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Effect of ovarian suppression with gonadotropin-releasing hormone agonist on glucose disposal and insulin secretion

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Toth MJ, Cooper BC, Pratley RE, Mari A, Matthews DE, Casson PR. Effect of ovarian suppression with gonadotropin-releasing hormone agonist on glucose disposal and insulin secretion. *Am J Physiol Endocrinol Metab* 294: E1035–E1045, 2008. First published April 15, 2008; doi:10.1152/ajpendo.00789.2007.—Several lines of evidence suggest that ovarian hormones influence glucose homeostasis, although their exact role in humans has not been clearly defined. In the present study, we sought to test the hypothesis that ovarian hormones regulate glucose homeostasis by examining the effect of pharmacologically induced ovarian hormone deficiency on glucose disposal and insulin secretion. Young, healthy women with regular menstrual patterns were studied during the follicular and luteal phases of their cycle at baseline and after 2 mo of treatment with gonadotropin-releasing hormone agonist (GnRHa; $n = 7$) or placebo ($n = 6$). Using hyperglycemic clamps, in combination with stable isotope-labeled (i.e., ^{13}C and ^{2}H) glucose tracers, we measured glucose disposal and insulin secretion. Additionally, we assessed body composition and regional fat distribution using radiologic imaging techniques as well as glucoregulatory hormones. Ovarian hormone suppression with GnRHa did not alter body composition, abdominal fat distribution, or thigh tissue composition. There was no effect of ovarian suppression on total, oxidative, or nonoxidative glucose disposal expressed relative to plasma insulin level. Similarly, no effect of ovarian hormone deficiency was observed on first- or second-phase insulin secretion or insulin clearance. Finally, ovarian hormone deficiency was associated with an increase in circulating adiponectin levels but no change in leptin concentration. Our findings suggest that a brief period of ovarian hormone deficiency in young, healthy, eugonadal women does not alter glucose disposal index or insulin secretion, supporting the conclusion that ovarian hormones play a minimal role in regulating glucose homeostasis. Our data do, however, support a role for ovarian hormones in the regulation of plasma adiponectin levels.

TISSUE INSULIN SENSITIVITY and pancreatic β-cell responsiveness decrease with age (1, 25), contributing to worsening glucose tolerance and, in some individuals, development of type 2 diabetes. In women, these age-related changes may accelerate following menopause (30, 37, 56, 57), leading to the hypothesis that ovarian hormone deficiency impairs insulin secretion and/or action. Supporting this notion are studies demonstrating that replacement of ovarian hormones in postmenopausal women enhances glucoregulation (4, 10, 18, 29, 30, 54). In fact, large clinical trials have shown that postmenopausal hormone replacement reduces the risk of developing diabetes (26, 31, 41). Collectively, these findings suggest that ovarian hormones regulate glucose homeostasis in a manner that may confer protection against the subsequent development of diabetes.

There is a considerable amount of evidence, however, that contradicts this conclusion. For instance, some investigations comparing pre- and postmenopausal women have found no differences in insulin sensitivity (47) or greater insulin sensitivity in postmenopausal women (56). Moreover, other studies have shown either no effect or deleterious effects of ovarian hormone replacement therapy on glucose homeostasis in postmenopausal women (17, 21, 42, 43, 53, 55) that is corrected upon cessation of therapy (43). Finally, studies performed at different times of the menstrual cycle that correspond with relative ovarian hormone deficiency and excess have suggested detrimental effects of ovarian hormones on glucose homeostasis (13, 38, 50). The reason(s) for differing results among studies are not clear but probably relate to the variety of experimental paradigms employed, the nature of the hormonal stimulus (e.g., endogenous vs. exogenous), and differences in the populations studied with respect to age, adiposity, activity level, and other factors. Regardless of an explanation for these disparities, on balance there is no clear consensus that emerges regarding the role of ovarian hormones in the regulation of glucose homeostasis.

The primary goal of this study was to examine the role of ovarian hormones in the regulation of glucose disposal and insulin secretion. To accomplish this objective, we studied young, healthy, nonobese women with normal menstrual cyclicity before and after 2 mo of treatment with gonadotropin-releasing hormone agonist (GnRHa) or placebo. GnRHa administration downregulates the production and release from the pituitary of luteinizing hormone and follicle-stimulating hormone, rapidly inducing a state of hypogonadotropic hypogonadism with consequent reductions in ovarian hormones to postmenopausal levels. This experimental paradigm provides the unique opportunity of studying the effects of ovarian hormone deficiency by use of a within-subjects design. We chose a short treatment period of 2 mo to minimize the effects of ovarian hormone deficiency on other physiological/metabolic systems, such as blood flow (8) or adiposity (49), that might confound our ability to detect an effect of the hormones on glucose homeostasis. Insulin secretion and clearance and intracellular pathways of glucose disposal were measured during hyperglycemic clamps from insulin and C-peptide levels, stable isotope-labeled glucose tracer kinetics, and indirect

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calorimetry. We hypothesized that ovarian hormone suppression with GnRHa would reduce glucose disposal by decreasing flux through the nonoxidative pathway and decrease insulin secretion. In addition, to examine whether ovarian suppression might influence glucose homeostasis through modulation of other hormonal systems, we assessed the effect of GnRHa administration on concentrations of circulating leptin and adiponectin, glucoregulatory hormones that are thought to be influenced, in part, by sex steroids (20, 24).

MATERIALS AND METHODS

Materials. D-[U-^{13}C]glucose (98% ^{13}C), D-[6,6-$^{2}H_2$]glucose (98% ^{13}C), and sodium [^{13}C]bicarbonate (99% ^{13}C) were obtained from Cambridge Isotope Laboratories (Andover, MA). Leuprolide acetate (Lupron Depot; 3.75 mg) was obtained from TAP Pharmaceuticals (Lake Forest, IL).

Subjects. Fourteen healthy young women were recruited and 13 women, ranging in age from 22 to 37 yr (mean ± SE; 29 ± 1 yr), completed the study. Women were nonobese (BMI <28 kg/m^2; 23.4 ± 0.8 kg/m^2), had a stable body weight (±2 kg) for 6 mo prior to study, were healthy on the basis of medical history, physical examination, and routine blood tests, were glucose tolerant (glucose <7.77 mmol/l 2 h following a 75-g oral glucose load), had no history of tobacco use, and were not on any medication that could affect glucose metabolism or ovarian/reproductive function. None of the volunteers had been exposed to any form of hormone-based contraceptive therapy for at least 6 mo prior to study and reported having at least two spontaneous cycles in the 3 mo prior to recruitment and a cycle length of between 25 and 32 days. The nature, purpose, and possible risks of the study were explained to each subject before she gave written consent to participate. The experimental protocol was approved by the Committee on Human Research at the University of Vermont.

Experimental protocol. Each volunteer underwent an outpatient screening visit at which time medical history, physical examination, biochemical laboratory tests, an exercise stress test, and an oral glucose tolerance test were performed. Volunteers that met the eligibility criteria were randomized using a stratified (age and BMI) block approach to receive the GnRHa leuprolide acetate (n = 7; Lupron Depot; 3.75 mg im; 28 ± 2 yr) or placebo (n = 7; 0.9% saline; 30 ± 2; P = 0.439). Prior to study, each volunteer's menstrual cycle was monitored for at least two cycles using menstrual diaries, ovulation prediction kits (Ovu-Quick One-Step; Quidel, San Diego, CA) and midluteal phase blood draws to discern length of the cycle and follicular and luteal phases.

