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A Randomized Controlled Trial of Ovarian Suppression in Premenopausal Women: No Change in Free-living Energy Expenditure

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Abstract

Objective—To determine whether suppression of ovarian function (Gonadotropin-releasing hormone agonist, GnRH_{AG}) for 24 weeks in premenopausal women approaching menopause causes changes in body composition and a decline in free living physical activity energy expenditure (PAEE) and if endurance exercise training attenuates the changes.

Methods—Premenopausal women who were approaching the menopause (mean [SD]: age 46 [3] yrs, body mass index 26.3 [4.8] kg/m²) were randomized to 24 weeks of GnRH_{AG} (n=14), GnRH_{AG}+Exercise (GnRH_{AG}+Ex; n=11), or placebo (n=9). Endurance exercise was performed four days per week with the goal of expending 200–300 kcal per session. Primary outcome measurements included body composition by dual-energy x-ray absorptiometry (DXA), total daily energy expenditure (TDEE), and PAEE by doubly labeled water (DLW), and resting energy expenditure (REE) by indirect calorimetry.

Results—Changes in TDEE, PAEE, REE or body composition were not different between groups. However, within the GnRH_{AG} group, fat mass increased (mean [SE]: total 1.7 [0.4] kg, trunk 0.9 [0.2] kg, leg 0.6 [0.2] kg) and leg fat-free mass decreased (mean [SE]: –0.4 [0.2] kg) significantly.

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Conclusions—In premenopausal women approaching menopause, ovarian hormone suppression resulted in increased adiposity without alterations in TDEE, PAEE, or REE.

Keywords

Estradiol; energy expenditure; body composition; fat mass; lean body mass

Introduction

Menopause, whether surgical or natural, is a consequence of aging that all women experience. With life expectancy increasing, women now live many years in the postmenopausal state. Importantly, women have increased cardiovascular and metabolic disease risk after menopause (1–3), which is attributed, in part, to the changes in body composition and body fat distribution (4–7). Although the loss of ovarian estrogens has been identified as a factor regulating these changes, the specific mechanisms by which estradiol (E_2) results in alterations in adiposity remain an area of investigation.

Reduced energy expenditure (EE) is one mechanism by which low E_2 could increase adiposity. Both acute (8) and chronic (9) suppression of ovarian hormones using gonadotropin-releasing hormone (GnRH) analogs in premenopausal women reduced resting energy expenditure (REE), which was prevented with concurrent E_2 therapy. Furthermore, chronic GnRH agonist ($GnRH_{AG}$) therapy also reduced 24 hour EE as measured by whole room calorimetry (9). Whether ovarian hormone suppression would have the same effect on free living total daily energy expenditure (TDEE) is not known.

One component of EE that has not been carefully studied in humans is physical activity energy expenditure (PAEE). Leisure-time physical activity level declines 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 (10). Evidence from studies of laboratory animals demonstrates that spontaneous physical activity is regulated by gonadal function, and specifically by sex hormones (11–14). However, the specific role of E_2 in reduced physical activity over the menopausal transition has not been determined in women (5). Preliminary evidence suggests physical activity levels are higher in women undergoing pharmacological suppression of ovarian function with E_2 add-back when compared with women treated with placebo add-back (15) and that exercise EE is reduced with ovarian hormone suppression (9), but whether PAEE decreases in free-living conditions is unknown.

The primary goal of this study was to determine whether suppression of ovarian function ($GnRH_{AG}$) for 24 weeks in premenopausal women causes a decline in free living PAEE and alterations in body composition. Furthermore, because programmed exercise mitigates metabolic consequences of gonadectomy in animals (16), a secondary aim was to determine whether the consequences of $GnRH_{AG}$ are mitigated through a programmed, supervised exercise training intervention ($GnRH_{AG}+Ex$). Our hypotheses were that 24 weeks of $GnRH_{AG}$ would result in a significant decrease in PAEE and total daily energy expenditure (TDEE) and an increase in adiposity and that programmed exercise would attenuate these changes.

