Plasma metastin levels are negatively correlated with insulin resistance and free androgens in women with polycystic ovary syndrome

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Objective: This study was designed to: [1] measure, for the first time, metastin (kisspeptin) levels in women with polycystic ovary syndrome (PCOS), a condition associated with hypersecretion of LH and hyperandrogenemia; and [2] investigate the possible correlations between metastin and PCOS-related reproductive and metabolic disturbances.
Design: Clinical study.
Setting: University hospital.
Patient(s): Twenty-eight obese and overweight (body mass index [BMI] >25 kg/m^2) women with PCOS, 28 normal weight (BMI <25 kg/m^2) women with the syndrome, and 13 obese and overweight controls (ovulatory women without clinical or biochemical hyperandrogenemia) were selected.
Intervention(s): Blood samples were collected between day 3 and day 6 of a spontaneous bleeding episode in the PCOS groups and a menstrual cycle of the controls, at 9:00 AM, after an overnight fast.
Main Outcome Measure(s): Circulating levels of LH, FSH, PRL, T, Δ_4-androstenedione (A), DHEAS, 17α-OH-P, sex hormone-binding globulin (SHBG), insulin, glucose, and metastin were measured.
Result(s): Both normal weight women with PCOS and obese controls were less insulin resistant and had significantly higher metastin levels, compared to obese and overweight women with the syndrome. Plasma kisspeptin levels were negatively correlated with BMI, free androgen index, and indices of insulin resistance.
Conclusion(s): These results indicate that metastin is negatively associated with free androgen levels. The PCOS-associated insulin resistance and consequent hyperinsulinemia probably contribute to this effect by [1] stimulating androgen synthesis by the polycystic ovary (PCO) and [2] suppressing SHBG production in the liver. (Fertil Steril® 2006;85:1778–83. ©2006 by American Society for Reproductive Medicine.)

Key Words: Metastin, kisspeptin, PCOS, obesity, insulin resistance

Metastin is a 54-amino-acid peptide, which was first isolated from the human placenta in 2001 (1). It is encoded by the Kiss-1 gene, therefore, it is also known as Kiss-1 peptide (kisspeptin) (2–4). Metastin action is exerted by a *trans*-membrane G-protein-coupled receptor, named GPR54, AXOR12, or HoT7T175 (1, 5, 6). Kiss-1 expression has been associated with antimetastatic activity in malignant melanoma (4), breast (7), papillary thyroid (3), esophageal (8) and bladder (9) carcinoma. Furthermore, kisspeptin has also been found to suppress the invasion of human trophoblast cells (10), a process sharing common pathways with tumor metastasis (11–13). A possible physiological role of this peptide in the control of trophoblast growth into adjacent tissues could also account for the dramatic increase of plasma metastin levels in pregnancy (14).

Intriguingly, loss of Kiss-1 function is associated with hypogonadotropic hypogonadism in humans (15) and in animal models (16, 17). Administration of the Kiss-1 peptide (metastin/kisspeptin) has been shown to induce maturation of the female reproductive system and precocious puberty in rodents (18). Moreover, there is strong evidence that metastin exerts these effects by stimulating GnRH release from the hypothalamus, and, as a consequence, pituitary LH secretion (19–22). It is also interesting that a negative feedback loop might also exist, as kisspeptin has been reported to be down-regulated by sex steroids (23).

The polycystic ovary syndrome (PCOS) is a common heterogeneous disorder of women of reproductive age, and the most frequent cause of hyperandrogenism combined with

Received March 24, 2005; revised and accepted November 6, 2005.
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0015-0282/06/$32.00
doi:10.1016/j.fertnstert.2005.11.044

anovulatory infertility (24, 25). Its complex pathogenesis involves [1] hypothalamic–pituitary disturbances in gonadotropin secretion, specifically increased LH levels (26, 27); [2] increased resistance to insulin (28) and compensatory hyperinsulinemia, which has been associated with enhanced androgen production (29) and reduced synthesis of sex hormone-binding globulin (SHBG) (30); and [3] disturbed gonadal steroidogenesis (31).

Given the complex relationship between the novel peptide metastin and the hypothalamic–pituitary–gonadal axis, the present study was designed to: [1] measure metastin levels in women with PCOS, a condition associated with aberrant gonadotropin secretion and hyperandrogenemia; and [2] investigate the possible correlations between kisspeptin and PCOS-related reproductive and metabolic disturbances.