Each woman underwent metabolic testing on three occasions: two prior to treatment and one following treatment. Baseline testing occurred during the early- to midfollicular phase (cycle days 3–8) and during the midluteal phase (cycle days 19–25). The order of baseline metabolic testing with respect to cycle phase (follicular-luteal or luteal-follicular) was randomized. Following baseline testing, GnRHa or placebo was administered by intramuscular injection during the midluteal phase. On average, the second injection was given 30 days following the first injection in the GnRHa group and 29 days following the first injection in the placebo group. Posttreatment metabolic testing was performed on average 56 days following the first injection in the GnRHa group and 58 days following the first injection in the placebo group. Posttreatment testing in the placebo group was performed during the same phase of the cycle as the second baseline testing period. Women in the placebo group underwent evaluations in one of two testing orders: follicular-luteal-luteal or luteal-follicular-follicular. Thus, posttreatment testing was randomized in volunteers in the placebo group in accordance with baseline testing order. Directly preceding each bout of metabolic testing, volunteers were provided 3 days of a weight maintenance, standardized diet (20% protein, 25% fat, and 55% carbohydrate) by the General Clinical Research Center (GCRC) Metabolic Kitchen. The diet was designed to provide at least 1 g protein/kg body wt and 200 g carbohydrate/day and was identical for each bout of testing.

Insulin secretion and glucose metabolism measurements were performed under hyperglycemic conditions the morning following an overnight visit to the GCRC. Volunteers were fasted after 1900 the evening of admission. At ~0600, catheters were placed in an antecubital vein for infusion and retrograde in a dorsal hand vein for blood draws. Baseline blood and breath samples were taken and primed (3.46 and 0.131 mg/kg), continuous (0.048 mg·kg^{-1}·min^{-1} and 1.82 μg·kg^{-1}·min^{-1}) infusions of [6,6-$^{2}H_2$]- and [U-^{13}C]glucose were started and maintained for 240 min. The bicarbonate pool was primed (10.6 μmol/kg) with sodium [^{13}C]bicarbonate. At 120 min, a variable-rate priming dose of 20% dextrose was started (from 120 to 140 min), followed by a variable rate infusion (from 140 to 240 min), with the goal of obtaining a steady-state plasma glucose level of 125 mg/dl above fasting glucose level. Both [6,6-$^{2}H_2$]- and [U-^{13}C]glucose were added to the 20% dextrose infusate prior to the study to preserve steady-state plasma enrichments. Plasma glucose level was monitored every 2 min during the first 20 min of the clamp and every 5 min thereafter, and the dextrose infusion rate was adjusted to achieve the hyperglycemic target. All infusions were stopped at 240 min except for the dextrose infusion, which was continued and tapered until no longer required to maintain normal glycemia. Problems with intravenous access in one patient in the placebo group during luteal phase and posttreatment testing rendered portions or all of her clamp study data unusable for analysis. Because of this, the final sample sizes were n = 6 for placebo and n = 7 for GnRHa for all analyses.

Blood and breath samples were drawn at 90, 100, 110, and 120 min for measurement of basal and at 210, 220, 230, and 240 min for measurement of clamp glucose kinetics. Blood samples were drawn at 2-min intervals from 120 to 140 min and then at 15-min intervals thereafter for the determination of plasma insulin and C-peptide levels. Oxygen consumption and carbon dioxide production rates were determined at 60 and 210 min, using the ventilated hood technique (DeltaTrac, Yorba Linda, CA). Oxygen consumption data were not available on two volunteers during the baseline clamp measurements (one during follicular- and one during luteal-phase evaluation) because of technical problems.

Body composition. Body mass was measured on a metabolic scale (Scale-Tronix, Wheaton, IL). Fat mass, fat-free mass, and bone mineral mass were each measured by dual-energy X-ray absorptiometry using a GE Lunar Prodigy densitometer (GE Lunar, Madison, WI). Bone mineral mass data are not presented.

Computed tomography. Abdominal adipose tissue areas and midthigh fat and muscle areas were measured by computed tomography with a Phillips Brilliance 40 or 64 computed tomography scanner (Phillips Medical Systems, Cleveland, OH), as described previously (48). For the midthigh scan, the midpoint between the anterior superior illiac crest and the proximal aspect of the patella was measured using external landmarks and the midpoint marked on the patients thigh. For all scans, the mark for the midthigh scan was placed at the same point. Images from these scans were analyzed using NIH Image software (Image J 1.36b) to determine adipose tissue and muscle areas, as described previously (48). Midthigh tissue composition measurements were not performed in two patients (one in each group) posttreatment because of logistical problems.

Analytic methods. Serum insulin was measured by radioimmunoassay (Linco, St. Louis, MO). The intra- and interassay coefficients of variation (CV) for insulin were 3.2 and 4%, respectively. Plasma C-peptide levels were determined by radioimmunoassay (Linco). The intra- and interassay CV were 4.6 and 4.9%, respectively. Plasma glucose concentrations were measured by a glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH).

Plasma [6,6-$^{2}H_2$]glucose enrichment was measured by electron impact ionization gas chromatography-mass spectrometry (model

5971A; Hewlett-Packard, Palo Alto, CA), as described previously (19). Prior to measurement, glucose was derivatized to the butylboronate acetate derivative. Injections of butylboronate acetyl glucose were made isothermally while monitoring the $[M\text{-}57]^+$ ions at a mass-to-charge ratio (m/z) of 297 and 299 for unlabeled and [6,6-$^2H_2$]glucose, respectively.

For plasma glucose ^{13}C measurements, following precipitation and removal of plasma proteins, the methylboronate glucose derivative was prepared (52) as described previously (23). The methylboronate acetate glucose derivatives were measured for ^{13}C content by gas chromatography-combustion-isotope ratio mass spectrometry (23) using a DELTA-Plus instrument with a GCC-III unit (Thermo-Fisher Finnigan, Bremen, Germany), and these data were transformed into mole fraction abundance of ^{13}C as described (23). Enrichment of ^{13}C was calculated as the difference between the F_{13C} of the sample minus the F_{13C} of a baseline sample taken prior to administration of the isotope and was adjusted for the number of carbons added to the glucose by the derivatization procedure.

The enrichment of expired CO_2 was measured by isotope ratio mass spectrometry (PDZ Europa 20/20 ABCA-NT; Crewe, Cheshire, UK).

Calculations. Glucose tracers were used in this study to monitor endogenous glucose production ([6,6-$^2H_2$]glucose) and glucose oxidation ([U-^{13}C]glucose). The following section details calculations used to derive these two estimates from tracer kinetic data.

The rate of appearance of glucose (R_a) can be calculated from the [6,6-$^2H_2$] tracer as

$$R_a = i(E_i/E_p - 1) \quad (1)$$

where i is the rate of the 6,6-$^2H_2$ tracer infusion, E_i is the enrichment of the glucose tracer in the infusate, and E_p is the mean enrichment of the glucose tracer in plasma. During the hyperglycemic clamp, the equation above must be modified to account for the fact that the glucose tracer was infused into the body from two sources: the [6,6-$^2H_2$]glucose tracer infusion and the 20% dextrose infusion. Thus, the glucose R_a during the clamp ($R_{a\ 2H}$) from the [6,6-$^2H_2$]glucose tracer is calculated as

$$R_{a\ 2H} = [(E_i - E_p) \cdot i + (E_{i\ Dex} - E_{p\ clamp}) \cdot i_{Dex}]/E_{p\ clamp} \quad (2)$$

where i, E_i, and E_p are as defined above, $E_{i\ DEX}$ is the enrichment of [6,6-$^2H_2$]glucose in the 20% dextrose infusate, $E_{p\ clamp}$ is the enrichment of the tracer in the plasma, and i_{DEX} is the infusion rate of the 20% dextrose. The glucose R_a data derived from the [6,6-$^2H_2$]glucose tracer during the clamp was used to determine endogenous glucose production during the clamp. Total glucose disposal during the clamp was calculated from data collected during 210–240 min as the 20% dextrose infusion rate plus the $R_{a\ 2H}$ from the [6,6-$^2H_2$]glucose tracer. Total glucose disposal was then expressed relative to plasma insulin concentration during the same time period and is referred to as the glucose disposal index.