Methods

This was a randomized controlled study in which premenopausal women were randomized to undergo 24 weeks of GnRH_{AG}, GnRH_{AG}+Ex, or Placebo ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01712230) identifier [NCT01712230](https://clinicaltrials.gov/ct2/show/study/NCT01712230)). The study was approved by the Colorado Multiple Institutional Review Board and all volunteers provided written informed consent form to participate. All procedures took place at the Clinical and Translational Research Center (CTRC) on the University of Colorado Anschutz Medical Campus (CU-AMC). Recruitment started in November 2012 and all study follow up was completed by February 2018.

Participants

Participants were healthy, eumenorrheic, premenopausal women nearing the menopause transition, aged 40 to 54 years. Eumenorrheic status was defined as not yet reaching the Early Menopausal Transition Stage –2 by the Stages of Reproductive Aging Workshop (STRAW) criteria (17), characterized by a change in cycle length of 7 or more days (e.g., regular cycles every 24 days instead of 31 days). After providing informed consent, volunteers underwent screening, including a medical history and physical examination, and clinical laboratory evaluation (comprehensive metabolic panel, TSH, complete blood count, and urine pregnancy test). Because GnRH_{AG} treatment may decrease bone mineral density (BMD), dual-energy x-ray absorptiometry (DXA) scans of the proximal femur and lumbar spine were conducted to exclude volunteers with T-scores of <-2.0 . A graded exercise treadmill test (GXT) was conducted to exclude volunteers with evidence of ischemic heart disease or serious arrhythmias. Additional exclusion criteria included: follicle-stimulating hormone (FSH) >25 mIU/mL measured during the first 5 days of the menstrual cycle; taking or planning to take hormonal contraceptive or menopausal therapy; pregnant or planning to become pregnant; lactating; known hypersensitivity to GnRH or leuprolide acetate; Center for Epidemiologic Studies Depression Scale (CES-D) score >16 ; thyroid stimulating hormone (TSH) <0.5 or >5.0 mU/L; uncontrolled hypertension ($>150/90$ mmHg); history of venous thromboembolism; orthopedic or other problems that would interfere with exercise; BMI >40 kg/m².

Randomization and Blinding

Volunteers were informed they would be randomized to placebo or active GnRH_{AG} and to exercise or no exercise. They were not informed that only women on active GnRH_{AG} would be randomized to exercise. Although GnRH_{AG} was administered to participants in a blinded manner, we acknowledge that participants could have been aware of treatment status because of the cessation of menses that typically occurs. Participants were informed that menstrual cycles may stop either because of the drug intervention or because they were approaching the age of menopause. Participants were also informed that menstrual cycles may not resume or may be irregular after the completion of the intervention because they were approaching menopause.

Randomization into the three groups was stratified by age (40–46, 47–54 y), with assignment to placebo stopped at $n=11$ to maximize the number of women assigned to the active GnRH_{AG} arms. Randomization sequences were generated by the biostatistician.

Treatment assignment was determined once study eligibility was confirmed and was given to the research nurse who oversaw the administration of the placebo and GnRH_{AG} injections. All study outcome data were collected by research staff members who remained blinded to treatment group.

Interventions

Study Drugs.—Intramuscular injections of placebo (saline) or leuprolide acetate (3.75 mg for depot suspension; Abbott Laboratories; Abbott Park, IL) were administered every 4 weeks for 24 weeks. Absence of pregnancy was confirmed before each dosing. The first injection of leuprolide acetate produces an initial stimulation (up to ~2 wk) followed by a prolonged suppression of pituitary gonadotropins and ovarian hormone secretion; repeated monthly dosing maintains the suppression.

Exercise Training.—Women randomized to GnRH_{AG}+Ex came to the Exercise Research Laboratory (ERL) at CU-AMC for a supervised endurance exercise program four days per week for 22 weeks. Exercise training was stopped one week before post-intervention testing to avoid acute residual effects of exercise on metabolic measurements. Individualized, endurance-based exercise was performed for 20–60 minutes at a target range of 70–80% maximum heart rate determined during screening GXT to increase EE by 200–300 kcal per session (18). Training was primarily treadmill, although cycle ergometry and elliptical were also permitted. Participants were considered non-compliant to the exercise intervention, and thus excluded from the analysis, if they completed <70% of the prescribed exercise sessions (n=5).

