit, participants will undergo proximal femur and lumber spine DXA scans to exclude volunteers who have severe low bone mass or osteoporosis (BMD t-scores of <-2.0) because BMD may decrease during $GnRH_{AG}$ treatment. A urine pregnancy test will be done prior to the DXA scan. Afterwards a resting BP and 12-lead ECG will be recorded and then a graded exercise test (GXT), with monitoring of BP and 12-lead ECG, will be administered by research personnel and supervised by a clinician. After screening tests are completed, an enrollment checklist that includes all enrollment criteria will be completed for each volunteer and reviewed by a study clinician and another investigator to verify eligibility.

2.d.6. Randomization and blinding

Volunteers will be informed that they will be randomized to placebo or active $GnRH_{AG}$ and to exercise or no exercise. They will *not* be informed that only women on active $GnRH_{AG}$ will be randomized to exercise. A permuted block randomization with random block sizes will be used for subject assignment into the 3 treatment groups. Randomization will be stratified by age (40-46, 47-60 y). The randomization sequences will be generated by the biostatistician, who will determine the treatment assignment for an individual once study eligibility has been confirmed. The treatment assignment will be given to the research nurse, who will oversee the administration of the placebo (saline) and $GnRH_{AG}$ injections. The nurse will not be responsible for acquisition of study outcomes data; such data will be collected by research staff members who are blinded to drug status. Randomization will occur after eligibility for the study has been determined.

Although the $GnRH_{AG}$ will be administered to participants in a blinded manner, it is acknowledged that participants may be aware of treatment status because of the cessation of menses that typically occurs. Participants will be informed that menstrual cycles may stop either because of the drug intervention or because they are approaching the age of menopause. Participants will be further informed that menstrual cycles may not resume or may be irregular after the completion of the intervention because they are approaching the age of menopause. We are aware of one study suggesting that menstrual cycle function may not resume in older pre-/perimenopausal women following $GnRH_{AG}$ therapy if the pretreatment serum FSH level measured during days 3 to 5 of the follicular phase is >25 mIU/mL.[67] We will exclude volunteers who meet this criterion because of the possibility that this may be an early harbinger of the menopause.

2.d.7. Interventions

Drug intervention. The drug intervention will be monthly intramuscular injections of placebo (saline) or leuprolide acetate (3.75 mg for depot suspension; Abbott Laboratories; Abbott Park, IL) for 24 weeks. One-third of participants will be randomized to receive placebo and two-thirds will receive $GnRH_{AG}$. Absence of pregnancy will be confirmed before each dosing. A single injection of leuprolide acetate produces an initial stimulation (for up to ~2 wk) followed by a prolonged suppression of pituitary gonadotropins and ovarian hormone secretion; repeated monthly dosing maintains the suppression of ovarian function. Leuprolide acetate has been used clinically for up to 1 year without serious adverse events, although there may be a decrease in BMD that recovers slowly following cessation of treatment.[68,69] Side effects are those typical of estrogen deficiency. 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.[70,71] Return of menses typically occurred

within 2-3 mo of cessation of therapy. The **rationale** for the drug regimen is that this standard clinical dose suppresses ovarian function and results in fat gain after 24 weeks of treatment.[9,10]

Injections will be administered in a blinded manner, but participants on $GnRH_{AG}$ will likely know their drug assignment because of the induction of amenorrhea. For this reason, volunteers will not be informed during the consenting process that the primary aim of the study is to determine whether $GnRH_{AG}$ causes a decline in physical activity (i.e., such knowledge may change behavior). Rather, women will be informed that the loss of normal ovarian function may alter how the body uses energy or have other metabolic effects. Participants who do not tolerate $GnRH_{AG}$ therapy can refuse further treatment after each monthly dosing. Among the first 64 premenopausal women treated with $GnRH_{AG}$ in the PI's ongoing study (AG018198), this occurred only once. Participants who undergo at least 2 months of therapy but not 6 months will be asked to undergo testing at the end of their final month of therapy. Should this occur, data analyses will include covariate analysis to adjust for duration of intervention.

Exercise intervention. If the loss of ovarian function generates a biological drive toward sedentary behavior, it may be possible to override this through programmed exercise. In laboratory animals, E_2 add-back and programmed exercise (i.e., treadmill running) each prevented, to a large extent, the OVX-related changes in body composition and biomarkers of disease risk (Fig 5).[25-29] The intent of **SA4** is to determine whether programmed exercise can offset the decline in PAEE in response to $GnRH_{AG}$ (PAEE: $GnRH_{AG}$ < $GnRH_{AG}$+exercise). Although it may seem logical to assume this will occur, it is possible that programmed exercise will cause a further decrease in other physical activities, such that programmed exercise produces no net gain in PAEE.

Women randomized to $GnRH_{AG}$+exercise will come to the Exercise Research Laboratory (ERL) for a supervised exercise program. The exercise facility in the ERL is open for research participants 6:30-8:30am, 11:30am-1:30pm, and 4:30-6:30pm Mon through Fri. The goal of the exercise program will be to generate an increase in EE of ~1400 kcal/wk to offset the projected decrease in PAEE. Women will be instructed to come in at least 3 days per week so that regular oversight and encouragement for exercise can be provided. The center-based program will be augmented with recommendations for home-based exercise to meet the weekly exercise goals. Individualized, endurance-based (e.g., treadmill, elliptical, rowing, cycling) exercise prescriptions will be designed to increase energy expenditure by 200-300 kcal per session. As an example, a 75-kg woman with a maximal aerobic power (VO_2max) of 25 mL/min/kg exercising at 65% of VO_2max would have to exercise ~40 min to increase energy expenditure by ~200 kcal. Exercise duration and intensity will be modified according to individual preferences to enhance compliance.

2.d.8. Compliance

All participants must come to the Clinical and Translational Research Center (CTRC) every 4 weeks for 24 weeks for $GnRH_{AG}$ and placebo injections. Compliance to the drug intervention will be tracked by the research nurse, who will deliver all of the injections. If the delivery of a dose is delayed (e.g., participant misses an appointment), it will be administered as soon as possible.

Compliance to center-based exercise will be monitored directly by the staff of the ERL that supervises exercise sessions. Participants will be contacted by the research staff when 3 consecutive sessions are unexpectedly missed. Exercise logs will be used to record the activities performed at the ERL; this information will be computerized so that the supervised exercise exposure can be calculated. Home-based exercise will be captured for all participants through the measurements of TEE by DLW and physical activity by accelerometry.

