Testosterone deficiency in young women with 46,XX spontaneous premature ovarian failure

Sophia N. Kalantaridou, M.D., Ph.D.,[a] *Karim A. Calis, Pharm.D., M.P.H.,*[b] *Vien H. Vanderhoof, C.R.N.P.,*[a] *Vladimir K. Bakalov, M.D.,*[a] *Emily C. Corrigan, B.S.,*[a] *James F. Troendle, Ph.D.,*[c] *and Lawrence M. Nelson, M.D.*[a]

[a] Section on Women's Health Research, Developmental Endocrinology Branch and [c] Biometry and Mathematical Statistics Branch, National Institute of Child Health and Human Development; and [b] Clinical Research Center, National Institutes of Health, Bethesda, Maryland

Objective: To determine whether women with 46,XX spontaneous premature ovarian failure have lower serum free-T levels than do control women.
Design: Cross-sectional.
Setting: National Institutes of Health Clinical Research Center.
Patient(s): Women with 46,XX spontaneous premature ovarian failure (n = 130).
Intervention(s): Evaluation while off any estrogen therapy and then again after receiving a standardized hormone regimen. Regularly menstruating control women (n = 65) were sampled during the midfollicular phase.
Main Outcome Measure(s): Serum total T by RIA after extraction and column chromatography, free T by equilibrium dialysis, and sex hormone–binding globulin by immunoradiometric assay.
Result(s): While off estrogen therapy patients had a median serum free-T concentration that was statistically significantly lower than controls (2.2 vs. 3.3 pg/mL). This dropped significantly lower to 1.9 pg/mL while the patients were on physiologic transdermal E_2 therapy. This is despite the fact that sex hormone–binding globulin levels did not change. While on E_2 therapy, 13% of women (95% confidence interval, 7.9%–20.3%) had serum free-T levels below the lower limit of normal (<1.1 pg/mL).
Conclusion(s): As a group, young women with 46,XX spontaneous premature ovarian failure have reduced circulating free-T levels, both during an interval off of estrogen therapy and while on physiologic transdermal E_2 therapy. (Fertil Steril® 2006;86:1475–82. ©2006 by American Society for Reproductive Medicine.)

Key Words: Free testosterone, total testosterone, premature ovarian failure, sex hormone–binding globulin, equilibrium dialysis, ovarian insufficiency

The role that T may play in maintaining a woman's health has been receiving increasing attention, but still there are no clear guidelines regarding the diagnosis of T deficiency in women (1). There is considerable controversy as to whether female androgen-deficiency syndrome in fact exists and whether such a syndrome has clinical consequences for women such as effects on bone, quality of life, or libido (1–21).

Received January 19, 2006; revised and accepted April 12, 2006.
Supported by the Intramural Research Program of the National Institute of Child Health and Human Development, National Institutes of Health (Bethesda, MD), and by Procter and Gamble, Inc. Cincinatti, OH, as part of an approved US Federal Cooperative Research and Development Agreement. Under this agreement, both sponsors participated in the design and conduct of the study. National Institutes of Health investigators were responsible for the collection, management, analysis, and interpretation of the data and for the preparation, review, and approval of the manuscript. The seventh author (L.M.N.) had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Authors V.H.V. and L.M.N. are commissioned officers in the United States Public Health Service.
Reprint requests: Lawrence M. Nelson, M.D., Developmental Endocrinology Branch, National Institutes of Health, CRC 1-3330, 10 Center Drive, MSC-1103, Bethesda, Maryland 20892-1103 (FAX: 301-402-0574; E-mail: lawrence_nelson@nih.gov).

Spontaneous premature ovarian failure affects approximately 1% of women and is characterized by the development of amenorrhea, estrogen (E) deficiency, and menopausal serum gonadotropin levels in women younger than 40 years of age (22–24). It has become clear that the pathophysiology of spontaneous premature ovarian failure differs from the normal menopausal process. Many of these women experience intermittent ovarian function, with remissions and even pregnancies possible even decades after the diagnosis (23–25). Recent evidence suggests that there is a gradual decline in circulating serum androgen levels as women age and that there is no independent effect of a natural menopause causing a further decline (26–28). However, there is uncertainty as to whether spontaneous premature ovarian failure is a cause of clinically significant T deficiency (29–33). Part of this uncertainty stems from the fact that the ideal methods by which to measure free and total T in women have been controversial (34–36).

To our knowledge, no studies have used the preferred methods of equilibrium dialysis or the mass-action equation approach (34, 35) to evaluate levels of free T in women with 46,XX spontaneous premature ovarian failure. We undertook this study by using these rigorous methods to determine whether women with 46,XX spontaneous premature ovarian

failure have reduced serum free-T levels compared with women of similar age with normal ovarian function. We examined this question both during an interval when they were off estrogen therapy (ET) and also during transdermal E_2 therapy, the more clinically relevant question.