Procedures

Baseline testing was initiated in the follicular phase of the menstrual cycle for all but nine women, who were in the luteal phase (Placebo, n=2; GnRH_{AG}, n=4; GnRH_{AG}+Exercise, n=3). After completion of baseline testing, participants started the drug/exercise intervention and repeated all testing again during weeks 23–24 of the intervention.

Body composition.—Body composition was measured using a Hologic Discovery-W instrument (software Apex 4.0.1; Hologic, Waltham, MA). Total body scans were analyzed for total and regional fat mass and fat-free mass (FFM). Intra-instrument coefficients of variation (CV) completed on women and men, aged 23–86 y, for FFM and fat mass (FM) were $0.5 \pm 0.3\%$, and $2.0 \pm 1.7\%$, respectively. Regional analyses were conducted as specified by the manufacturer. Visceral fat mass was estimated from DXA as previously described (19). Scans were completed by two trained and experienced technicians and reviewed by a study investigator to ensure correct acquisition and analysis.

VO₂peak.—VO₂peak was measured by indirect calorimetry during treadmill exercise as previously described (20). Baseline results guided exercise prescription for the GnRH_{AG}+Ex group.

REE.—Participants reported to the CTSC at approximately 0700 h after an overnight fast. After lying quietly for 30 min, REE was measured using standard indirect calorimetry with

the ventilated hood technique (TrueOne 2400, ParvoMedics, Sandy, UT). Respiratory gas exchange was measured for 30 minutes, and values from the last 20 minutes were used to determine REE (9, 21).

TDEE.—Free-living EE was measured utilizing the free-living doubly labeled water (DLW) technique over a nine-day period (22). Participants reported to the CTRC in the morning between 0630 and 0700 h after at least an eight-hour fast. Upon arrival, participants were instructed to completely empty their bladder and a baseline urine specimen was collected for background isotopic abundance measurement (^2H and ^{18}O). Participants then consumed 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 ^2H (ISOTEC INC; Sigma Aldrich, Miamisburg, OH) following procedures developed by Schoeller (23). TBW was estimated as 73% of total fat-free mass measured by DXA. The dose container was rinsed with 10 mL of tap water twice, which the participant consumed. The bladder was emptied again one hour after dosing and 8 oz of tap water was consumed. No additional food or drink was allowed until after the final urine collection and participants were instructed to remain as inactive as possible during the equilibration period. Urine samples for the measurement of $^2\text{H}_2^{18}\text{O}$ were obtained four and five hours after dosing and again nine days after dosing at the same time of day. Urine aliquots were stored in cryogenic tubes at -80°C until shipment for analysis at the Isotope Ratio Mass Spectrometry (IRMS) Laboratory directed by Dr. Schoeller at the University of Wisconsin-Madison. TDEE was calculated as previously described (24–26). PAEE was calculated as $\text{TDEE} - (\text{REE} + (0.1 \times \text{TDEE}))$, which assumes that the thermic effect of food is 10% of TDEE.

Circulating factors.—Blood samples were obtained through an antecubital IV catheter between 0700 and 0730 h after an overnight fast. After collection, samples were stored at -80°C . estradiol (E_2), progesterone, FSH, and sex hormone binding globulin (SHBG) were measured by chemiluminescent immunoassay (Beckman Coulter). The respective within- and between-day CVs were, 4.3% and 8.2% for E_2 (sensitivity 36.7 pmol/L), 4.4% and 7.9% for progesterone (sensitivity 0.318 nmol/L), 1.8% and 3.8% for FSH (sensitivity 0.11 mIU/mL), and 3.6% and 5.7% for SHBG (sensitivity 3 nmol/L). Testosterone was measured via 1-step competitive assay (Beckman Coulter) with CVs of 2.1% and 5.1% (sensitivity 0.59 nmol/L).

Statistics

Sample size.—The expected effect of ovarian hormone suppression on PAEE was conservatively set at -25% , based on the range observed in preclinical studies of -30% to -80% (11–14). Baseline PAEE was assumed to be 800 kcal/d with a SD of 330 kcal/d (27). A sample size of 10 in each of the three groups would achieve $>80\%$ power to detect a 200 kcal/d (SD 148 kcal/d) difference between groups in the change in PAEE using a *t*-test at the 0.05 significance level.