2.d.9. Tests and procedures

2.d.9.1. Baseline testing schedule and methods

Figure 9 depicts the baseline testing schedule. Given the intensive nature of the testing, some flexibility will be necessary to accommodate the schedules of the participants. However, the general plan is to initiate visit 1 during the early follicular phase of the menstrual cycle so that all testing can be completed before the end of the cycle. This will facilitate starting the $GnRH_{AG}$ intervention in the early part of the next menstrual cycle.

Visit 1- RMR, EI, PA, VO_2max. This visit will occur in the early follicular phase. Participants will report to the lab at approximately 0700h after an overnight fast.

Resting metabolic rate (RMR). After lying quietly for 30 min, RMR will be measured for 15-20 min by indirect calorimetry using the ventilated hood method (Parvo Medics metabolic cart) as previously described.[37] RMR will be used to estimate the energy needed to maintain energy balance.

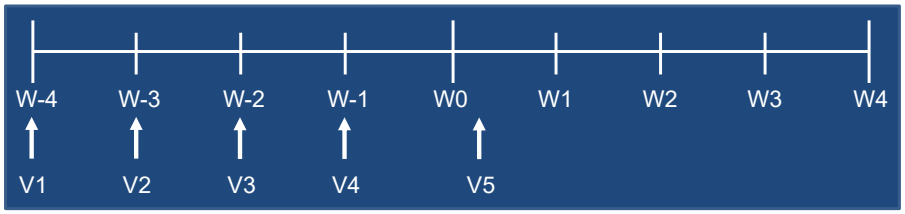
Measurement of physical activity. Physical activity will be measured using ActivPal monitors. The ActivPal categorizes free-living activity into periods spent sitting/lying, standing, and walking. This information can be used to estimate daily energy expenditure and track changes in free-living activity profile. There is no display to provide feedback to the participant. Participants will be instructed to wear the monitor for 7 consecutive days at all times except when sleeping or participating in water-based activities (e.g. showering, swimming). Activity and step counts will be summed over the day and further analyzed to calculate the minutes per days spent in resting, ambulation, or lifestyle activities using a two-regression model.[73] The **rationale** for assessing PA by accelerometry is to compare the changes with those observed in women as they approach the natural menopause.[12]

VO_2max. VO_2max will be measured by indirect calorimetry during treadmill exercise as previously described.[58] The **rationale** for this test is that it will guide the prescription of exercise intensity for the GnRH+exercise group and will reveal whether decreases in PAEE are accompanied by a decrease in cardiovascular fitness.

Visit 2 – TEE by DLW and body composition by DXA. This visit will initiate the measurement of TEE by DLW. The pre-intervention 10-d test will start during the mid-follicular phase of the menstrual cycle. Participants will report to the lab in the morning after an overnight fast. A urine specimen will be collected and the participant will then consume an oral dose of water containing 1.8 g/kg total body water (TBW) of 10 atom percent excess (APE) ^{18}O and 0.12 g/kg TBW of 99.9 APE ^{2}H following procedures developed by Dr. Schoeller.[74] Urine samples for the measurement of $^{2}H_2{}^{18}O$ will be obtained approximately 3 and 4 hours after dosing. Two urine samples will also be collected 10 days after dosing, 1 hour apart. Sample aliquots of 4 mL each will be stored in cryogenic tubes and frozen at -10C. Samples will be decolorized with 200 mg/5 mL of dry carbon black and filtered (0.45 micron). The stable isotope abundances will be measured by isotope ratio mass spectrometry and TEE will be calculated as previously described.[75-77] The **rationale** for the DLW procedure is that it is the most accurate and precise measure of TEE under free-living conditions.[78,79] The major advantages of the DLW method are its objectivity, minimal interference with daily activities, accuracy, and precision. It is objective because the body water acts as a metabolic recorder; the participant does not have to keep logs or report a history. The tracer elimination rates are determined from spot urines collected on the day the tracer is given by mouth and again at the end of the period, so there is little disruption of daily activities. The DLW method has a coefficient of variance of ±1%.[80]

Body composition by DXA. Body composition will be measured by DXA (Hologic Discovery). The lumbar spine and proximal femur scans will not be repeated at this visit; baseline values will be obtained at screening. The operation of the DXA instrument in the PI's lab includes daily measurements of the spine phantom, weekly air scans, and tissue bar scans at least once per month. The reproducibility of measurements has

Figure 9. Baseline testing schedule (W = week; V = visit); weeks 0 and -4 correspond roughly with the start of menstrual cycles



V1 – RMR; meet with dietician, research technician; start 3-d food record and 7-day recording of physical activity by accelerometry; VO_2max test

V2 – start 14-d assessment of physical activity by DLW; body composition by DXA; return food record and accelerometer (at V2 or V3)

V3 – 7-d urine sample for DLW (can be obtained/stored at home)

V4 – 14-d urine sample for DLW; 24-h stay in metabolic chamber; OGTT; tissue biopsies

V5 – first placebo/$GnRH_{AG}$ injection; repeated every 4 weeks for a total of 6 doses

been assessed in 40 women and men, aged 23-86 y, measured on 3 occasions over 1 mo. The coefficients of variation (CV) for total mass, FFM, and fat mass were 0.8±0.8%, 0.8±0.8%, and 2.3±2.7%, respectively. The CVs for BMD of the lumbar spine, total hip, femoral neck, and trochanter were 1.3±0.7%, 1.1±0.9%, 1.9±1.0%, and 1.4±1.0%, respectively. Total body scans will be analyzed for total fat mass and FFM, and regional (i.e., legs, arms, and trunk) fat mass. An increase in the ratio of trunk-to-leg fat mass in response to $GnRH_{AG}$ will be interpreted as a shift in fat distribution pattern toward increased abdominal adiposity.[9,10]

At visit 2 participants will also return the ActivPal and be given nutrition handouts instructing them on how to prepare for their OGTT by maintaining a diet that includes at least 150g/day of carbohydrates.

Visit 4 – urine sample for DLW, chamber stay, OGTT, tissue biopsies, OPTIONAL Vascular Sub-study. These tests may be conducted over 2 visits if scheduling does not permit the chamber stay to occur on day 10 of the DLW interval.