Using the [U-^{13}C]glucose tracer, we partitioned glucose disposal into oxidative and nonoxidative pathways. To accomplish this, the fraction of [U-^{13}C]glucose tracer infused that was oxidized during the last 30 min of the hyperglycemic clamp ($f_{ox\ clamp}$) was calculated as

$$f_{ox\ clamp} = F_{13CO2\ clamp}/i_{13C} \cdot 6 \quad (3)$$

where $F_{13CO2\ clamp}$ is the rate of $^{13}CO_2$ excretion calculated as the breath $^{13}CO_2$ enrichment times the CO_2 production rate derived from indirect calorimetry, i_{13C} is the infusion rate of the ^{13}C from the [U-^{13}C]glucose tracer and 20% dextrose infusate, and 6 is a constant that accounts for the fact that there are 6 ^{13}C labels in the [U-^{13}C]glucose tracer. The bicarbonate retention factor for the ^{13}C tracer was assumed to be 1.0 during the hyperglycemic clamp. The $f_{ox\ clamp}$ was then multiplied by the R_a for the [U-^{13}C]glucose tracer ($R_{a\ 13C}$) to derive the rate of glucose oxidation during the clamp. $R_{a\ 13C}$ was calculated as

$$R_{a\ 13C} = [(i_{13C} \cdot E_{i\ 13C}) + (i_{Dex} \cdot E_{Dex})]/E_{p\ 13C} \quad (4)$$

where i_{13C} is the infusion rate of the [U-$^{13}C_6$]glucose tracer, $E_{i\ 13C}$ is the enrichment of the [U-$^{13}C_6$]glucose infusate, i_{DEX} is as defined above, E_{DEX} is the enrichment of the [U-$^{13}C_6$]glucose in the 20% dextrose infusate, and $E_{p\ 13C}$ is the plasma enrichment of [U-$^{13}C_6$]glucose. As with total glucose disposal, oxidative and nonoxidative disposal were expressed relative to plasma insulin levels.

Insulin secretion was calculated by deconvolution according to the method of van Cauter et al. (51), using C-peptide concentrations. First-phase insulin secretion was defined from 120 to 128 min and second-phase secretion from 128 to 240 min and is expressed as the area under the curve during these time periods. Insulin clearance was calculated as the ratio of insulin secretion rate to the corresponding plasma insulin concentration from 140 to 240 min and is expressed as the mean of the ratios at each time point.

Hormone measurements. Serum levels of estrone, estradiol, testosterone, androstenedione, and dehydroepiandrosterone were measured by radioimmunoassay. Prior to measurement, steroids were extracted from serum with hexane-ethyl acetate (3:2). Androstenedione, dehydroepiandrosterone (DHEA), and testosterone were then separated by Celite column partition chromatography using increasing concentrations of toluene in trimethylpentane. Estrone and estradiol were separated in a similar fashion using ethyl acetate in trimethylpentane. Dehydroepiandrosterone sulfate (DHEA-S) and sex hormone-binding globulin were measured by direct chemiluminescence immunoassays using the Immulite analyzer (Diagnostic Products, Inglewood, CA). Free estradiol and testosterone were calculated using their respective total serum concentration, sex hormone-binding globulin levels, and an assumed constant for albumin in a validated algorithm (44). Intra- and interassay CV for steroid hormones and their binding proteins varied from 4 to 8 and from 8 to 13%, respectively. The limit of detection for each hormone was as follows: estrone: 4 pg/ml; estradiol: 3 pg/ml; testosterone: 15 pg/ml; DHEA: 30 pg/ml; DHEA-S: 30 ng/ml; androstenedione: 30 pg/ml; sex hormone-binding globulin: 0.2 nmol/l. Plasma leptin was measured by ELISA (Linco). The intra- and interassay CV for leptin were 5 and 4.9%, respectively. Plasma adiponectin levels were determined by ELISA (R&D Systems, Minneapolis, MN). This assay detects total plasma adiponectin levels, which includes, but does not distinguish between, all molecular weight species. The interassay CV ranges from 9 to 13%.

Statistics. Paired *t*-tests were used to compare data between baseline follicular and luteal phase measurements in the entire cohort. For comparison with posttreatment data, follicular and luteal phase measurements were averaged and are referred to as "baseline" values. A 2 × 2 repeated-measures analysis of variance (RM-ANOVA) model was used with treatment group (GnRHa vs. placebo) as the between-subjects factor and time (baseline vs. posttreatment values) as the within-subjects factor. If a significant group × time interaction effect was found, a post hoc analysis was performed to assess the unique effect of time within each group through an analysis of the simple effects. For steroid hormone data, several variables (estrone, estradiol, testosterone) were not normally distributed (Shapiro-Wilk test, $P < 0.05$) and remained so after attempts at transformation (e.g., $\log_{10}$). Thus, we evaluated the normality of the distribution of the difference in steroid hormone data between baseline and posttreatment evaluations. For all calculated differences, the assumption of normality was fulfilled. Thus, the calculated difference was compared between groups using an unpaired *t*-test. In addition, Wilcoxon signed rank tests were used to test the difference of estrone and estradiol levels from the each menstrual cycle phase at baseline to posttreatment. All analyses were conducted with SPSS software (SPSS v. 15.0; Chicago, IL).

RESULTS

Baseline and posttreatment body composition and fat distribution data are shown in Table 1. Comparing average baseline

Table 1. *Effect of GnRHa administration on total and regional body composition*

	Baseline		Posttreatment	
	GnRHa	Placebo	GnRHa	Placebo
Weight, kg	60.2±3.3	69.2±3.5	60.0±3.3	69.6±3.8
Fat mass, kg	17.4±2.5	22.5±1.9	17.9±2.6	22.9±2.4
Body fat, %	28.7±3.0	33.5±2.0	29.9±2.9	33.5±2.4
Fat-free mass, kg	41.3±1.2	44.7±2.5	40.5±1.3	45.0±2.6
Appendicular fat-free mass, kg	17.6±0.6	19.2±1.2	17.2±0.6	19.5±1.2
Total abdominal fat area, cm^2	243±45	299±35	253±45	303±37
Subcutaneous abdominal fat area, cm^2	194±42	249±33	199±43	251±36
Intra-abdominal fat area, cm^2	48±4	50±7	54±6	52±7
Mid-thigh fat area, cm^2	113±18	153±22	127±24	150±24
Midthigh muscle area, cm^2	111±6	118±10	107±6	116±11

Data are means ± SE. Baseline data represent the average of follicular and luteal phase evaluations. Sample sizes are $n = 7$ and $n = 6$ for gonadotropin-releasing hormone agonist (GnRHa) and placebo groups, respectively, except for midthigh tissue composition data, where sample sizes are $n = 6$ and $n = 5$, respectively.

values between the two groups, women in the placebo group tended ($P = 0.09$) to weigh more than those in the GnRHa group due to the fact that they tended ($P = 0.07$) to be taller (GnRHa 161 ± 3 vs. placebo 171 ± 3 cm). No differences were found, however, in BMI (GnRHa 23.0 ± 1.2 vs. placebo 23.9 ± 1.3 kg/m^2, $P = 0.64$). Similarly, no differences were found between GnRHa and placebo groups in any index of whole body or regional composition at baseline. Groups were similar at baseline for peak aerobic capacity on an absolute basis (GnRHa 2.33 ± 0.18 vs. placebo 2.45 ± 0.22 l/min, $P = 0.68$) or when statistically adjusted for fat-free mass (GnRHa 2.43 ± 0.17 vs. placebo 2.33 ± 0.18 l/min, $P = 0.72$). Similarly, there was no effect of time on body mass in either group when considered across all three evaluations (GnRHa 59.8 ± 3.2 vs. 60.6 ± 3.4 vs. 60.0 ± 3.3 kg, $P = 0.81$; placebo 69.3 ± 3.4 vs. 69.1 ± 3.6 vs. 69.6 ± 3.8 kg, $P = 0.321$, for follicular, luteal, and posttreatment evaluations, respectively). Finally, comparing baseline and posttreatment data, no group × time interaction effects were found for any whole body or regional tissue composition measure.