Analyses.—Baseline differences between the three groups were tested using ANOVA for numeric variables and chi-square test for non-numeric variables. The repeated measurements of the EE outcomes (i.e., PAEE, TDEE, and REE) were modeled using a general linear

mixed model with maximum likelihood estimation, controlling for baseline values of age, E₂, and FM. To control for effects of changes in body composition on EE outcomes a second model, also controlling for changes in FM and FFM, was generated. This approach is conceptually identical to repeated measures analysis of variance but has the advantage of using all available data; estimates are unbiased under the assumption that missing data were missing at random. A compound symmetric covariance structure was chosen for the model based on using Akaike Information Criterion (AIC) to compare various covariance structures. A cell means model was used to estimate the mean EE level for each combination of treatment and study visit. Linear contrasts were then constructed to estimate all effects of interest, such as the within-group changes across visits or the between-group treatment differences. The significance of each effect was determined by performing a *t*-test on the contrast estimate. Differences in change between groups for sex hormones and body composition variables were evaluated using ANOVA with Tukey's HSD post-hoc test when the global F-statistic reached significance. The standard convention of controlling the type 1 error rate at 0.05 was used. All analyses were completed using SAS version 9.4 M5 (SAS Institute Inc, Cary, NC). Data are reported as means $\pm$ SEM or mean (95% CI), unless otherwise specified.

Results

Forty-seven women were randomized and seven were lost to follow up (Figure 1). Five women did not meet the criterion for compliance to the exercise intervention (i.e., completed <70% of the prescribed exercise sessions) and were excluded from the final analysis. Of the 34 women who completed the intervention as prescribed, nine were randomized to placebo, 14 to GnRH_{AG}, and 11 to GnRH_{AG}+Ex.

There were no group differences at baseline in race/ethnicity, age, VO₂peak, or body composition (Table 1). Sex hormones were also similar across the groups, with the exception of testosterone, which was elevated in the GnRH_{AG}+Ex group due to one elevated result (3.5 nmol/L).

E₂ decreased in the GnRH_{AG} and GnRH_{AG}+Ex groups, but did not change in the placebo group (Table 2). There was no main effect of group for progesterone, testosterone, or SHBG. VO₂peak increased in the GnRH_{AG}+Ex group (Table 3), but did not change in the Placebo or GnRH_{AG} groups.

There were no between group differences in any outcomes, including PAEE, TEE, REE, or body composition variables. This was true for EE outcomes with (Figure 2) or without (Table 4) adjustment for changes in body composition. However, total, leg, and trunk fat mass all increased and leg FFM decreased in the GnRH_{AG} group (Table 4, Figure 3).

Discussion

The primary aim of this study was to determine if suppression of ovarian function in premenopausal women causes a decline in components of free-living EE and an increase in adiposity. The secondary aim was to determine if programmed endurance exercise could attenuate these alterations. We found after 24 weeks there were no differences between

GnRH_{AG}, GnRH_{AG}+Ex or placebo in the changes in any component of free living EE or body composition demonstrating no effect of the ovarian hormone suppression or exercise training interventions. However, in support of our hypothesis, GnRH_{AG} alone resulted in within group changes including increased total and regional FM and decreased leg FFM.

The approach to and transit through menopause results in alterations in body composition characterized by increased FM, particularly abdominal, and decreased FFM, and no change or an increase in body weight (5, 7). However, studying the mechanisms underlying alterations in body composition across the menopausal transition is difficult because of its long duration and unpredictable nature. Furthermore, identifying changes specifically resultant to alterations in circulating sex steroids is challenging because other aging-related changes also occur over that time course. Therefore, we studied premenopausal women who were approaching the age of menopause before and after 24 weeks of GnRH_{AG} therapy. This pharmacological model of gonadal hormone suppression provides an approach of isolating the effects of loss of gonadal hormones from the usual consequences of aging.