Chamber stay. Participants will report to the CTRC at 0700h after an overnight fast. A urine sample for DLW will be obtained, if necessary. An IV catheter will be positioned in an antecubital vein and a fasted blood sample will be obtained. Participants will enter the chamber at 0800h. A reclining chair will be positioned next to the port in the chamber through which blood samples are obtained. Participants will rest for the first hour. At 0900h, they will drink a 75-g glucose beverage and remain semi-recumbent for the next 2 hours for the TEF measurement. To mimic typical activity outside the calorimeter, two 30-min bouts of treadmill walking at 3 mph will be performed at 1500h and 1630h. This activity should produce a physical activity level (PAL; TEE/REE) of ~1.5 (unpublished observations, Melanson and Kohrt). All urine will be collected and analyzed for urine nitrogen, urea nitrogen, and creatinine for substrate oxidation calculations. Core temperature will be measured during the chamber stay using an ingestible thermistor capsule (length 23 mm, diameter 8.6 mm) and a VitalSense telemetric monitor. TEE will be calculated as previously described.[53] Stepping EE will be measured during the exercise bout. SEE will be measured from 0100h to 0400h.[81] Pre-study diet and exercise are known to affect 24-h fat oxidation.[82,83] Subjects will be asked to refrain from structured exercise the day before the chamber stay. Diet will not be controlled because of the possibility that energy intake (and possibly macronutrient preference) is influenced by ovarian function. The energy composition of the diet for the chamber stay will be 30% fat, 55% carbohydrate, and 15% protein, with the 75g glucose beverage accounting for breakfast and about 25% of total energy needs, while both lunch and dinner will each account for about 30-35% of total energy needs. 'Extra' calories will be provided in the form of a snack box that participants will have free access to from the time the lunch meal is delivered until lights out. Participants will be instructed to eat what they want and that it is not necessary to eat all the food provided. This approach may provide insight on whether the suppression of ovarian function promotes hyperphagia. Data from each calorimeter stay will be evaluated for state of energy balance.

The **rationale** for the chamber stay is to **1)** obtain a prolonged measure of basal metabolic rate for the calculation of PAEE, **2)** obtain a measure of TEE under controlled conditions, **3)** determine whether TEF is influenced by $GnRH_{AG}$, and **4)** determine whether EE during a specific physical task (stepping activity) is influenced by $GnRH_{AG}$. PAEE will be the difference between TEE measured by DLW (adjusted -10% for TEF) and SEE measured during the chamber stay, as previously described.[14] The OGTT will be performed in the calorimeter to avoid a separate visit for participants and to assess whether the TEF is influenced by $GnRH_{AG}$.[84]

Blood samples during the chamber stay. Blood samples will be obtained before and 30, 60, 90 and 120 min after glucose ingestion. These will be used for the measurement of insulin resistance (product of the glucose and insulin areas under the curve) and inflammatory cytokines (IL-6, hsCRP) for **SA3**. The assays will be performed by the CTRC core laboratories. The extent to which changes in disease risk could be explored was limited by the resources available under the P50 mechanism. Measures of insulin resistance and mild chronic inflammation were selected because of the prominent role these factors play in metabolic dysfunction.[85]

Chamber stay alternative**.** Heavy use of the metabolic chamber for multiple research protocols has created scheduling difficulties. Subjects who cannot complete the 24-hr metabolic chamber stay in a timely manner will be offered the option of completing this alternative approach. Subjects will report fasted to the CTRC at 7:00am and will have vitals measured and an IV catheter inserted for the OGTT. At 7:30am they will consume a 75-g glucose beverage and remain semi-recumbent for the next 3 hours for the OGTT and TEF measurements. Blood samples will be drawn before and 30, 60, 90 and 120 min after glucose ingestion. The IV will be removed after the last blood sample. TEF will be measured through indirect calorimetry using the ventilated

hood method (Parvo Medics metabolic cart). Measurements will be taken at 30-minute intervals, and each measurement will last for approximately 10 minutes. Subjects will remain in bed for the duration of the measurements, except to use the bathroom. If the subject needs to use the bathroom, this will be done immediately after a measurement, with the patient then returning to bed as soon as possible. PAEE will be calculated exactly as it is being done in chamber studies, TEE (DLW) - (RMR + TEF).

Tissue biopsies. This research project is a component (Project I) of a Specialized Center of Research (SCOR) on Sex Differences (PI, Wendy Kohrt) sponsored by the NIH Office of Research on Women's Health. The topic of the UCAMC SCOR is *Bioenergetic and Metabolic Consequences of the Loss of Gonadal Function*. **SCOR Project II** (PI, Paul MacLean) is a preclinical study (animals) of *Effects of Pre-existing Obesity on Consequences of the Loss of Ovarian Function*. **SCOR Project III** (PI, Dwight Klemm) is a basic study (animals) on *Sex Hormones Differentially Regulate Production of Distinct Adipocyte Populations*. The tissue samples to be obtained from participants in **Project I** (below) will address specific aims from **Project I** as well as **Projects II and III.**

Tissue will be obtained from vastus lateralis muscle tissue for **SCOR Project II** and abdominal and femoral subcutaneous adipose tissue for **SCOR Project I** and **SCOR Project III**. Percutaneous muscle samples will be obtained from the vastus lateralis. Following lidocaine injection, a small incision will be made in the fascia of the vastus lateralis and 100-200 mg of muscle tissue will be removed using a Bergstrom side-cut biopsy needle with suction applied. Tissue will be flash frozen with liquid N_2**.** Subcutaneous adipose samples will be collected from the lateral thigh region adjacent to the muscle biopsy site and the periumbilical region of the abdomen. An infusion cannula is inserted through a small skin incision (same incision as muscle biopsy for femoral collection) to infiltrate a 50-100 cm^2 area of subcutaneous adipose tissue with 50 ml of normal saline containing 0.0015% lidocaine (15 ml 1% lidocaine per 100 ml saline). An aspiration cannula will be used to collect 10 cc of a mixture of fluid and adipose tissue using Coleman's manual vacuum ("mini liposuction") technique.[86] Collected tissue will be separated from the fluid and processed for analysis. The incision will be closed with a suture, covered with a bandage, and an ice compress applied for 10 min.

Vascular sub-study procedures.