As expected, there were differences in estrone (55 ± 13 vs. 104 ± 14 pg/ml, $P < 0.01$) and estradiol (92 ± 30 vs. 223 ± 27 pg/ml, $P < 0.01$) levels between the follicular and luteal phases of the cycle, respectively. All other hormone levels did not differ significantly with menstrual cycle phase.

The effect of ovarian suppression on serum steroid levels and binding proteins is shown in Table 2. Testosterone, free testosterone, and androstenedione decreased with treatment in the GnRHa group compared with placebo ($P < 0.05$), whereas other hormone levels were unaffected. Because estrone and estradiol differed by menstrual cycle phase, we also evaluated the difference between each hormone measured at follicular and luteal phase evaluations compared with the posttreatment evaluation. In the GnRHa group, posttreatment estrone and estradiol levels were lower than either follicular- or luteal-phase evaluations ($P < 0.05$ for all). In the placebo group, no difference was found between follicular and posttreatment estrone and estradiol levels ($P = 0.917$ for both), whereas estrone level was lower posttreatment compared with luteal phase ($P < 0.05$), and estradiol level tended to be lower ($P = 0.08$). Importantly, ovarian suppression was confirmed in all volunteers in the GnRHa group 10 days following the first injection, as indicated by plasma estradiol level <50 pg/ml, and was confirmed at posttreatment testing (range 4–15 pg/ml).

The effect of menstrual cycle phase on glycemia (*top*), insulin (*middle*) and c-peptide (*bottom*) levels during the hyperglycemic clamp are shown for follicular and luteal phase evaluations in Fig. 1. No effect of cycle phase was found on the fasting glucose level [follicular (F) 76 ± 1 vs. luteal (L) 76 ± 1 mg/dl, $P = 0.97$], insulin (F: 9.4 ± 1.2 vs. L: 9.2 ± 1.0 μU/ml, $P = 0.822$) or C-peptide (F: 1.8 ± 0.3 vs. L: 1.8 ± 0.2 ng/ml, $P = 0.80$) level. There was a small, but significant, difference in average plasma glucose level during the final 30 min of the clamp (F: 197 ± 1 vs. L: 192 ± 2 mg/dl, $P < 0.01$).

Glucose disposal indexes and insulin secretion data for follicular and luteal phases of the menstrual cycle are shown in Fig. 2. There was no effect of menstrual cycle phase on total glucose disposal normalized to insulin level [F: 12.6 ± 2.2 vs. L: 12.0 ± 1.6 (mg/min)/(μU/ml), $P = 0.628$] or when glucose disposal was not normalized to insulin levels (F: 616 ± 50 vs. L: 684 ± 63 mg/min, $P = 0.133$). Similarly, there was no effect of cycle phase on residual endogenous glucose production [F: 0.41 ± 0.16 vs. L: 0.40 ± 0.17 (mg/min)/(μU/ml), $P = 0.903$], nonoxidative glucose disposal [F: 11.3 ± 2.2 vs. L: 10.9 ± 1.6 (mg/min)/(μU/ml), $P = 0.748$] or oxidative glucose disposal [F: 1.8 ± 0.5 vs. L: 1.5 ± 0.2 (mg/min)/(μU/ml), $P = 0.402$]. Expression of glucose disposal data relative to body mass or fat-free mass yielded no differences between menstrual cycle phases (data not shown). There was a 10% greater area

Table 2. *Effect of GnRHa administration on steroid hormone and binding protein levels*

	Baseline		Posttreatment	
	GnRHa	Placebo	GnRHa	Placebo
Estrone, pg/ml	69±10	92±24	19±2	60±11
Estradiol, pg/ml	129±15	190±51	9.8±1.4	122±28
Free estradiol, pg/ml	2.98±0.35	4.32±0.98	0.24±0.05	2.83±0.64
Testosterone, ng/dl	38±2	36±4	20±2*	34±8
Free testosterone, ng/dl	6.54±0.38	6.21±0.59	3.87±0.58*	6.15±1.47
DHEA, nmol/l	6.53±1.24	6.46±0.44	7.39±2.30	5.73±0.70
DHEA-S, μg/dl	123±25	112±19	124±28	93±14
Androstenedione, ng/ml	1.37±0.05	1.27±0.11	0.72±0.11*	1.06±0.15
Sex hormone-binding globulin, nmol/l	57±4	56±6	51±7	54±7

Data are means ± SE. DHEA, dehydroepiandrosterone; DHEA-S, dehydroepiandrosterone sulfate. Sample sizes are $n = 7$ for GnRHa and $n = 6$ for placebo. *$P < 0.05$, change with treatment different between GnRHa and placebo groups.

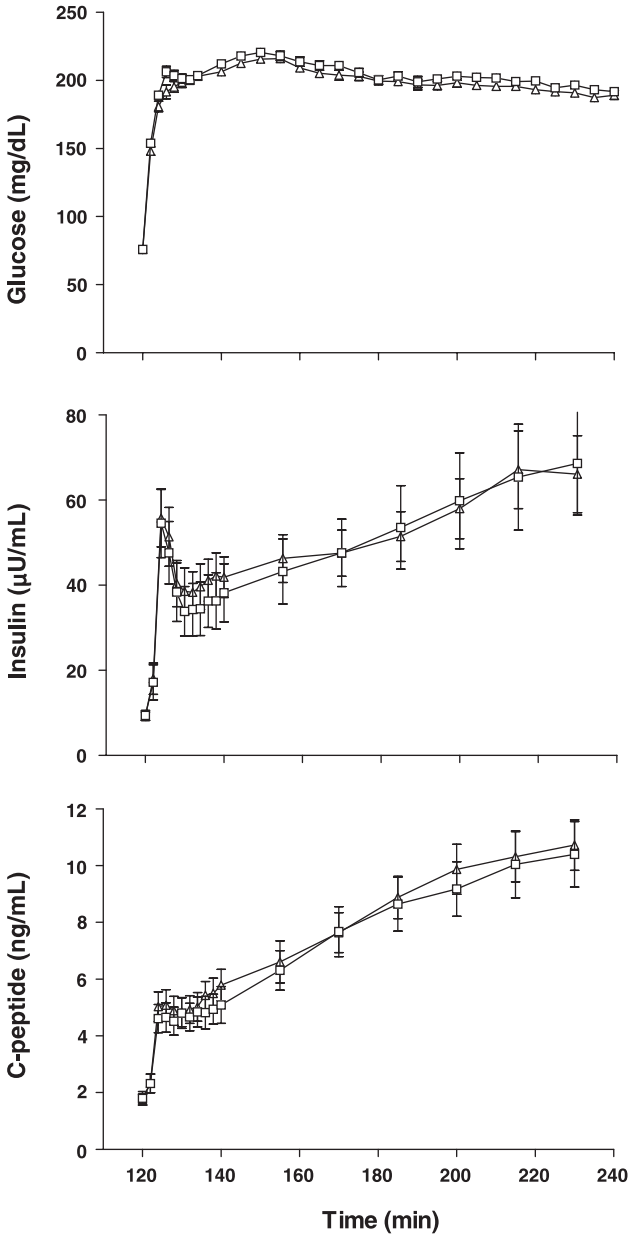

Fig. 1. Plasma glucose, insulin, and C-peptide levels during the hyperglycemic clamp for follicular (□) and luteal (△) phases evaluations (n = 13). Data are means ± SE.