MATERIALS AND METHODS

Subjects

Fifty-six women with PCOS, aged 15–37 years, were recruited from the outpatient endocrine infirmary of our clinic, with at least one of the following signs: oligomenorrhea, fertility problem, hirsutism, acne, or male pattern alopecia. Diagnosis of PCOS was based on the presence of chronic anovulation (less than six cycles in 12 months) and hyperandrogenemia. Other common causes of hyperandrogenism or menstrual disorders (prolactinoma, congenital adrenal hyperplasia, Cushing syndrome, and virilizing ovarian or adrenal tumors) were excluded, in accord with the criteria proposed in 1990 by the National Institutes of Health–National Institute of Child Health and Human Development (NIH-NICHHD) (32), and revised in 2003 by the Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group (33). Women with PCOS were further divided into two groups, based on body mass index (BMI) values: obese and overweight (BMI >25 kg/m^2) (n = 28) and normal weight (BMI <25 kg/m^2) women with the syndrome (n = 28).

Thirteen obese and overweight healthy women, aged 18–31 years, volunteered as controls. All controls had normal ovulating cycles (28 ± 2 days, blood P levels >10 ng/mL in two consequent cycles), and no signs of hyperandrogenism. None of the women studied had galactorrhea, or systemic disease that could affect their reproductive physiology. Furthermore, no woman reported use of any medication that could interfere with the normal function of the hypothalamic–pituitary–gonadal axis, during the past 6 months. Informed consent was obtained from all 69 women and the study was approved by the local ethical committee.

Hormonal and Biochemical Measurements and Calculations

Blood samples were collected between day 3 and day 6 of a spontaneous bleeding episode in the PCOS groups and a menstrual cycle of the controls, at 9:00 AM, after an overnight fast. In all women, basal serum levels of FSH, LH, T, Δ_4-androstenedione (A), and DHEAS were measured. Fasting concentrations of PRL, 17α-OH-P, sex hormone-binding globulin (SHBG), glucose, insulin, and metastin were also measured. Free androgen index (FAI) was calculated according to the equation: T (nmol/L) × 100/SHBG (nmol/L) (34). Homeostatic model assessment–insulin resistance (HoMA-IR) was derived from the equation: Glucose (mmol/L) × Insulin (μIU/mL)/22.5 (35). The glucose-to-insulin ratio was also calculated as a reliable index of insulin resistance (36). Hyperandrogenemia was defined as T levels >60 ng/mL. This value was derived from the mean value ± 2SD of 100 control women. On the same day that blood samples were collected, transvaginal ultrasonographic evaluation was performed.

Assay Methods

Plasma glucose concentrations were measured with the glucose oxidase technique using an auto-analyzer (Roche/Hitachi 902; Roche Diagnostics GmBH, Manheim, Germany). LH, FSH, PRL, androgen, and 17α-OH-P levels were measured with the RIA method, whereas SHBG levels were measured with the IRMA method, using commercial kits (FSH: Radioisotopic Kit, Nichols Institute Diagnostics, San Juan Capistrano, CA; LH: Radioisotopic Kit, Nichols Institute Diagnostics; PRL: Radioisotopic Kit, Nichols Institute Diagnostics; T: Radioisotopic Kit, Diagnostic Systems Laboratories, Webster, TX; Δ_4A: Radioisotopic Kit, Diagnostic Systems Laboratories; DHEAS: Radioisotopic Kit, Diagnostic Systems Laboratories; 17α-OHP: Radioisotopic Kit, Diagnostic Systems Laboratories; SHBG: Immunoradiometric Assay (IRMA) Kit, Diagnostic Systems Laboratories). Serum insulin levels were measured by enzyme immunoassay (ELISA Kit, Mercodia AB, Uppsala, Sweden). Metastin levels were also measured with an enzyme-linked immunoassay kit (ELISA kit, Phoenix Pharmaceuticals Inc., Belmond, CA), after extraction with Phoenix Peptide sep-columns (RK-Sepcol-2). An intra-assay variation of 4.5% was observed.

Statistical Analyses

Values are presented as mean ± standard error for mean (SEM). The Kolmogorov-Smirnov test was used to test the normality of distribution. Comparison of means between women with PCOS and controls was performed with the Mann-Whitney U test for non-normal values. Comparisons between the three groups were performed with multivariate general linear model-based one-way analysis of variance (ANOVA), after log-transformation of non-normal values. Post-hoc analysis was performed with Dunnette's T3 test. Calculation of the Spearman coefficient was used to assess the correlation of plasma kisspeptin to each parameter. All analyses were performed by SPSS software (v.13.0 SPSS, Inc., Chicago, IL). The level of statistical significance was set at 5%.