Resting Arterial Blood Pressure. At baseline and after Phases 2 and 3 brachial artery blood pressure will be measured both sitting (after 10 minutes of rest) and supine under quiet, comfortable ambient laboratory conditions. Blood pressure will be measured in triplicate using an oscillometric technique (Dinamap).

*Arterial Stiffness***.** Common carotid artery diameter will be measured from the images derived from an ultrasound machine (GE Vivid i) equipped with a linear array transducer as previously described in CTRC protocols 1574, 1618, 1673 and 1466. Carotid diameter image will be analyzed using image analysis software (Carotid Analyzer). The pressure waveform and amplitude will be obtained from the common carotid artery using arterial applanation tonometry, a high-fidelity strain gauge transducer (Millar Instruments). The combination of ultrasound imaging of the common carotid artery with simultaneous applanation tonometric-obtained arterial pressure waveforms from the contralateral artery permits noninvasive determination of carotid artery compliance.

Endothelial Function. Flow-mediated dilation (FMD) of the brachial artery will be used as a non-invasive measure of endothelial function, as previously described in CTRC protocols 1574, 1618, 1466 and 1673. Briefly, a pediatric cuff will be placed on the upper forearm and the brachial artery will be located 2-3 cm above the antecubital fossa. After obtaining concurrent measures of baseline brachial artery diameter and blood flow velocity, reactive hyperemia will be produced by inflating the cuff up to ~250 mmHg of pressure for 5 minutes followed by rapid deflation. After the release of the arterial occlusion, Doppler blood flow velocity and B-mode ultrasound brachial artery diameter images will be measured continuously and concurrently for 2 minutes. Brachial artery diameter and flow velocity will be acquired and analyzed using a commercially available software package (Vascular Analysis Tools 5.5.1, Medical Imaging Applications, LLC, Iowa City, IA).

Visit 5 – start placebo/$GnRH_{AG}$ intervention. The intervention will start in the early follicular phase of the next menstrual cycle to avoid the induction of another menstrual cycle. Absence of pregnancy will be confirmed.

Participants will receive a total of 6 injections at 4-week intervals. They will complete 3 questionnaires at this visits: the Menopausal Symptom List (MSL) on the frequency and severity of menopause-related symptoms (e.g., hot flashes, depressed feelings),[87] the Eating Behavior Questionnaire (EBQ) to monitor dietary restraint, disinhibition, and hunger,[88] and the Pittsburgh Sleep Quality Index (PSQI) to monitor sleeping behavior.[89]

2.d.9.2. Follow-up testing

Monthly visits. Participants will meet with the study research nurse every 4 weeks. They will complete the MSL, EBQ, PSQI, an exercise motivation questionnaire, and a health status questionnaire that inquires about changes in use of medications (prescription and over-the-counter), changes in health (e.g., doctor visits, hospitalizations), and any study-related problems/concerns over the past 4 weeks. This will provide a regular mechanism by which adverse events will be queried. They will pick up a physical activity monitor to wear for 7 days and return by mail or in person. Following confirmation of the absence of pregnancy, a placebo or $GnRH_{AG}$ dose will be delivered.

Intervention weeks 10 to 12. Study visits V1 to V4 (Fig 9) will be repeated during weeks 10 to 12 of the intervention. The **rationale** for intermediate (12 weeks) and final (24 weeks) testing intervals is that previous studies of $GnRH_{AG}$ therapy in premenopausal women found that body composition is not significantly changed after 12 weeks of treatment[90,91] but is significantly changed after 24 weeks.[9,10,36,92] One concern is that women may begin to adopt compensatory behavioral changes (e.g., decreased food intake, increased physical activity) when changes in body composition are sensed. Thus, the greatest change in PAEE may be apparent at the intermediate testing interval. However, the final testing interval will enable us to determine whether the disruption in energy balance does, indeed, lead to the expected changes in body composition. This testing plan is congruent with Dr. Schoeller's recommendation that at least 10 weeks should separate serial measurements of TEE by DLW (personal communication).

Intervention weeks 22 to 24. Study visits V1 to V4 (Fig 9) will be repeated during weeks 22 to 24 of the intervention. One modification is that the DXA visit will include the measurement of spine and hip BMD in addition to the measurement of body composition to determine the impact of $GnRH_{AG}$ on BMD.

Annual follow-up exams. Participants who complete the intervention may be asked to return for annual follow-up visits through the end of the award period (and longer if a competing renewal is successful). This will enable us to assess the bioenergetic and metabolic consequences of women who undergo the natural menopause. This will serve two major purposes: **1)** It will facilitate a comparison of the findings with the only other study to evaluate such outcomes in women through the menopausal transition, which was carried out in Louisiana;[12] and **2)** It will help us to determine whether changes measured in response to an experimental model of menopause ($GnRH_{AG}$) are consistent with those that occur during the natural menopause. This will have important implications for future research on the health consequences of the loss of ovarian function. **If there are sufficient funds to pursue such long-term follow-up, we will update the protocol and create a second Consent Form which identifies specific study visits/ outcomes and their associated risks.**

Limitation of the follow-up testing schedule. For women assigned to the placebo treatment arm, the follow-up tests will not necessarily occur at the same point in the menstrual cycle as the baseline test. Testing will occur during specific time intervals (weeks 10-12, 22-24) to be consistent with the $GnRH_{AG}$ arms. Because this may increase the variance of changes in bioenergetics in the placebo arm, the sample size calculations relied on conservative assumptions (e.g., ability to detect a 25% change in PAEE; use of $\rho = 0.9$; power of 95%).

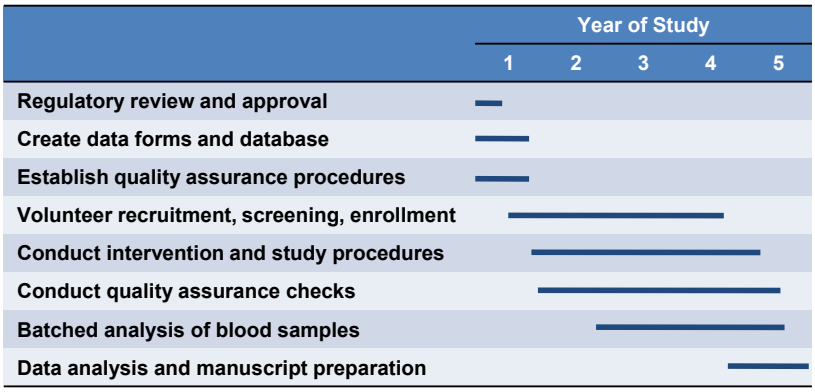
2.d.10. Data analysis

General Analysis Plan. Data analysis will occur in 4 steps: **1)** Descriptive analyses (including boxplots, scatterplots, profile plots to examine change over time) will be conducted for quality assurance and to assess need for data transformation; **2)** demographic and baseline characteristics of the groups will be compared; **3)** primary and secondary analyses will be completed; and **4)** findings will be assessed for their robustness to issues including the effects of any imbalances in important baseline covariates and the effects of missing data.