MATERIALS AND METHODS

Study Population

The study was approved by the Institutional Review Board of the National Institute of Child Health and Human Development, National Institutes of Health (Bethesda, MD). All women provided written informed consent. We recruited controls by local advertisement and patients by letters to physicians, notices in medical journals, and by the Internet. From January 2000 to November 2004, we recruited 131 women with 46,XX spontaneous premature ovarian failure and 65 control women of similar age who had normal ovarian function. We recruited patients and controls as part of a screening process for enrollment in a 3-year prospective double-blind randomized placebo-controlled trial (designed to evaluate the effect of transdermal E_2-T therapy on bone density).

Study Design

The design was cross-sectional and controlled. Women with spontaneous premature ovarian failure were seen for two visits: [1] a baseline screening evaluation at which time they had been off any E or progestin hormone therapy (HT) for ≥2 weeks, and [2] after receiving a standardized hormone regimen for ≥3 months consisting of a 100-μg E_2 patch and cyclic oral medroxyprogesterone acetate (10 mg for 12 d/mo). Blood samples were drawn for the determination of serum free T, total T, E_2, sex hormone–binding globulin (SHBG), FSH, and LH levels. For women with spontaneous premature ovarian failure, these were drawn at baseline (off any E or progestin therapy for ≥2 weeks) and after ≥3 months on the replacement regimen (during the E-only phase of the hormone cycle; not while on progestin). These samples were drawn during the E_2-only phase of the replacement to avoid potential confounding effects of progestin administration. For control women, blood samples were drawn during the midfollicular phase of their menstrual cycle (days 5–12).

Women With Premature Ovarian Failure

To be eligible for the study, patients had to meet the following inclusion criteria: [1] diagnosis of 46,XX spontaneous premature ovarian failure before the age of 40 years (i.e., ≥4 mo of amenorrhea and 2 FSH levels in the menopausal range [as defined by the laboratory reports obtained from the referring clinician, confirmed on 2 separate occasions, ≥1 mo apart]), [2] age between 18 and 42 years, [3] no iatrogenic cause of ovarian failure or known chromosomal abnormality, and [4] no contraindication for HT. Screening baseline evaluation was as described elsewhere (37).

Controls

Control women were healthy, nonpregnant, and regularly menstruating (cycles between 21 and 35 d). They did not smoke more than two cigarettes per day or use alcohol (<2 drinks per day). They were taking no chronic medications and were not using hormonal contraception. They were compensated according to National Institutes of Health guidelines.

Hormonal Assessment

Total T Blood samples were drawn from fasted subjects at 8 AM, separated within 1 hour, and frozen. We measured serum total T by RIA after extraction chromatography (Esoterix Endocrinology, Calabasas Hills, CA) (38). Samples were extracted with hexane-ethyl acetate, and the extract was applied to Al_2O_3 microcolumns. Testosterone was eluted by using hexane containing ethanol. Duplicate aliquots were then taken for RIA. Purified samples were incubated with an antibody made to a T–3-oxime–bovine serum albumin conjugate. Free and antibody-bound T were separated with ammonium sulfate. The concentration of T in each sample was calculated from a curve generated by purified T standards. Sensitivity of the method is 3 ng/dL. The intra-assay coefficient of variation (CV) was 6.1% at 11.4 ng/dL, 2.9% at 35.4 ng/dL, 3.9% at 236.9 ng/dL, and 8.0% at 521.6 ng/dL. The interassay CV was 15% at 14.4 ng/dL, 9% at 37 ng/dL, 9% at 242 ng/dL, and 8% at 469 ng/dL. The normal premenopausal range is 10 to 55 ng/dL, with a mean of 32 ng/dL.

Sex Hormone–Binding Globulin

We measured serum SHBG by using an immunoradiometric assay that had been developed at Esoterix Endocrinology. A monoclonal antibody made to human SHBG was coated onto plastic beads. The sample and a different SHBG monoclonal antibody labeled with ^{125}I were incubated overnight with the antibody-coated beads. Sex hormone–binding globulin in the sample or in the standard forms bead–antibody–SHBG–antibody^{125}I sandwiches, attaching the label to the beads. The beads are washed to remove unbound label and are counted. The intra-assay CV was 2.4% at 47.9 nMol/L, 3.5% at 89.8 nMol/L, and 3.9% at 178 nMol/L. The interassay CV was 7.8% at 49 nMol/L, 8.1% at 95 nMol/L, and 10.6% at 177 nMol/L. The normal adult range for women is 36 to 185 nMol/L, with a mean of 84 nMol/L.

Free T

We determined serum free-T concentrations by using equilibrium dialysis (39). Serum containing tritium-labeled T was placed inside a semipermeable dialysis cell and dialyzed against a buffer. The percentage of free T was calculated from the ratio of radioactivity outside the cell vs. inside the cell. The percentage free T, multiplied by the total T in the serum and corrected for units, results in the concentration of free T in the serum. Protein levels were checked in the