Association of anti-mullerian hormone levels with obesity in late reproductive-age women

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Objective: To describe anti-mullerian hormone (AMH) levels in healthy late reproductive age women and test the hypothesis that AMH levels are lower in obese compared to non-obese women.
Design: Cross-sectional study of AMH levels. Longitudinal analysis of a subgroup with 10 AMH measures over 8 years to support the cross-sectional results.
Setting: A population-based cohort of healthy late reproductive-age women.
Participants: Selected from the cohort to provide comparisons of body mass index (BMI), menopausal status, age and race (n = 122).
Interventions: AMH levels were determined from blood samples collected in the parent study.
Main Outcome Measure: Serum levels of AMH.
Results: AMH levels were 65% lower in obese women compared to non-obese women (0.016 ng/mL and 0.046 ng/mL, respectively). The geometric mean ratio was 0.35; 95% CI 0.13, 0.92, P=.034. AMH levels were significantly lower in the menopausal transition compared to premenopausal women and were significantly lower in all age groups ≥40 years compared to the 35–39 year-old women. BMI remained significantly associated with AMH levels in multivariable models that included adjustments for menopausal status, age, race and cycle day. In the longitudinal analysis of a subgroup, obese women had significantly lower mean AMH levels over the 8-year interval compared to the non-obese women (0.459 ng/mL; CI 0.28, 0.75 and 0.566 ng/mL; CI 0.34, 0.94, respectively; P=.016), corroborating the cross-sectional study results.
Conclusions: Obese women have lower AMH levels compared to non-obese women in the late reproductive years. The findings offer further evidence of the complex relationships between obesity and reproductive hormone levels in women. (Fertil Steril® 2007;87:101–6. ©2007 by American Society for Reproductive Medicine.)

Key Words: AMH, anti-mullerian hormone, obesity, BMI, menopause, ovarian aging

INTRODUCTION

Anti-mullerian hormone (AMH), also known as Mullerian inhibiting substance, is a key factor in embryonic sex differentiation in males. In postnatal females, AMH is secreted by the primary, secondary and antral follicles and appears to regulate early follicular development (1, 2). The exact role of AMH in the ovary is not entirely clear, but the proposed functions include inhibition of follicular recruitment, inhibition of aromatase activity, and reduced sensitivity of follicles to FSH (3, 4).

AMH is emerging as an early predictor of decreased ovarian reserve (5–9). Specifically, serum AMH levels have been shown to decrease with age (5, 6, 10) and also to be a stronger and more consistent correlate of age than the number of antral follicles, inhibin B or FSH levels (6). Studies using transvaginal ultrasonography have demonstrated that there is a correlation between AMH levels and the number of small antral follicles (11). Undetectable levels of AMH in women following ovariectomy as well as natural menopause confirm its ovarian source (7).

We previously found that BMI was related to the change in inhibin B levels in the menopausal transition, with a significant association between decreasing inhibin B levels and increased body mass index (BMI) independent of race (12). These findings in healthy late reproductive age women extended previous

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reports of an inverse association of BMI with inhibin B levels in women with polycystic ovary syndrome and anovulatory premenopausal women (13, 14). Although the mechanism for the negative effect of BMI on inhibin B is unclear, it may reflect the negative effect of obesity and insulin resistance on granulosa cell function. We have now examined the association of obesity with AMH levels to support our previous findings of a negative effect of obesity in ovarian function. We hypothesized that AMH levels were lower in obese women compared to non-obese women in the late reproductive years.

MATERIALS AND METHODS

Subjects

One hundred twenty two participants in the Penn Ovarian Aging Study (12, 15, 16), an ongoing population-based cohort study of late reproductive-age women, were studied. At enrollment in the cohort, the women were ages 35–47 years, had menstrual cycles in normal range (22–35 days) for the previous three months, an intact uterus and at least one ovary. Exclusion criteria included current use of hormonal or psychotropic medications, hysterectomy, pregnancy, breast feeding, or serious health problems known to compromise ovarian function. The study was approved by the Institutional Review Board of the University and written informed consent was obtained.

AMH levels were determined from blood samples that were collected from 102 women, primarily at Period 9, approximately 7 years after enrollment in the cohort. The frozen blood samples were selected for the AMH assays in order to provide similar distributions for each of three variables: menopausal status (premenopausal, early transition and postmenopausal), body mass index (BMI $\geq$30 and BMI $<$30) and race (African American or Caucasian). We then added blood samples from another 20 women who had longitudinal measures of AMH levels for a total of 122 women. Only the baseline measure from each woman in this subgroup was used in the cross-sectional analysis.

The subgroup with longitudinal measures (n = 20) had 10 assessments of AMH, starting with the first assessment in the cohort through the 10th assessment period approximately 8 years later. At the first assessment, all women in the subgroup were premenopausal and 35–37 years of age; 7 had a BMI $\geq$30 and 13 had a BMI $<$30; 8 were African American, 13 Caucasian.

Measures

Non-fasting blood samples for the hormone assays were collected from all participants between days 1-6 of the menstrual cycle in cycling women. On average, the specimens were obtained on cycle day 4. The blood samples were centrifuged and frozen in aliquots at -70 degrees C.

AMH was assayed at Specialty Laboratories (Valencia, CA) using RUO-labeled kits (DSL-14400 Active AMH/MIS) from Diagnostic Systems Laboratories and performed according to the manufacturer's guidelines. There were two levels of kit controls at 1.00 +/$-$0.12 ng/mL and 4.20 +/$-$ 0.38 ng/mL. These manufactured assay control materials covered the reportable range of the assay from 0.05 through 15.0 ng/mL. The levels and imprecision studies are described in detail in the manufacturer's kit insert. The intra- and interassay coefficients of variation were less than 4% and less than 6%, respectively. The lower limit of detection was 0.05 ng/mL. The AMH data were reported in ng/mL (mean of duplicates).

Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m). Height was measured without shoes to the nearest 0.25 inch; weight was measured in light clothing to the nearest 0.50 pound. Each measurement was taken twice, and the average of the two measures was used in the calculation of BMI at each study period.

Menstrual cycle lengths were determined from the menstrual date at each study interview (interviews were conducted within 6 days of bleeding) and the two previous menstrual dates recorded in the interview at each assessment period. Confirmatory dates were obtained from the menstrual diaries recorded by the participants for one menstrual cycle at each assessment period.

The definitions of menopausal stages were adapted from the staging system for reproductive aging in women (STRAW) that we have validated in previous reports (16, 17). At each assessment, the participant was assigned to one of the following categories based on menstrual bleeding patterns at that assessment: Premenopausal: regular menstrual cycles in the 22–35 day range; Late premenopausal: change in cycle length $\geq$7 days in either direction compared to the participant's personal baseline at enrollment in the cohort and observed for one cycle in the study; Early transition: change in cycle length $\geq$7 days in either direction from the participant's personal baseline at enrollment in the cohort and observed for at least 2 cycles in the study. Late transition: 3–11 months amenorrhea during the study; Postmenopausal: $\geq$12 months amenorrhea with no hysterectomy.

AMH levels in the late transition and postmenopause were generally below the level of detection and were considered censored in the analyses. To accommodate these groups in the analyses, we considered menopausal status as a two-group variable: premenopausal and transition.

Statistical Analysis

Distributions of hormone values were first examined and values were plotted with BMI, menopausal status and race to elucidate relationships of these variables. Because the hormone values were not normally distributed, the natural logarithm of AMH was used in all analyses to accommodate modeling assumptions. AMH values below the limit of detection were censored in the analytic models. Geometric means of hormone levels are shown in the table and figures.