Primary Analysis. The primary analysis (**SA1**) will be an intention-to-treat comparison of the differences in the change in PAEE measured after 12 weeks of placebo or $GnRH_{AG}$ therapy. This comparison will be conditioned on the stratification variable, age group (40-46, 47-60y). Statistical inference regarding the difference between treatment groups will be based on the estimated coefficient for a treatment group indicator variable in a linear regression model with 24-week change from baseline as the response variable, and explanatory variables,

including treatment group, age group, and baseline PAEE; the latter is included to improve the precision/power of the inference about treatment differences. The conclusion about statistical significance will be drawn from the linear contrast comparing the placebo and $GnRH_{AG}$ groups.

Timeline



Secondary Analyses. To address **SA2** and **SA3**, 24-week differences in other outcome measures (24-h TEE, REE, SEE, TEF, PA by accelerometry, measurement of biomarkers in adipose tissue samples, glucose and insulin response to OGTT, serum concentrations of inflammatory cytokines, and body composition will be analyzed as above. Secondary analyses will be evaluated for their consistency with the conclusions on the primary endpoint. We anticipate that changes in the secondary outcomes will be consistent (i.e., in terms of biological plausibility) with changes in the primary outcome so that significant differences in the primary endpoint will be reinforced by the secondary analyses. Failure to find biological consistency in primary and secondary endpoints will be taken as evidence that the effects of hormone suppression are not clear and that further study is necessary to resolve inconsistencies. This approach will reduce the risk of false-positive conclusions resulting from multiple statistical tests. **SA4** will be evaluated by ANCOVA as described above, regressing change in PAEE or REE on baseline, age group, and treatment group, adding a linear contrast to compare the $GnRH_{AG}$ and $GnRH_{AG}$ +exercise groups. **Exploratory Aim.** Will be evaluated by ANCOVA as described above, regressing change in vascular outcomes (arterial stiffness or FMD) on baseline and treatment group, adding a linear contrast to compare the $GnRH_{AG}$ and $GnRH_{AG}$ +exercise groups.

The characteristics of the time trajectory (at 12 and 24 weeks) for treatment effects will also be determined in secondary analyses. These analyses will evaluate the biological plausibility of the time trajectory (i.e., a biologically-meaningful time trend) to rule out false positive conclusions in the primary analysis. Evaluation of the time trajectory will also motivate future studies of mechanisms and methods to improve longer-term outcomes. The analytic methods to be used for evaluation of the time trajectory include a mixed-effects model with time as a fixed covariate to estimate the mean effect at each measurement time,[93] conditioned on the stratification variable and the baseline value of the outcome variable.

2.d.11. Data management

Data management will be coordinated by the PI, with direct management by the Center on Aging data manager and guidance from the study biostatisticians (Ms. Wolfe). Whenever possible, study data will be transferred electronically into the Center on Aging SQL database. Double entry will be used for manually entered outcome data to track entry errors. Manual data entry will use the Research Electronic Data Capture (REDCap) interface, which is a secure web application. It provides user-friendly case report forms, real-time data entry validation (e.g., for data types and range checks), audit trails, and a de-identified data export mechanism to common statistical packages. The database is hosted at the University of Colorado Development and Informatics Service Center (DISC), which will be used as a central location for data processing and management. Data back-up and security measures are those expected of an Informatics Core at a major academic research institution. Data quality checks will be ongoing, but will formally occur (e.g., checks for duplicate entries, missing data) at least annually when progress reports are prepared.

2.d.12. Timeline

Because the PI has conducted studies that used $GnRH_{AG}$ and exercise training interventions, the regulatory approval process should be completed within 3 to 4 months and recruitment will commence shortly thereafter. The subject accrual rate will be approximately 1.5 per month over 44 months. The primary factor limiting the accrual rate is the availability of the metabolic chamber for study visits.

2.d.14. Potential Limitations

A limitation of the study design is that $GnRH_{AG}$ therapy may not mimic the bioenergetic and metabolic

consequences of natural menopause. It is different from menopause in that is causes a relatively 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). With respect to the latter, it is not the OVX-induced increase in gonadotropins that causes the decline in PAEE and increase in fat mass in rodents. $GnRH_{AG}$ therapy (low gonadotropins) reduced wheel-running activity to the same extent (~45%) as OVX [94] and OVX and $GnRH_{AG}$ generate similar increases in fat mass.[95] With respect to whether the abruptness of the loss of ovarian function is an important determinant of the bioenergetic consequences, there is a relatively novel animal model of ovarian failure that appears to recapitulate the perimenopausal transition. The ovotoxic chemical 4-vinylcyclohexene diepoxide (VCD) destroys ovarian follicles by accelerating the natural process of follicular atresia and mimics perimenopausal acyclicity; other tissues are not affected.[96] Mice treated with VCD have persistent diestrus within ~2 months and progress to a 'postmenopausal' state by ~4 months.[97] VCD appears to have similar bioenergetic and metabolic consequences as OVX. Animals treated with VCD have a pronounced increase in abdominal fat and insulin resistance.[98] This limited preclinical evidence suggests that the bioenergetic and metabolic responses to $GnRH_{AG}$ therapy are likely to be similar to the effects of natural menopause. The use of $GnRH_{AG}$ therapy in the proposed study may be a limitation because it does not perfectly mimic the menopause, but it is also a major strength. In women, the menopause is a process, not an event, which occurs over a variable and unpredictable number of years, making it difficult to study except in large cohort studies. Such studies are typically not experimentally controlled or mechanistically driven. Because the menopause occurs during mid-life, at a time when other chronological aging effects are also occurring, it is difficult to isolate the consequences of ovarian failure. The $GnRH_{AG}$ model will allow us to look specifically at the loss of ovarian function as a trigger for bioenergetic and metabolic changes that could increase disease risk in women. To the extent resources will allow (see budget justification), women in the proposed study will be studied at annual visits after they complete the intervention to capture the bioenergetic and metabolic changes of natural menopause.