The main finding of this study was that total, trunk, and leg FM increased in the GnRH_{AG} group, but not placebo or GnRH_{AG}+Exercise groups. This was consistent with previous GnRH_{AG} interventions in premenopausal women, which found increases in FM of 0.0 to 2.0 kg over 16 to 24 weeks (28–32). However, none of the previous studies included a placebo control group. We previously conducted a 20 week GnRH_{AG} intervention in a cohort of younger premenopausal women (36 ± 9 y, range 20–49 y) and unexpectedly found no change in total fat mass (32). Although we did not observe an increase in total FM in that study, abdominal visceral fat area increased by ~12% in the GnRH_{AG}+PL group, and this was prevented in the GnRH_{AG}+E₂ group. In the current study, we enrolled older premenopausal women approaching menopause (46 ± 3 y, 40–54 y) finding a 1.7 kg increase in total FM, falling directly within the range of previous studies. Excess fat accumulation appears to begin a few years before the final menstrual period (5, 7). Thus, the relative proximity to the menopausal transition of the women in the current study may have increased their susceptibility for alterations in body composition compared to a younger cohort.

No measures of FM changed significantly in the placebo or GnRH_{AG}+Exercise groups. These results suggest that endurance exercise attenuated the GnRH_{AG}-related increase in FM by almost 90%. However, because the between-group differences in changes in markers of adiposity were not statistically significant, the attenuating effect of the exercise intervention must be interpreted cautiously and should be confirmed. Nonetheless, exercise is beneficial for women throughout life, particularly with menopause as it reduces chronic disease risk and maximizes functional life span and quality of life (33). The Study of Women's Health Across the Nation found that regular physical activity has beneficial effects on changes in body composition and body fat distribution in peri- and postmenopausal women, including less central adiposity (34).

FFM decreases during aging and across the menopause. Indeed, a decline in ovarian function has an independent effect on FFM (35–37). There were nonsignificant decreases in total FFM in the GnRH_{AG} and GnRH_{AG}+Ex groups and a significant decrease in leg FFM in

the GnRH_{AG} group. Notably, the 0.7 kg loss of total FFM, although not statistically significant, was similar to that previously observed by us (−0.6 kg) (32) and others in response to ovarian suppression (−0.7 to −1.2 kg) (28–31). When considered with previous studies, our findings suggest that the loss of gonadal hormones, possibly through direct effects in the muscle, results in reduced FFM in women.

Decreased physical activity may contribute to the loss of FFM in aging women. The women in our GnRH_{AG}+Exercise group regularly performed endurance exercise throughout the 24 week intervention and did not demonstrate a significant change in either total or leg FFM. However, changes in FFM in the exercise group appeared more similar to GnRH_{AG} than placebo. Thus, endurance exercise at the volume and intensity prescribed here may not be sufficient to prevent the loss of FFM observed with gonadal hormone suppression. Because the between-group differences in changes in FFM were not statistically significant, the effect of the exercise intervention must be interpreted cautiously and should be confirmed. Interestingly, a recent systemic review and meta-analysis found no beneficial effect of hormone therapy on muscle mass retention in postmenopausal women (38). This emphasizes the need to more thoroughly understand the role of estrogen in maintenance of FFM in women and to develop strategies for preventing loss in aging women.

In contrast to our hypothesis, there were no significant decreases in PAEE or other components of free-living EE in response to GnRH_{AG}, with or without adjustment for changes in body composition. In a prospective cohort study of women as they progressed from pre/perimenopausal to postmenopausal status, Lovejoy et al. found that physical activity EE, measured by 4 days of accelerometry, decreased by 50% over the 4 years before postmenopausal status was reached (5). Those results are consistent with murine studies, which demonstrate regulation of spontaneous physical activity by gonadal function, and specifically by sex hormones. Compared with intact animals, ovariectomy (OVX) results in a dramatic decline in physical activity that is not attributable to the effects of surgery (11–14). Further, physical activity can be rescued in OVX animals treated with E₂ (11–14). We previously published proof-of-concept evidence supporting the hypothesis that physical activity is maintained at a higher level in women undergoing pharmacological suppression of ovarian function with E₂ compared to placebo add-back (15), providing the first evidence for the regulation of physical activity by E₂ in women. However, we did not observe differences in non-exercise physical activity or sedentary behavior between the groups in this intervention (data not shown). Furthermore, here we report estimates of PAEE from the doubly-labeled water technique measured over two timepoints separated by 24 weeks, not by accelerometry, which could contribute to the conflicting results. The large variability in the DLW measurement over the relatively short timeline of our intervention may have limited our ability to observe changes that appear to occur over a longer timeline.