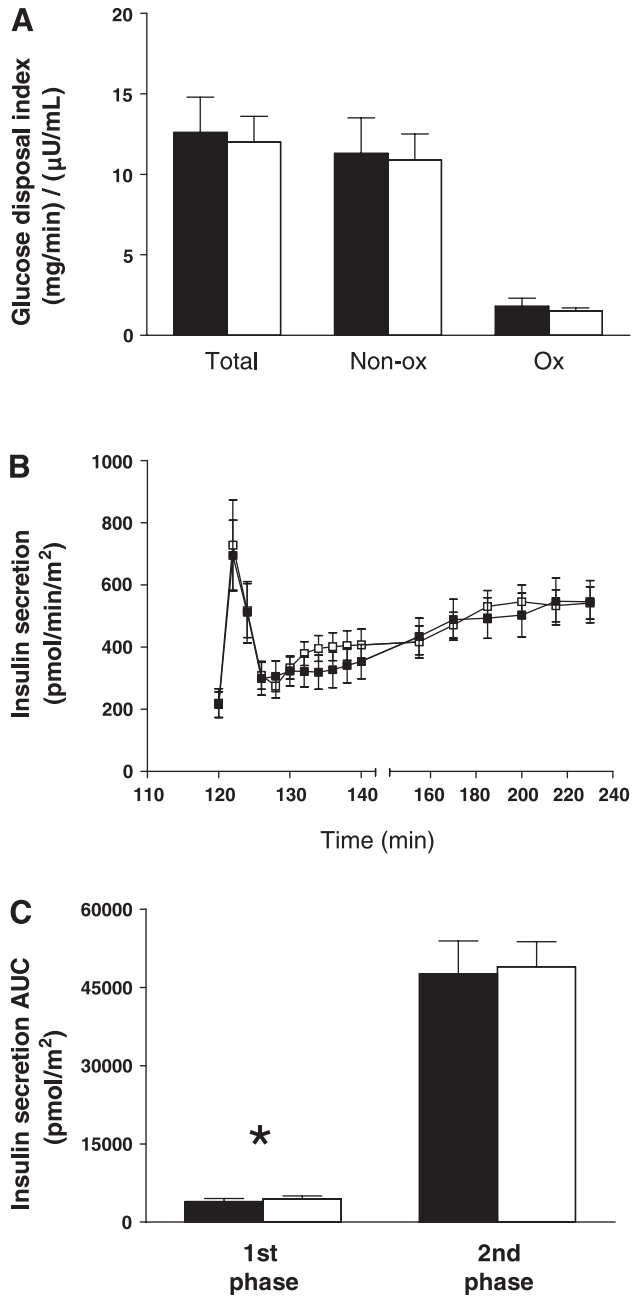

Fig. 2. Glucose disposal index (*A*), insulin secretion (*B*), and first- and second-phase insulin-secretory response (*C*) during follicular (closed bar/symbols) and luteal (open bar/symbols) phase evaluations (n = 13). Total glucose disposal data represent the average glucose infusion rate during the last 30 min of the clamp plus residual endogenous glucose disposal derived from [$^2H_2$]glucose kinetics and are expressed relative to the average insulin level during the same period. Total glucose disposal was divided into nonoxidative (Non-ox) and oxidative (Ox) disposal pathways. Non-ox was calculated as the difference between total glucose disposal and Ox disposal measured using the [$^{13}C_6$]glucose tracer. For Non-ox and Ox disposal data, n = 12 due to the absence of indirect calorimetry data in 2 patients. Insulin-secretory dynamics are calculated from plasma insulin and C-peptide levels, as described in MATERIALS AND METHODS. Area under the curve (AUC) was calculated for 1st-phase (120–128 min) and 2nd-phase (128–240 min) insulin-secretory responses. Data are means ± SE. *$P < 0.02$.

under the curve for the first-phase insulin-secretory response in the luteal vs. the follicular phase of the cycle (F: 3,957 ± 554 vs. L: 4,420 ± 570 pmol/m^2, $P < 0.01$) but no cycle effect on the area under the curve of second-phase insulin secretion (F: 47,667 ± 6,243 vs. L: 48,956 ± 4,849 pmol/m^2, $P = 0.552$). Because of these cycle-dependent differences, in analyses below, we also examined the effect of GnRHa treatment by comparing posttreatment data to baseline follicular and luteal phase evaluations individually. No effect of cycle phase was found on insulin clearance (F: 1.74 ± 0.19 vs. L: 1.63 ± 0.13 l·min^{-1}·m^{-2}; $P = 0.33$; data not shown in Fig. 2).

Pre- and posttreatment glucose (*top*), insulin (*middle*) and C-peptide (*bottom*) levels during the hyperglycemic clamp are shown in Fig. 3 for GnRHa and placebo groups. There were no

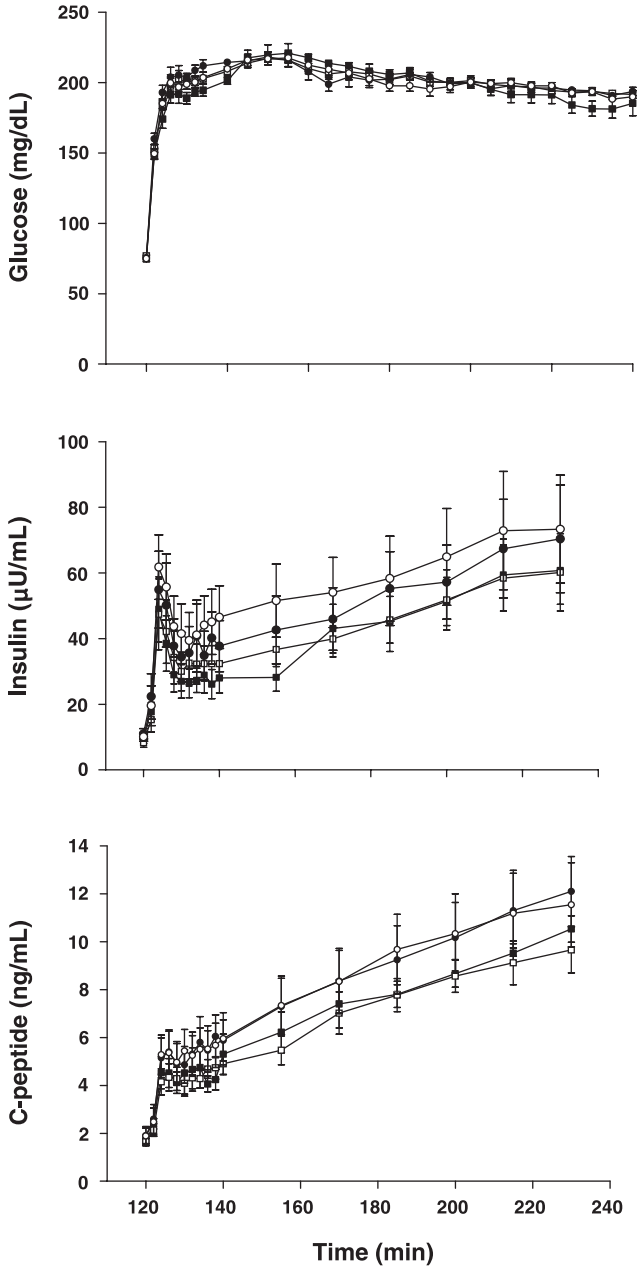

Fig. 3. Plasma glucose, insulin, and C-peptide levels during the hyperglycemic clamp in GnRHa (circles; $n = 7$) and placebo (squares; $n = 6$) for baseline (open symbols) and posttreatment (closed symbols) evaluations. Data are means ± SE.

group × time interaction effects noted for fasting plasma glucose (GnRHa 75 ± 2 to 76 ± 1 vs. placebo 77 ± 1 to 75 ± 2 mg/dl, $P = 0.298$), insulin (GnRHa 10 ± 1 to 11 ± 2 vs. placebo 8 ± 1 to 10 ± 2 μU/mL; $P = 0.677$), C-peptide (GnRHa 1.9 ± 0.4 to 1.9 ± 0.3 vs. placebo 1.7 ± 0.1 to 1.7 ± 0.2 ng/ml, $P = 0.887$) or mean plasma glucose level during the last 30 min of the clamp (GnRHa 194 ± 1 to 195 ± 2 vs. placebo 194 ± 5 to 187 ± 6 mg/dl, $P = 0.238$).

