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"Evidence for Insulin Suppression of Baseline LH in Normal and PCOS Women"

Supplemental Material

Subjects and Methods

Procedure

The first subset of subjects was admitted to the General Clinical Research Center at the University of California, San Diego, for 2 d of study. The PCOS subjects were tested at random. Normal control subjects were studied during the mid-follicular phase defined as d 6–8. On the morning of day 1, blood samples were obtained from an indwelling i.v. cannula in the dorsal hand warmed at 60C at 10-min intervals for 12 hr. On d 2 of study three successive doses of GnRH (2 μg, 10 μg, and 20 μg (Factrel, Wyeth Pharmaceuticals) were administered by a second i.v. cannula in an antecubital vein at 4-h intervals over a continuous 12-h period. The sequence of GnRH dosing was not randomized to evaluate the baseline increases in serum LH after each GnRH dose. Blood samples were obtained before and for up to 120 min after each dose of GnRH. At a minimum interval of 1 month the protocol was repeated during a euglycemic hyperinsulinemic clamp. Studies were performed in the morning after a 12-h overnight fast. An i.v. infusion of insulin (Humulin; Eli Lilly, Indianapolis, IN) diluted in 0.15 M saline containing 1% wt/vol human albumin was begun at a rate of 80 mU/m^2/min 2 h before the first GnRH dose and continued for 12 h. Potassium and phosphate were given i.v. to maintain normal blood levels. Variable infusion of 20% glucose was delivered to maintain a plasma glucose concentration of 4.72 M (85 ng/dl). The measured glucose disposal rate was 8.3 mg/kg/min ± 0.8 and 5.2 ± 1.0 mg/kg/min for PCOS subjects, which was significantly different between groups ($p<0.05$).

The second subset of subjects were admitted to the General Clinical Research Center at the University of California, San Diego and after an overnight fast were prepared as above for successive days of infusion with saline or insulin to maintain a serum concentration of 1600 μU/ml insulin for a 4 hour duration. An insulin infusion was initiated at 0900 and frequent blood samples were obtained every 10 minutes for 8 hours. At 1200, an injection of GnRH, 10 ug, was administered and insulin infusion was halted at 1300. At a later date each subject returned to the GCRC and the protocol was repeated with the exception that insulin was infused at a different rate designed to achieve a physiological concentration of 60 μU/ml. Blood sampling was repeated as described above. The amounts of insulin infused during each admission were was calculated according to the body surface area of the subjects using 20mU/m^2/min and 320 mU/m^2/min to achieve a steady state level of approximately 60 μU/ml (I-60) and 1600 μU/ml (I-1600), respectively. Blood glucose levels were monitored at 5 minute intervals and variable infusion of 20% glucose was delivered to maintain a plasma glucose concentration of 4.72 M (85 ng/dl).

Statistical Analysis

Analyses were performed using the statistical software packages JMP Version 6.03 (SAS, Carey, North Carolina) and R (http://www.r-project.org/), version 2.4.1.

Comparison of Baseline Hormone Levels Between PCOS and Normal Women:

The analysis of group differences between PCOS and normal subjects were tested under the null assumption that no differences would be detected between groups. Endocrine values from both groups were pooled, transformed to correct for non-normality, and compared by two-sided t-test to determine group differences. Variables were examined for heteroscedasticity and variance homogeneity by testing for normal distribution of sample data and variance residuals. Deviations

from normality were corrected by optimal transformation using the method of Box and Cox as implemented in JMP. Correction of deviation was confirmed by reexamination of residual distribution and normality testing. Transformation of all variables improved the distribution of variance residuals as demonstrated by an increased W test statistic. PCOS and normal groups were analyzed as pooled groups as well as analyzed separately to examine the relationship between each factor and the composite 12h mean LH levels using Pearson's product moment correlation. Regression models were also constructed using the transformed data to test the relationship between the factors identified as significant in the univariate analysis and the outcome in a multivariable approach.

LH Response to Varying Doses of GnRH during a Fixed Rate of Insulin Infusion:

Descriptive statistics of baseline LH and change in LH (peak LH – baseline LH) with and without insulin infusion were computed for each diagnosis group (normal and PCOS) and each GnRH dose level (2, 10, and 20 micrograms). For each dose level, two-sided paired t-tests were conducted to compare baseline LH with/without insulin infusion for each diagnosis group separately. Paired t-tests were also conducted to compare change in LH with or without insulin infusion for each diagnosis group separately.

To determine the effect of insulin infusion on the baseline LH response across GnRH doses for both diagnosis groups, a linear mixed-effects model was used. This approach adjusts for repeated measures (doses and with or without insulin infusion) and variation in baseline among patients. The following linear mixed model was used to model pre-injection baseline LH for the jth dosage measurement from patient i: $Y_{ij} = \alpha + \gamma_i + \delta^T X_{ij} + \varepsilon_{ij}$. The γ_i is a random effect which represents individual patient differences in baseline LH and is assumed to be normally distributed with mean 0 and variance σ_γ^2. δ denotes the fixed effects of covariates X_{ij}, which include a

dichotomous variable indicating insulin infusion (with insulin infusion or without insulin infusion), a dichotomous variable for diagnosis group (PCOS or control), an interaction term for insulin infusion and diagnosis group, and a GnRH dosage variable. In addition, BMI and testosterone were added in as potential confounding covariates. Other interaction terms were considered in the model-building process, but removed due to insignificance. The error terms, ε_{ij}, are normally distributed and independent across subjects with mean 0 and variance σ^2. To study the effect of insulin infusion on the change in LH (peak LH-baseline LH) across GnRH doses for both diagnosis groups, the same linear mixed-effects model above was considered with baseline LH added in as a covariate.

LH Response to a Fixed Dose of GnRH during Low- and High-Dose Insulin Infusion:

Descriptive statistics of mean preinjection baseline LH and change in LH (peak LH – baseline LH) with vehicle or insulin infusion at each dose were computed. Data were analyzed for heteroscedasticity and variance homogeneity by testing for normal distribution of sample data and variance residuals. Deviations from normality were corrected by optimal transformation using the method of Box and Cox as implemented in JMP. Correction of deviation was confirmed by reexamination of residual distribution and normality testing using the W statistic as above. Comparisons between treatment conditions were normalized by subject by inclusion as a covariable in the model. Post hoc paired comparisons to control were made with Students T test.