Notably, although not significant, the magnitude of decrease in TDEE (93 kcal/day) and REE (59 kcal/day) in the GnRH_{AG} group, are consistent with our previously published 20 week GnRH_{AG} intervention (9). It is likely that the high variability and small sample size limited our ability to detect changes in these components in the free-living environment in the current study. Controlled measurements in the metabolic chamber would help to determine if more strictly measured components would reveal the hypothesized changes.

The potential physiological relevance of the observed decreases in EE after gonadal hormone suppression should not be discounted. A decrease of nearly 100 kcal per day for total EE, if not compensated with decreased energy intake or increased EE is a relatively large disruption in energy balance that would be expected to promote fat gain.

Although the exercise intervention was successful at increasing $\text{VO}_{2\text{peak}}$, we did not observe between-group differences related to exercise for any body composition or EE outcomes. The goal of the exercise intervention was to increase EE 200–300 kcal per session. This was not reflected in the post-intervention DLW measurements because the exercise intervention was stopped one week prior to follow-up testing to avoid acute residual effects of exercise on metabolic measurements. Although the exercise intervention increased aerobic fitness, we cannot conclude that it was effective at preventing changes in body composition during ovarian suppression. Further research is warranted.

Although this was a rigorously conducted randomized controlled trial, the study design does not mimic the menopausal transition and has some limitations. GnRH_{AG} treatment results in a relatively rapid decline in ovarian hormone levels (3–4 weeks), and FSH and luteinizing hormone levels are suppressed rather than elevated. The potentially important role of FSH in observed changes in body composition and EE with menopause was recently brought to the forefront by murine studies conducted by Liu et al. (39). Compared with controls, mice treated with an FSH antibody had decreased visceral and subcutaneous adiposity and increased REE during light hours. Notably, body composition changes were evident in both both sham and OVX animals, suggesting FSH can regulate fat mass even when E_2 is normal. In addition, although we had a placebo group, we did not have a GnRH_{AG}+ E_2 add-back group to isolate the effects of E_2 from FSH or other ovarian hormones. Yet, the between-group difference in change for sex hormone levels was only significant for E_2 . Although testosterone and SHBG also decreased significantly in the GnRH_{AG}+Exercise group, changes in these hormones were not different between groups. Thus, our results support a role for E_2 in the changes in FM observed with ovarian suppression. This does not exclude a potential role for FSH in changes in adiposity, but does demonstrate elevated FSH is not essential for these changes to occur. Finally, our study design was not powered to detect changes in body composition.

Conclusion

Changes in body composition are a hallmark of aging in women. Although not a perfect mimetic for menopause, we demonstrated that the loss of circulating ovarian hormones with GnRH_{AG} therapy resulted in increased FM and loss of FFM that was not related to decreased PAEE. Exercise is a potential non-hormonal lifestyle intervention that could attenuate adverse changes in body composition in peri- and postmenopausal women promoting healthy aging. Larger trials must be conducted before firm conclusions can be made.

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Study Importance

What is already known?

- The decline in ovarian function over the menopausal transition is associated with increased adiposity and chronic disease risk.
- Gonadotropin releasing hormone (GnRH) analog models are useful in isolating the effects of ovarian failure from effects of aging and also in isolating effects of specific ovarian hormones.
- Previous GnRH analog studies demonstrate that decreased energy expenditure may be a mechanism contributing to the propensity for fat gain across the menopause.

What are the new findings?

- Ovarian hormone suppression in premenopausal women approaching the age of menopause results in increased total and abdominal adiposity.
- Ovarian hormone suppression did not result in a significant change in any component of free-living energy expenditure, although the magnitude of decrease in total and resting energy expenditure was similar to changes observed in previous studies.

How might your results change the direction of research?