The effect of ovarian suppression on glucose disposal is shown in Fig. 4. No group × time interaction effects were noted for total [GnRHa 11.7 ± 2.5 to 13.4 ± 2.7 vs. placebo 12.2 ± 2.5 to 13.7 ± 3.4 (mg/min)/(μU/ml), $P = 0.863$], oxidative [GnRHa 1.36 ± 0.25 to 1.23 ± 0.25 vs. placebo 1.76 ± 0.38 to 1.68 ± 0.43 (mg/min)/(μU/mL), $P = 0.783$] or nonoxidative [GnRHa 10.1 ± 2.3 to 12.2 ± 2.5 vs. placebo 10.5 ± 2.2 to 12.0 ± 3.0 (mg/min)/(μU/ml), $P = 0.756$] glucose disposal expressed relative to plasma insulin level. Similarly, there were no group × time interaction effects for residual endogenous glucose production [GnRHa 0.33 ± 0.22 to 0.21 ± 0.18 vs. placebo 0.44 ± 0.20 to 0.54 ± 0.31 (mg/min)/(μU/ml), $P = 0.514$; data not shown in Fig. 4]. No group × time interaction effects were found for any glucose disposal data when posttreatment data were compared with either follicular or luteal phase measurements individually (range of P values 0.459 to 0.960). Finally, no group × time interaction effects were noted when glucose disposal data were expressed relative to body mass or fat-free mass (data not shown).

The effect of GnRHa administration on insulin secretion, first- and second-phase insulin-secretory responses, and insulin clearance are shown in Fig. 5. No group × time interaction effect was found for the area under the curve for the first-phase (GnRHa 4,745 ± 866 to 4,497 ± 849 vs. placebo 3,539 ± 639 to 3,687 ± 724 pmol/m^2, $P = 0.201$) or second-phase (GnRHa 54,327 ± 9,412 to 54,286 ± 8,081 vs. placebo 41,294 ± 3,802

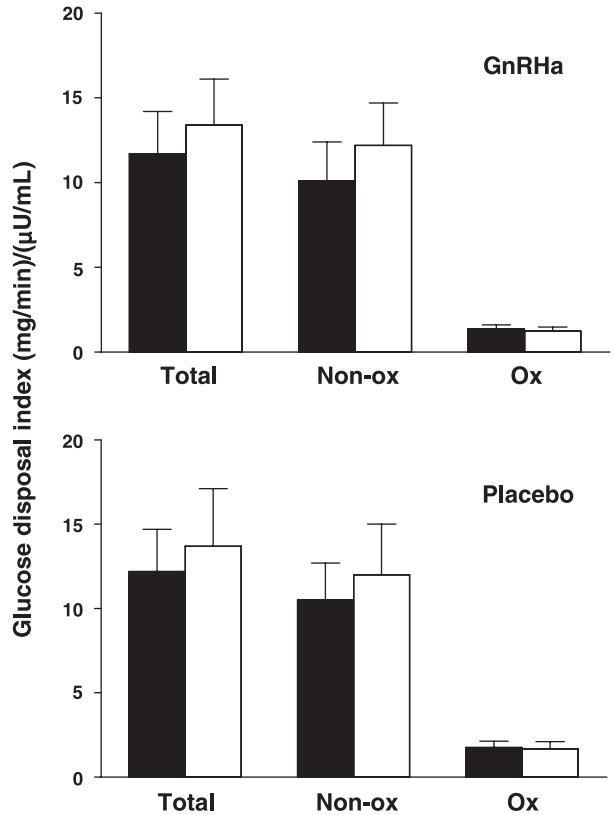

Fig. 4. Glucose disposal index (*A*), insulin secretion (*B*), and insulin clearance (*C*) data in GnRHa ($n = 7$) and placebo ($n = 6$) groups for baseline (closed bar/symbols) evaluations. Total glucose disposal data represent the average glucose infusion rate during the last 30 min of the clamp plus residual endogenous glucose disposal derived from [$^2H_2$]glucose kinetics and are expressed relative to the average insulin level during the same period. Total glucose disposal during this period was divided into Non-ox and Ox disposal pathways. Non-ox was calculated as the difference between total glucose disposal and Ox disposal measured using the [$^{13}C_6$]glucose tracer. Data are means ± SE.

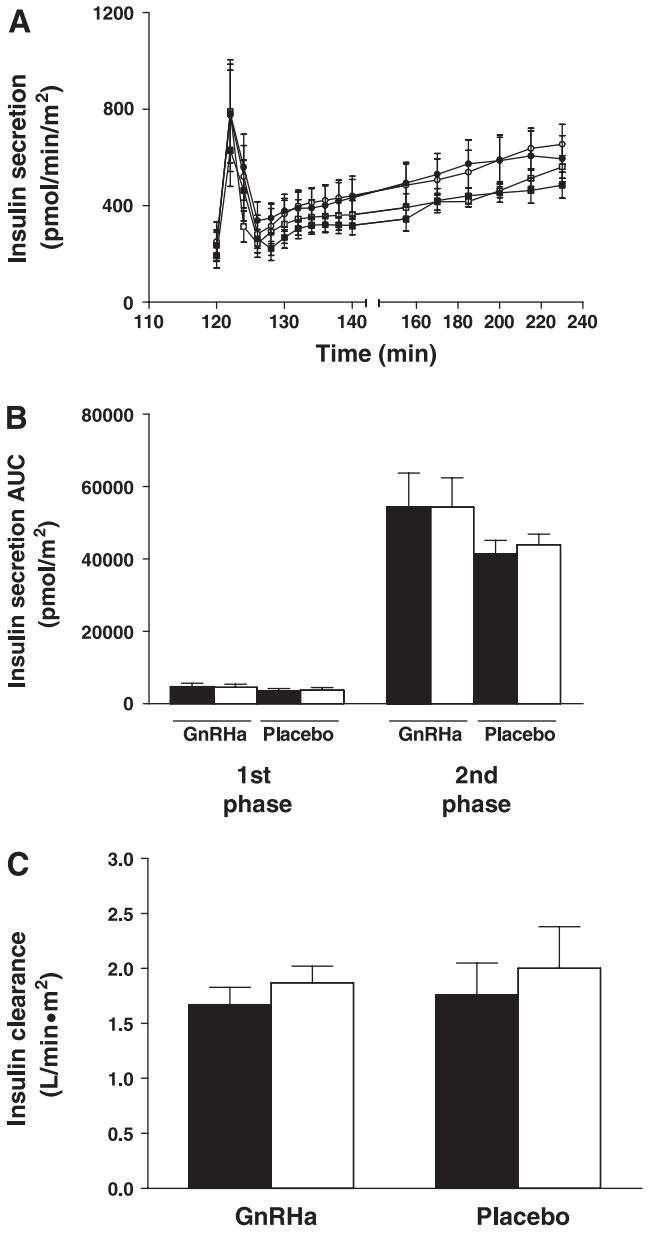

Fig. 5. Insulin secretion (*A* and *B*) and clearance (*C*) data in gonadotropin-releasing hormone agonist (GnRHa; $n = 7$) and placebo ($n = 6$) groups for baseline (open bars/symbols) and posttreatment (closed bars/symbols) evaluations. Insulin secretion data are calculated from plasma C-peptide levels, as described in MATERIALS AND METHODS. AUC was calculated for 1st-phase (120–128 min) and 2nd-phase (128–240 min) insulin-secretory responses. Insulin clearance was calculated as the ratio of insulin secretion rate to the corresponding plasma insulin concentration from 140 to 240 min and is expressed as the mean of the ratios at each time point. Data are means ± SE.

to 43,905 ± 2,905 pmol/m^2, $P = 0.485$) insulin-secretory response or insulin clearance rates (GnRHa 1.67 ± 0.16 to 1.87 ± 0.15 vs. placebo 1.76 ± 0.29 to 2.00 ± 0.38 l·min^{-1}·m^{-2}, $P = 0.807$). Because there were menstrual cycle differences in the first-phase insulin-secretory response, we also examined the effect of GnRHa administration by comparing the individual menstrual cycle phases to the posttreatment evaluation. However, there was still no group × time interaction effect noted for first-phase insulin secretion when post-treatment data were compared with either follicular ($P = 0.170$) or luteal ($P = 0.385$) phases individually.