Women in the placebo group may progress to peri-menopausal status during the 6-month intervention (they cannot become postmenopausal by definition of absence of menses for 12 months) and may, therefore, have a decline in PAEE. This was taken into account by using conservative for statistical power and sample size calculations. Secondary data analyses will also be performed that include only women who maintain premenopausal status over the period of intervention.

The potential effects of $GnRH_{AG}$ therapy to reduce PAEE could be mediated by a number of factors not being thoroughly studied in this project (e.g., sleep disturbance secondary to the expected increase in menopausal symptoms in women on $GnRH_{AG}$). The Ancillary Projects program of the SCOR could support the addition of some outcomes measures, but careful consideration will be given to the impact on subject burden before any ancillary projects are approved.

3. Protection of human subjects

The proposed study meets the definition of a clinical trial in that it is a prospective biomedical research study of human subjects that involves random assignment to pharmacologic and behavior intervention groups. However, we consider it to be clinical research on a relatively small number of subjects, rather than a 'clinical trial' because it is designed to reveal mechanistic consequences of the loss of ovarian function. It is not a Phase III clinical trial.

3.1 Risks to human subjects

a. Human subjects involvement, characteristics, and design

Volunteers who are eligible and willing to participate will undergo the intervention protocol and procedures described in the Research Plan. We plan to enroll 66 volunteers in the study. They will be healthy, eumenorrheic premenopausal women, aged 40 to 60 years. Eumenorrheic premenopausal status is defined as not yet reaching the Early Menopausal Transition Stage -2 by the Stages of Reproductive Aging Workshop (STRAW) criteria.[99] Stage -2 is characterized by a change in cycle length of 7 or more days (e.g., regular cycles every 24 days instead of 31 days). Accordingly, the medical history will query for cycle regularity in the past year and whether cycle length has changed. The investigators debated whether to have an exclusion criterion for participation in regular exercise. In the absence of knowledge as to whether women who exercise

regularly may be more or less susceptible to the potential effects of gonadal function to regulate physical activity, it was decided that this should not be too restrictive. Thus, only women who exercise at least 30 minutes per day at a moderate to vigorous intensity most days of the week (defined as >4 d/wk) will be excluded. The prospective cohort study that supports a decline in PAEE during the menopausal transition did not have an eligibility criterion based on physical activity level.[12] The investigators also debated whether there should be an exclusion criterion for body mass index (BMI). The prospective cohort study did not have one.[12] That paper did not report BMI, but fat mass averaged ~40% of body weight. In our experience, that falls into the 'obese' category by BMI. Based on this, we chose the exclusion threshold as a BMI $\geq$40kg/m^2.

Inclusion criteria

Volunteers will be women aged 40 to 60 years who are still experiencing regular menstrual cycles. They must be willing to be randomized to placebo or $GnRH_{AG}$ therapy for 24 weeks. They must be physically able and willing to be randomized to participate in a supervised exercise training program. Volunteers for the Vascular sub-study will be recruited from the larger group of participants in the parent study.

Exclusion criteria

Volunteers will be excluded from participation for the following:

- irregular menstrual cycles defined as 2 or more missed cycles in the previous year
- serum FSH >25 mIU/mL measured during the first 5 days of the menstrual cycle
- on hormonal contraceptive or menopausal therapy
- positive pregnancy test
- intention to become pregnant or start hormonal contraceptive therapy during the period of study
- lactation
- known hypersensitivity to GnRH or leuprolide acetate
- CES-D score $\leq$16 OR CES-D score >16 with clinician follow-up and BDI-II score <18
- severe osteopenia or osteoporosis (i.e., proximal femur or lumbar spine t scores < -2.0)
- abnormal vaginal bleeding
- thyroid dysfunction, defined as an ultrasensitive TSH <0.5 or >5.0 mU/L; 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
- cardiovascular disease; subjective or objective indicators of ischemic heart disease (e.g., angina, ST segment depression) or serious arrhythmias at rest or during the graded exercise test (GXT) without follow-up evaluation; follow-up evaluation must include diagnostic testing (e.g., thallium stress test) with interpretation by a cardiologist
- history of venous thromboembolic event
- orthopedic or other problems that would interfere with participation in the exercise program
- BMI $\leq$40 kg/m^2

The proposed studies do not involve special classes of subjects that may be considered vulnerable populations. There are no additional exclusion criteria for the Vascular sub-study

Study group assignment

The approach by which eligible volunteers will be randomly assigned to a study group is described in section 2.d.6.

Justification for dose and frequency of study drug administration

The rationale for the study drug regimen is discussed in section 2.d.7.

b. Sources of materials

The research materials that will be collected from human subjects include:

- medical records – only when needed to follow-up on health status
- blood and tissue samples – for screening and for study-specific outcomes, as described in the research strategy
- data – from all the study procedures, as described in the research strategy

Only members of the research team will have access to protected health information (PHI) collected from the volunteers. Confidentiality of electronic data will be maintained by using only patient identification numbers for data entry. Samples sent to collaborators within the institution (Drs. Klemm and MacLean) will be de-identified. The master document linking patient names with identification numbers will be maintained in a separate password-protected file with restricted access. Paper records will be kept in individual study charts that are stored either in a locked cabinet or in a locked office. Data records will be maintained for at least 7 years after the publication of results. At that time, the data records may be destroyed by the PI by deleting them from electronic media and by shredding paper documents, but some of the data records may be retained indefinitely.

Sharing of data among investigators within the institution is typically facilitated either by providing a password for the database or by electronic transmissions. All members of the research group have individual computers that are part of the institution network with institutional oversight of security. Files that contain data that could jeopardize blinding to treatment code are password-protected to restrict access. Sharing of data with investigators or monitoring personnel outside the institution is typically facilitated by electronic transfer, in a manner that is compliant with HIPAA regulations.

c. Potential risks

Study drugs: All participants will be randomized to receive placebo (saline) or $GnRH_{AG}$ (leuprolide acetate) 3.75 mg/mo by intramuscular injection every 4 weeks for 24 weeks (6 doses).

leuprolide acetate

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.