- The role of ovarian hormones in the regulation of body composition and the components of energy expenditure deserves additional mechanistic research.

Figure 1.
CONSORT diagram

Figure 2.
Changes in components of free-living energy expenditure after 24 weeks of Placebo, GnRH_{AG}, or GnRH_{AG}+Exercise adjusted for changes in fat mass and fat-free mass.

Figure 3. Changes in components of body composition measured by DXA after 24 weeks of Placebo, GnRH_{AG}, or GnRH_{AG}+Exercise. * $p < 0.05$ for within-group change.

Table 1.

Baseline characteristics

	Placebo (n=9)	GnRH _{AG} (n=14)	GnRH _{AG} +Ex (n=11)	P-value
Ethnicity, N (%)				0.41
Not Hispanic or Latino	6 (67)	10 (71)	10 (91)	
Hispanic or Latino	3 (33)	3 (21)	1 (9)	
Unknown	0 (0)	1 (7)	0 (0)	
Race, N (%)				0.37
White	5 (56)	11 (79)	10 (91)	
Black/African American	2 (22)	2 (14)	1 (9)	
More than one Race	2 (22)	1 (7)	0 (0)	
Age, y	46.1 ± 1.8	47.2 ± 3.7	44.9 ± 2.9	0.19
BMI, kg/m ²	27.1 ± 5.1	25.3 ± 4.4	27.0 ± 5.1	0.57
Weight, kg	70.6 ± 14.4	67.7 ± 12.7	74.2 ± 10.7	0.45
Fat-free mass, kg	45.3 ± 4.8	45.2 ± 6.9	47.1 ± 4.6	0.67
Leg fat-free mass, kg	14.6 ± 1.9	14.6 ± 2.7	15.3 ± 1.7	0.74
Fat mass, kg	25.3 ± 10.5	22.5 ± 7.2	27.1 ± 6.7	0.47
Leg fat mass, kg	9.4 ± 3.9	9.0 ± 2.7	10.1 ± 2.2	0.68
Trunk fat mass, kg	12.2 ± 6.0	10.2 ± 4.1	13.3 ± 4.5	0.29
Estradiol, pmol/L	235 ± 261	686 ± 595	631 ± 492	0.12
Progesterone, nmol/L	6.9 ± 12.7	9.2 ± 16.2	9.7 ± 16.0	0.92
Testosterone, nmol/L	0.69 ± 0.21	0.80 ± 0.28	1.32 ± 0.90	0.04
SHBG, nmol/L	54 ± 26	61 ± 27	56 ± 31	0.85
FSH, IU/L	8.7 ± 3.7	10.0 ± 5.1	8.9 ± 3.6	0.73
VO ₂ max, mL/kg/min	30.4 ± 6.3	30.8 ± 6.3	28.2 ± 5.8	0.54

Values are mean ± SD unless otherwise indicated. GnRH_{AG}, gonadotropin-releasing hormone agonist; Ex, exercise; BMI, body mass index; y, years; SHBG, sex hormone binding globulin; FSH, follicle stimulating hormone.

Table 2.

Changes in sex hormones and sex hormone binding globulin (SHBG) in response to 24 weeks of Placebo, gonadotropin releasing hormone agonist (GnRH_{AG}) or GnRH_{AG}+Exercise

	Placebo (n=8)	GnRH _{AG} (n=13)	GnRH _{AG} +Ex (n=11)	Between-group difference in change (p-value)
Estradiol, pmol/L	-16.5 ± 55.7	-617 ± 176 ^{*†}	-559 ± 148 [*]	0.027
Progesterone, nmol/L	-1.8 ± 5.8	-8.7 ± 4.5	-9.1 ± 4.8	0.566
Testosterone, nmol/L	-0.05 ± 0.13	-0.20 ± 0.11	-0.40 ± 0.17 [*]	0.258
SHBG, nmol/L	-19.3 ± 11.5	-10.5 ± 5.6	-12.5 ± 4.3 [*]	0.687

Values are mean ± SEM. GnRH_{AG}, gonadotropin-releasing hormone agonist; Ex, exercise.

^{*} p<0.05 within group change,

[†] p<0.05 vs placebo.