The effects of the menstrual cycle and GnRHa treatment on circulating adipokine levels are shown in Fig. 6. No effect of menstrual cycle phase was found on circulating adiponectin (F: 9.54 ± 1.41 vs. L: 10.58 ± 1.98 μg/ml, $P = 0.269$), although a trend toward greater leptin level was observed in the luteal phase (F: 16.00 ± 3.06 vs. L: 18.91 ± 3.02 ng/ml, $P = 0.09$). A significant group × time interaction effect was observed for adiponectin ($P < 0.01$). Further examination of simple effects showed no change in adiponectin in the placebo group (Pre 9.84 ± 2.17 vs. Post

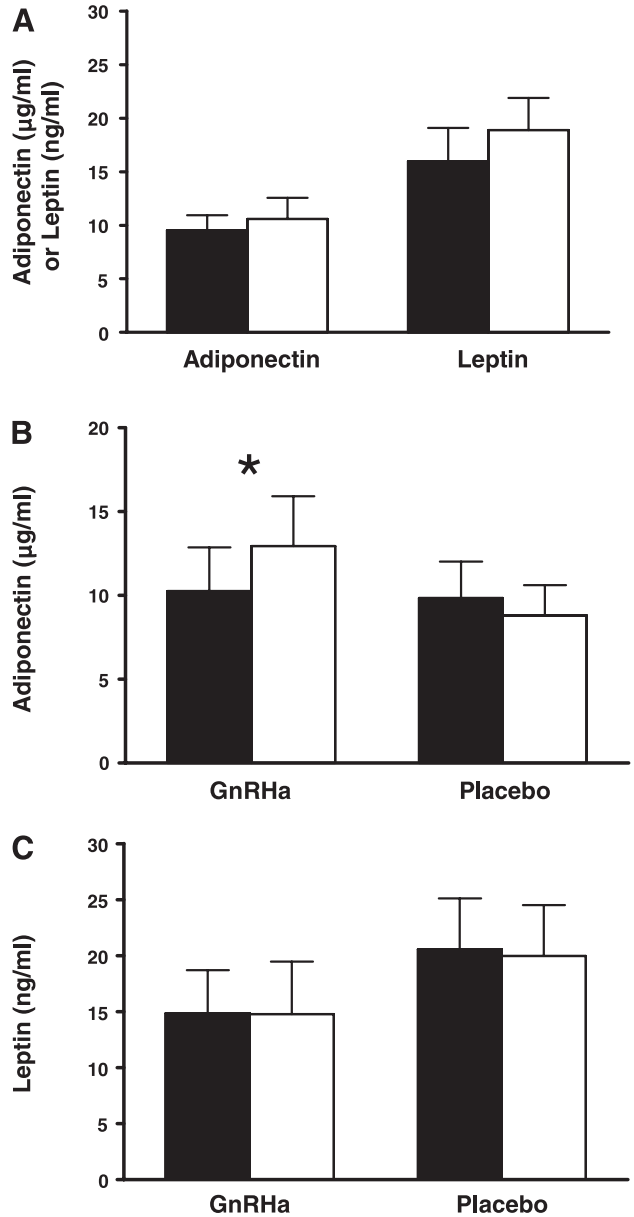

Fig. 6. Effect of menstrual cycle phase (*A*) and GnRHa administration (*B* and *C*) on circulating adipokine levels. For all measurements, total sample size is $n = 13$, with $n = 7$ for the GnRHa group and $n = 6$ for the placebo group. *A*: follicular phase evaluations are shown as closed bars and luteal phase as open bars. *B* and *C*: baselines are represented by open bars and posttreatment by closed bars. Data are means ± SE. *$P < 0.01$ group × time interaction effect, $P = 0.001$ simple effect of time within the GnRHa group.

8.80 ± 1.80 μg/ml, $P = 0.154$) but a significant increase in the GnRHa group (Pre 10.25 ± 2.61 vs. Post 12.94 ± 2.97 μg/ml; $P = 0.001$). No group × time interaction effect was observed ($P = 0.786$) for leptin when average baseline-to-posttreatment values were compared (GnRHa 14.86 ± 3.84 to 14.79 ± 4.67 ng/ml; placebo 20.58 ± 4.54 to 19.96 ± 4.55 ng/ml) or when follicular or luteal phase evaluations were compared with the posttreatment evaluation individually ($P = 0.733$ and 0.959, respectively).

DISCUSSION

To examine the physiological role of ovarian hormones in the regulation of glucose homeostasis, we measured glucose disposal index and insulin secretion in young, healthy, eugonadal women before and after pharmacological suppression of ovarian hormone production with GnRHa. To our knowledge, this is the first study to evaluate, using a randomized, controlled design, the effects of GnRHa treatment on glucose disposal and insulin secretion in healthy, young women with normal menstrual cyclicity. We hypothesized that ovarian suppression would reduce both glucose disposal and insulin secretion. Contrary to this hypothesis, however, we found no effect of 2 mo of GnRHa treatment, and the resulting ovarian hormone-deficient state, on either glucose disposal or insulin dynamics in healthy eugonadal women.

GnRHa treatment did not affect glucose disposal index in response to the hyperglycemic clamp stimulus. Our data agree with studies examining the effects of GnRHa on glucose disposal in healthy obese women evaluated using variable hyperglycemic hyperinsulinemic clamps (15) and in healthy lean women assessed by oral and intravenous glucose tolerance tests (3). Taken together with our prior results using the hyperinsulinemic clamp (7), these findings suggest that ovarian hormone suppression with GnRHa does not modulate tissue responsiveness to insulin (7) or to the combined effects of insulin and hyperglycemia (3, 15).

Subtle changes in the intracellular pathways of glucose disposal might not be discerned from measurements of total glucose disposal. This is particularly important in the context of ovarian hormones, since animal models have shown that hormone deficiency specifically reduces nonoxidative glucose disposal (27, 40). Thus, we partitioned total glucose disposal into oxidative and nonoxidative components by use of a combination of stable isotope-labeled glucose ([U-^{13}C]glucose) and indirect calorimetry. Similar to total glucose disposal, however, we found no effect of GnRHa on either oxidative or nonoxidative glucose disposal. The reason for disparities between data from humans and those from animal models is not clear. The effect of ovarian hormones in animals may relate to an indirect effect of ovarian hormone deficiency to induce hyperphagia and, in turn, increase adiposity (39), which would be expected to reduce nonoxidative glucose disposal (28). In contrast, GnRHa treatment in the present study did not alter body weight, adiposity or fat distribution (Table 1), and food intake was controlled for 3 days prior to glucose disposal measurements to eliminate any effects of GnRHa treatment on the antecedent diet. Thus, our results are likely unaffected by either acute or chronic alterations in energy balance. Together with our results using the hyperinsulinemic clamp (7), the current data provide further evidence that a brief period of ovarian hormone deficiency does not alter intracellular pathways of glucose disposal in humans.

GnRHa treatment did not alter first- or second-phase insulin secretion or insulin clearance. To our knowledge, this is the first study to directly examine the effect of GnRHa treatment on insulin secretion in humans by using a controlled hyperglycemic stimulus. Our results agree with those of Cagnacci et al. (3), who found no effect of 1 mo of GnRHa treatment on plasma insulin and C-peptide responses to either oral or intravenous glucose loads in women. Thus, in healthy women a brief period of ovarian suppression with GnRHa does not modulate insulin dynamics in response to either oral or intravenous glucose administration.