Convulsions have been observed in patients taking leuprolide acetate. These included patients with medical conditions or those taking medications associated with convulsions. Convulsions have also been reported in patients without any of these conditions.

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 disorder, reduction in breast size, and vaginal dryness. Bone loss may occur, although the amount lost over the 24-week intervention should not be clinically significant and 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.

Leuprolide acetate therapy for 24 weeks may cause an increase in fat mass (1-2 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.

Tissue biopsies: The risks associated with adipose tissue and muscle biopsies include brief, mild burning pain from the local anesthetic (lidocaine), more than mild discomfort during the acquisition of muscle tissue (about 10% of cases), and infection (less than 0.4% of cases). Allergy to the anesthetic (skin swelling or rash) occurs rarely. There may be persistent numbness in the biopsy area, but this is rare.

DXA: The DXA (to assess BMD and body composition) procedure involves exposure to ionizing radiation. All DXA measurements during the study (whole body 3 times, lumbar spine and proximal femur 2 times) during the study involve a total effective radiation exposure that is roughly equivalent to spending 5 days outside in Denver (<50 mrems), or about ~1% of the annual allowable exposure for radiation workers. This risk is minimized by having trained technicians conduct the DXA scans, thereby reducing the likelihood of needing repeat assessments.

Exercise Testing and Training: The risks associated with exercise include falls, pain in muscles or joints, dizziness, fainting, irregular heartbeats. Rarely, exercise testing can result in a stroke, heart attack, or death. The risk of death during or immediately after an exercise test is less than 1 in 10,000. The risk of a heart attack during or immediately after an exercise test is less than 4 in 10,000. The risk of a problem that would require hospitalization, such as chest pain, is less than 2 in 1000. The same discomforts and risks are present during the exercise training program.

Venipuncture and IV catheters: There is a small risk of local hematoma, infection, and thrombosis associated with intravenous blood sampling.

Arterial endothelial function test (Vascular sub-study): the 5 minutes of cuff occlusion may cause a tingling in your fingers and hands, a feeling of pins and needles, and may cause you to feel pain.

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.

3.2 Adequacy of protection against risks

a. Recruitment and informed consent

Volunteer recruitment

Recruitment of volunteers for the study will rely on strategies that have proven successful in attracting volunteers to other projects the PI has conducted, including projects involving $GnRH_{AG}$ and exercise interventions. The recruitment strategies will include (ranked from most to least successful):

- radio advertisements – utilization of radio stations that target a demographic consistent with the study population
- direct mailings – fliers sent to households meeting specified demographics in geographics regions proximal to UCAMC
- e-mail bulletins – system-wide (e.g., UCAMC and UCH) e-mail blasts
- suburban newspaper advertisements – there are many suburban journals in the Metro area that are issued on a weekly or monthly basis; we specifically advertise in those that target neighborhoods in relative proximity to UCAMC and those that target persons from under-represented minority groups
- major newspaper advertisements – the major publication in the Denver Metropolitan area has a Health and Fitness section that runs once per week; advertisements in these sections have targeted appropriate individuals for our previous studies
- study pamphlets – pamphlets describing the study will be printed for a variety of purposes (for study participants to give to family and friends who may be interested in participating; to place in the UCAMC Ob/Gyn Clinic and other relevant clinics; to place in community recreation centers; to hand out when

the investigators give community lectures)

- university advertisements – advertisements will be placed on a quarterly basis in the two UCAMC publications that target students/faculty/staff and alumni
- community lectures – the investigators participate in a UCAMC-sponsored health-related lecture series for the community as well as other lectures sponsored by local churches and organizations

Informed consent process

The consenting process is part of the first orientation session, which takes 60-90 min. Volunteers are either sent a copy of the consent form in advance or are given one at the start of the orientation; time is allowed for review of the consent form. Volunteers then meet with a member of the research team to a) have the study and what is expected of them explained in detail; b) discuss their reasons and motivation for wanting to participate to determine whether they are realistic; c) discuss any practical problems (e.g., scheduling conflicts, vacations) that could interfere with participation; d) have their questions answered; and e) 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. The research team member in charge of informed consent will fill out the documentation of informed consent standard checklist. Volunteers are given a signed copy of the consent form and a signed copy of the consent and the documentation of informed consent checklist will be maintained in the study chart for each participant. The consent form includes HIPAA authorization.

Members of the research group who obtain consent and HIPAA authorization will be in compliance with IRB and HIPAA education requirements. The PI will have the responsibility of ensuring that research personnel are prepared to convey study information to volunteers.

b. Protections against risk

Plans to minimize risks

Study drugs: 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 the study clinician and will undergo a maximal exercise stress test. Participants will be observed for 30 min after drug injection to monitor for new hypersensitivity reactions. Absence of pregnancy will be confirmed before placebo or $GnRH_{AG}$ are administered. Women will be instructed that they should not become pregnant while taking study drugs because of risks to the fetus. 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. The exercise training intervention that one-third of the participants will engage in is expected to minimize changes in body composition. Participants randomized to the non-exercise arms will be given the opportunity to undergo exercise training after the study to help reverse any changes in body composition that occurred.

DXA: The risk of radiation exposure is minimized by having trained technicians administer the procedures, thereby reducing the likelihood of needing repeat assessments. Pregnancy tests will be done prior to each DXA scan to reduce risk of fetal radiation exposure.

Exercise testing and training: The risks of exercise testing and training will be minimized by adhering to the following strategies:

Endpoints for exercise tests: In asymptomatic individuals who do not develop cardiovascular abnormalities, the endpoint for the maximal exercise tests will be severe fatigue that forces cessation of exercise. The criteria that will be used to stop the exercise test before volitional fatigue include the development of: (a) ST-segment depression of more than 0.2 mV that is either horizontal, downsloping, or slowly upsloping (less than 1 mV/sec) and lasts for 0.08 sec, or ST-segment elevation greater than 0.1 mV; (b) chest pain or discomfort; (c) serious arrhythmias, including multifocal PVCs, ventricular tachycardia, frequent (>10/min) PVCs or couplets, or sustained atrial tachyarrhythmias; (d) A-V block or other conduction defects; (e) a fall of systolic blood pressure of 10 mmHg or greater from the peak level with increasing exercise intensity; (f) diastolic blood pressure above 110 mmHg or systolic above 220 mmHg; (g) dizziness; (h) ataxic gait; and (i) pallor or cyanosis.