VO₂peak test results before and after 24 weeks of Placebo, gonadotropin releasing hormone agonist (GnRH_{AG}) or GnRH_{AG}+Exercise

Table 3.

	Placebo (n=6)		GnRH _{AG} (n=12)		GnRH _{AG} +Ex (n=11)	
	Baseline	24-weeks	Baseline	24-weeks	Baseline	24-weeks
VO ₂ peak, L/min	2.3 ± 0.1	2.3 ± 0.1	2.0 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	2.3 ± 0.1 *
VO ₂ peak, mL/kg/min	31.9 ± 2.7	31.0 ± 2.6	31.3 ± 1.9	31.0 ± 2.2	28.2 ± 1.7	31.1 ± 2.1 *
Maximal Heart Rate, bpm	181 ± 8	178 ± 5	171 ± 4	174 ± 3	175 ± 2	177 ± 3
Maximal RER	1.10 ± 0.03	1.07 ± 0.02	1.07 ± 0.02	1.08 ± 0.02	1.09 ± 0.01	1.07 ± 0.02

Data presented as mean ± SEM. Within group change determined by paired t-test.

* p<0.05 vs baseline in same group. RER, respiratory exchange ratio.

Body composition and energy expenditure outcomes before and after 24-weeks of Placebo, gonadotropin releasing hormone agonist (GnRH_{AG}) or GnRH_{AG}+Exercise

Table 4.

	Placebo (n=9)		GnRH _{AG} (n=14)		GnRH _{AG} +Ex (n=11)	
	Baseline	24 weeks	Baseline	24 weeks	Baseline	24 weeks
Body Composition						
BMI, kg/m ²	27.1 ± 1.7	27.4 ± 1.7	25.3 ± 1.2	25.6 ± 1.2	27.0 ± 1.5	27.2 ± 1.7
Weight, kg	70.6 ± 4.8	71.5 ± 4.8	67.7 ± 3.4	68.7 ± 3.6	74.2 ± 3.2	74.5 ± 3.7
Fat-free mass, kg	45.3 ± 1.6	45.4 ± 1.7	45.2 ± 1.8	44.5 ± 1.8	47.1 ± 1.4	46.5 ± 1.6
Leg fat-free mass, kg	14.6 ± 0.6	14.5 ± 0.7	14.6 ± 0.7	14.2 ± 0.7 [*]	15.3 ± 0.5	14.9 ± 0.6
Fat mass, kg	25.3 ± 3.5	26.1 ± 3.3	22.5 ± 1.9	24.2 ± 2.1 [*]	27.1 ± 2.0	28.0 ± 2.2
Fat mass, %	34.5 ± 3.0	35.4 ± 2.5	32.7 ± 1.6	34.5 ± 1.6 [*]	36.0 ± 1.4	37.0 ± 1.4
Leg fat Mass, kg	9.4 ± 1.3	9.9 ± 1.3	9.0 ± 0.7	9.6 ± 0.8 [*]	10.1 ± 0.7	10.1 ± 0.7
Trunk fat Mass, kg	12.2 ± 2.0	12.5 ± 1.9	10.2 ± 1.1	11.1 ± 1.2 [*]	13.3 ± 1.3	13.9 ± 1.5
VAT Mass, kg	0.57 ± 0.10	0.57 ± 0.10	0.41 ± 0.06	0.43 ± 0.06	0.55 ± 0.08	0.55 ± 0.08
Energy Expenditure						
PAEE, kcal/d	646 ± 102	721 ± 102	642 ± 97	633 ± 97	753 ± 99	814 ± 99
REE, kcal/d	1426 ± 53	1396 ± 53	1462 ± 49	1431 ± 49	1418 ± 50	1365 ± 50
TDEE, kcal/d	2303 ± 112	2351 ± 112	2338 ± 107	2293 ± 107	2412 ± 110	2421 ± 110

Values are mean ± SEM. Energy Expenditure data were adjusted for baseline age, estradiol level, and fat mass.

^{*} p<0.05 vs baseline in same group. BMI, body mass index; VAT, visceral adipose tissue; PAEE, physical activity energy expenditure; REE, resting energy expenditure; TDEE, total daily energy expenditure.