An important caveat to the present study and others (3, 7, 15) that have used the GnRHa model is that treatment is associated with mild reductions in circulating total and free testosterone, as well as androgenic precursors (Table 2). This complicates the interpretation of our findings if androgens regulate glucose homeostasis. Although pharmacological doses of androgens have minimal effects on insulin secretion, they have been shown to impair glucose disposal in women (11), and endogenous hyperandrogenemia is associated with insulin resistance (16). Moreover, in cross-sectional studies of eugonadal women, variation in serum total and free testosterone within the physiological range is negatively correlated with plasma insulin response during hyperglycemic clamps (22). Thus, one could postulate that our hypothesized effect of GnRHa treatment to reduce glucose disposal and insulin secretion could be masked by a reciprocal effect of reduced androgen levels to enhance these parameters. Although this scenario is plausible, it is unclear what effect, if any, a reduction in circulating levels of androgens might have on glucoregulation in women with normal androgen levels. In men, GnRHa treatment impairs glucose disposal under hyperglycemic clamp conditions but has minimal effects on insulin secretion (5). Normal circulating testosterone levels are significantly greater in men, and their decline in response to GnRHa (~400 ng/dl) is 20-fold higher than that observed in women in the present study (18 ng/dl). The question then becomes whether such small reductions in testosterone affect glucose disposal and insulin secretion in euandrogenemic women. Preliminary studies from our laboratory have shown, contrary to pharmacological and pathological hyperandrogenemia, that circulating androgens within the physiological range are positively associated with insulin-stimulated glucose disposal in postmenopausal women (Casson PR, unpublished observations), whereas other studies have shown no relationship between androgen levels and glucose disposal in young eugonadal women (22). Thus, rather than masking an effect of ovarian hormone deficiency on glucose disposal, GnRHa-induced reductions in testosterone levels may have no effect or could even enhance the suppressive effects of GnRHa treatment on glucose disposal. With respect to insulin secretion, although some studies have shown modest negative correlations between androgen levels and insulin secretion in eugonadal women (22), pharmacological administration of androgens to women has no effect on insulin secretion (11). Moreover, GnRHa treatment in men does not alter plasma insulin response to the hyperglycemic clamp stimulus (5). On the basis of these data, we believe that androgen levels probably had minimal effects on insulin secretion.

Our results regarding the effect of menstrual cycle phase on glucose metabolism deserve further comment. That menstrual cycle phase did not affect glucose disposal index is at odds with the only other study to use the hyperglycemic clamp to investigate cycle effects on glucose homeostasis, which found reduced glucose disposal during the luteal phase (13). The fact that studies using the hyperinsulinemic clamp from both laboratories (7, 12), as well as others (46, 59), show no effect of menstrual cycle phase on insulin-stimulated glucose disposal suggests that reduced glucose disposal in the luteal phase observed with the hyperglycemic clamp (12) may due to a diminished ability of hyperglycemia to stimulate glucose disposal (i.e., glucose-induced glucose disposal). Unfortunately, no study has directly measured the effect of ovarian hormones on glucose-induced glucose disposal. One study that attempted to experimentally reproduce luteal phase estradiol and progesterone levels by administration of oral micronized estradiol and progesterone failed to find an effect of either hormone alone or in combination on glucose disposal measured under euglycemic and hyperinsulinemic conditions (45), suggesting no effect of these hormones on glucose-induced glucose disposal. Thus, reasons to explain differences between the two studies are not readily apparent. The only notable differences are that the present study had a larger sample size (13 vs. 8) and controlled dietary intake for 3 days prior to the hyperglycemic clamps. With respect to the latter point, the luteal phase of the menstrual cycle is associated with increased energy intake and expenditure (2, 32). If intake exceeds expenditure during the luteal phase, this could result in a brief period of overfeeding that could impair glucose disposal. That this may occur is buttressed by the fact that women are susceptible to impaired glucoregulation in response to short-term energy excess (9). Thus, our attempt to control food intake prior to measurements could have diminished or prevented luteal phase hyperphagia and any corresponding reductions in glucose disposal secondary to energy imbalance. Perhaps most importantly, the absence of reduced glucose disposal index during the luteal phase would not impair our ability to detect an effect of GnRHa on glucose disposal. If anything, the absence of menstrual cycle differences in glucose disposal would enhance, not hinder, our ability to detect an effect of GnRHa.

In addition, we observed a small, but significant, increase in the first-phase insulin secretion during the luteal phase. Although to our knowledge no other study has measured insulin secretion rate during the menstrual cycle, our findings differ slightly from other studies that have failed to show altered plasma insulin levels in response to intravenous glucose (13, 38). Some studies have shown cycle-dependent differences in plasma insulin responses that were similar in magnitude to those in our study (38), albeit nonsignificant. Additionally, another study that attempted to experimentally reproduce luteal phase estradiol and progesterone levels by administration of oral micronized estradiol and progesterone found a similar magnitude increase in plasma insulin response to the hyperglycemic clamp (45). Importantly, we should stress that comparison of posttreatment insulin dynamics data to either baseline follicular or luteal phase measurements separately did not reveal an effect of GnRHa, suggesting that any cycle-dependent differences in insulin secretion would not impact the overall conclusions of our study.

A novel result in this study was the increase in plasma adiponectin in women treated with GnRHa. The fact that adiponectin increased in response to GnRHa-induced ovarian suppression is in keeping with cross-sectional data showing a negative relationship between estradiol and adiponectin (20) but contrasts with studies showing that transdermal estradiol increases adiponectin in obese postmenopausal women with the metabolic syndrome (6). We should note that, as with glucose metabolism, modest decreases in testosterone with GnRHa administration confound the interpretation of our results. Because testosterone decreases circulating adiponectin levels (34, 35, 58), the reduction in circulating testosterone in response to GnRHa could increase adiponectin (35, 58). These inhibitory effects of testosterone on adiponectin, however, have been observed in men, where changes in testosterone (35, 58) are considerably greater than those observed in the present study. If we assume that androgen receptors are present in adipocytes at similar levels in men and women (14) and have similar binding affinities, it seems unlikely that such small changes in androgen levels in women would provoke the observed changes in adiponectin.

Regardless of the hormonal mediator, as adiponectin is believed to sensitize tissues to the effects of insulin, it could be argued that the increase in adiponectin diminished the effect of GnRHa treatment to impair glucose disposal. The relative change in adiponectin in our study, however, was quite modest compared with alterations previously demonstrated to correlate to altered tissue insulin responsiveness (36). Moreover, the effect of adiponectin is thought to derive from alterations in hepatic insulin sensitivity (33, 36), whereas we found no alterations in the suppression of endogenous glucose production with GnRHa, an index of hepatic insulin sensitivity. Thus, we think that it is unlikely that changes in adiponectin influenced glucose disposal data.

Our study is limited by small samples sizes. The directionality of some of the observed changes in glucose disposal and insulin secretion, however, were not in accord with our hypotheses. In these instances, our data clearly argue against the notion that ovarian hormone deficiency is of importance for the pathogenesis of glucose intolerance with age. For other variables, such as first-phase insulin secretion, the number of volunteers needed to detect a group $\times$ time interaction effect concordant with our hypothesis is quite large ($>$50 per group). In these cases, the question then becomes whether such small changes in outcome variables are physiologically or pathophysiolgically relevant. In other words, if suppression of ovarian hormone concentrations to postmenopausal levels with GnRHa invokes such minor changes in glucose disposal or insulin secretion, one might conclude simply that the hormones play a relatively minor role in the regulation of glucose/insulin homeostasis.

In summary, our study suggests that 2 mo of ovarian hormone deficiency induced by GnRHa administration has no effect on glucose disposal or insulin dynamics. Although we acknowledge the limitation of extrapolating our findings using GnRHa in young eugonadal women to middle-aged women transitioning to the menopausal state, from a physiological perspective, our data suggest a minor role for endogenous ovarian hormones in the regulation of glucose disposal or insulin secretion. Similarly, we should note that our results are limited to eugonadal women, and the lack of effect of GnRHa

on glucose metabolism and insulin secretion may not apply to other populations, such as hyperandrogenic women. Our findings do, however, suggest a potential role for ovarian hormones in the regulation of plasma adiponectin levels.

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GRANTS

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