Training of personnel: Personnel assisting with the administration of screening treadmill tests will have BLS

certification and an experienced clinician will either conduct the test or be in the immediate vicinity (i.e., can be present within 3 minutes). A physician will interpret ECG and BP responses for all screening treadmill tests.

Screening of volunteers: To minimize risks of exercise testing in subjects, testing will be conducted only after the volunteer is examined by a clinician and after a resting ECG is obtained and evaluated. During exercise testing, the ECG is monitored constantly and BP is measured frequently. Exercise tests are terminated if any of the ACSM absolute or relative stopping criteria are met. The AHA-recommended emergency equipment and supplies will be available, including: automated electronic defibrillator; portable oxygen tank, nasal cannula, ventimask, non-rebreathing mask, and appropriate tubing to connect to the oxygen tank; oral airways; bag-valve-mask hand respirator; syringes and needles; IV tubing, solutions, and stand; and adhesive tape. The Exercise Research Laboratory operates as a 911 emergency response facility, as do all other UCH outpatient clinics.

Exercise training. The risks of exercise training will be minimized by the use of individualized exercise prescriptions and by having exercise professionals supervise the exercise training sessions.

Tissue Biopsies: Risks of the adipose and muscle biopsy procedures will be minimized by having a trained clinician perform the procedure. Persons with a prior history of allergies to local anesthetics will not be enrolled. Participants will return to the laboratory within one week of the biopsies to assess wound healing.

DXA: The acquisition of DXA data by trained research staff minimizes radiation exposure by reducing the need to perform repeated scans. Scans will not be performed in women who are pregnant.

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

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

Participants 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, concerns with study medications or exercise. 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. Ms. Quick will initiate event reports for both the programmed and spontaneous complaints.

3.3 Potential benefits to the subjects and others

The potential benefits to an individual participant in the study are not known. All participants will have an opportunity to participate in a supervised exercise training program either during or after the study intervention period that may trigger beneficial adaptations, but not all individuals respond favorably to exercise with such adaptations.

The potential benefits of the study to women in general could be significant. The study is expected to lead to the scientific discovery of the role of ovarian function in regulating energy balance, body composition, and disease risk. This may lead to the development of strategies to improve the health of postmenopausal women. In light of these potential benefits, the risks to subjects are acceptable.

3.4 Importance of 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 hormone therapy (HT) attenuates weight gain in postmenopausal women by an average of 40%. Further, in the absence of HT, 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 CAD 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. Currently, there is very limited knowledge (1 prospective cohort study) in humans of the potential gonadal regulation of spontaneous physical activity. In this context, the proposed study has the potential to reveal a consequence of the loss of ovarian function that has widespread health implications.

3.5 Data and safety monitoring plan

The NIH requirements for a Data and Safety Monitoring Board (DSMB) is as follows (from the SF424 guide):

“NIH specifically requires the establishment of Data and Safety Monitoring Boards (DSMBs) for multi-site clinical trials involving interventions that entail potential risk to the participants, and generally for Phase III clinical trials. Although Phase I and Phase II clinical trials may also need DSMBs, smaller clinical trials may not require this oversight format, and alternative monitoring plans may be appropriate.”

As discussed above, the proposed study is viewed as mechanistically driven clinical research but not a ‘clinical trial.’ In this context and based on the NIH DSMB recommendations, we propose that safety monitoring can be performed by the research team, with oversight by an independent local Safety Officer. However, in the event that the NIH requires the assembly of an independent, external DSMB, the general guidelines would be as described below.

Roles and responsibilities of the Safety Officer (or DSMB)

The Safety Officer (or DSMB, if required by the NIH) will: 1) monitor recruitment, enrollment, and adherence of study participants; 2) approve criteria for modifying or discontinuing the drug or exercise interventions for individual subjects; and 3) review serious adverse events (SAEs). Dr. David Weitzenkamp and Ms. Wolfe, the SCOR biostatisticians, will be responsible for preparing any data sets that the Safety Officer may ask to review. The Safety Officer will remain blinded to treatment status unless, for safety reasons, it is decided that knowledge of the treatment code is important.

The objectives of the Safety Officer will be to assess the safety of the interventions and to assure the highest degree of subject safety. The Safety Officer will: 1) review the protocol as funded and make suggestions for any changes (especially safety related); 2) determine appropriate adverse effect endpoints to be monitored and generate individual- and study-stopping rules; 3) review study progress and data quality; 4) determine formatting for data reports; 5) review endpoints for safety and efficacy; 6) submit reports and suggestions to the PI and the NIH; and 7) add to or modify this list of objectives.

Because of the nature of the study, the Safety Officer will need to monitor safety only and not efficacy. In the event the Safety Officer determines that the study or a phase of the study should be stopped for reasons of safety, this will be communicated to the PI and the NIH; the PI will then inform the UCAMC IRB.

Safety Officer meetings will address issues of protocol design (including any proposed changes in design, data management or analysis), recruitment, retention, data management, and data quality. The Safety Officer will summarize any information to be noted or acted on by the PI.

Defining and reporting serious adverse events (SAEs)

We will promptly notify the IRB (within 5 days of the occurrence) when unexpected SAEs (unanticipated problems) occur, defined as death, life threatening illness, hospitalization or prolongation of hospitalization, congenital anomaly/birth defects, and persistent/significant disability. Any SAE that is unexpected and related or possibly related to the drug or other research intervention will be reported. SAEs that are unrelated to the research intervention will not be reported to the IRB (however, we will report these to the Safety Officer and NIH if they wish to be informed). Risks that are described in the protocol and consent will not be reported promptly to the IRB unless they occur more frequently or are more serious than expected. One exception to this rule is in the case of a death. All deaths must be reported, whether or not the death was related to the research.

In addition to following the requirements above, we anticipate that the Safety Officer will define study-specific

SAEs that trigger the cessation of the intervention for an individual. We will suggest the following:

- Inability to tolerate $GnRH_{AG}$

Because the study interventions include a drug intervention that is used clinically (i.e., risks are known) and exercise training, we do not believe study-stopping rules are necessary.

3.6 ClinicalTrials.gov requirements

The proposed study is mechanistically driven clinical research, but is not a ‘clinical trial.‘ However, because it involves a randomized controlled intervention, it will be registered at ClinicalTrials.gov by the PI.

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