Serum insulin-like factor 3 is highly correlated with intratesticular testosterone in normal men with acute, experimental gonadotropin deficiency stimulated with low-dose human chorionic gonadotropin: a randomized, controlled trial

Mara Y. Roth, M.D.,[a] Kat Lin, M.D.,[b] Katrine Bay, M.Sc., Ph.D.,[c] John K. Amory, M.D., M.P.H.,[a] Bradley D. Anawalt, M.D.,[a] Alvin M. Matsumoto, M.D.,[a,d] Brett T. Marck, B.S.,[d] William J. Bremner, M.D., Ph.D.,[a] and Stephanie T. Page, M.D., Ph.D.[a]

[a] Center for Research in Reproduction and Contraception, Department of Medicine; and [b] Department of Obstetrics and Gynecology, University of Washington, Seattle, Washington; [c] University Department of Growth and Reproduction, Rigshospitalet, Copenhagen, Denmark; and [d] Geriatric Research, Education and Clinical Center, Veterans Affairs Puget Sound Health Care System, Seattle, Washington

Objective: To study the potential role for using serum biomarkers, including insulin-like factor 3 (INSL3), 17α-hydroxyprogesterone, antimüllerian hormone, and inhibin B, as correlates of intratesticular T (IT-T) concentrations in men.
Design: Prospective, randomized, controlled trial.
Setting: University-based medical center.
Patient(s): Thirty-seven healthy men aged 18–50 years.
Intervention(s): All men received the GnRH antagonist acyline, plus very low doses of hCG (0 IU, 15 IU, 60 IU, or 125 IU) SC every other day or 7.5 g T gel daily (75 mg delivered). The IT-T concentrations obtained by percutaneous testicular aspiration with simultaneous serum protein and steroid concentrations were measured at baseline and after 10 days of treatment.
Main Outcome Measure(s): Intratesticular and serum hormone and gonadotropin concentrations.
Result(s): After 10 days of gonadotropin suppression, serum INSL3 decreased by more than 90% and correlated highly with IT-T concentrations. In contrast, serum inhibin B, antimüllerian hormone, and 17α-hydroxyprogesterone did not correlate with IT-T. Serum INSL3 increased with the dose of hCG administered and returned to baseline after treatment.
Conclusion(s): Serum INSL3 correlates highly with IT-T and serum T concentrations during acute gonadotropin suppression in men. Human chorionic gonadotropin stimulates dose-dependent increases in INSL3 and IT-T in healthy men and might be a useful biomarker of IT-T concentration in some clinical settings.
Clinical Trial Registration Number: NCT# 00839319. (Fertil Steril® 2013;99:132–9. ©2013 by American Society for Reproductive Medicine.)
Key Words: Androgens, INSL3, male infertility, gonadotropins, intratesticular testosterone

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Reprint requests: Mara Y. Roth, M.D., Department of Medicine, University of Washington, 1959 NE Pacific Street, Box 357138, Seattle, Washington 98195 (E-mail: mylang@u.washington.edu).

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Male factor infertility accounts for 20% of infertile couples, and in as many as 60% of these cases the underlying cause of their infertility is unknown (1). Androgens play a vital role in spermatogenesis. Testosterone, secreted by testicular Leydig cells upon stimulation by LH, is present in very high concentrations within the testes, 100–1,000-fold higher than the concentrations found in the circulation (2–7). Infertile men with low gonadotropins, and in some cases men with idiopathic infertility, can be treated with exogenous gonadotropins in an effort to initiate or optimize spermatogenesis. Human chorionic gonadotropin, which stimulates Leydig cells similarly to LH, is used to increase T production in the testes before the addition of FSH. In men with hypogonadotropic hypogonadism, the dose of hCG is often adjusted to normalize serum T, yet the intratesticular T (IT-T) concentration is one of the keys to inducing spermatogenesis. In some men, normal serum T concentrations can be achieved by administering hCG without normalizing IT-T concentrations or stimulating spermatogenesis (3, 8, 9), raising the possibility that serum T may not be an accurate marker of IT-T. In addition, given that gonadotropin therapy to induce spermatogenesis often requires several years to achieve conception (10), identifying serum markers to potentially predict response to therapy would benefit infertility patients. Unfortunately, measurement of intratesticular androgens is invasive, requiring either percutaneous testicular aspiration or surgical testicular biopsy, so IT-T concentrations are not often evaluated in infertile men. A serum biomarker of IT-T might be useful in the diagnosis, prognosis, and treatment monitoring of some men with infertility.

Insulin-like factor 3 (INSL3), antimüllerian hormone (AMH), inhibin B (INHB), and the T precursor 17α-hydroxyprogesterone (17-OHP) are candidate substances, quantifiable in serum, and have the potential to serve as serum correlates of IT-T. Similar to T, INSL3 is a protein produced by Leydig cells and is known to play a key role in testicular descent during fetal development (11, 12). Expression of INSL3 increases during pubertal development (13) and is abundant in serum of adult men (14). Although the role of INSL3 in adults remains unclear, some studies suggest that serum concentrations of INSL3 are lower in men with infertility, even those with normal serum T concentrations (15). Production of INSL3 is clearly regulated by LH, although it has yet to be fully determined whether this regulation is direct or via IT-T (13, 16). Although T concentrations often vary widely in a given individual over the course of a day (17), INSL3 seems to be constitutively expressed in normal adult men and declines with age (18). Therefore, serum INSL3 has the potential to reflect steady-state IT-T levels without the confounding pulsatility of serum LH and T (19, 20).

Antimüllerian hormone and INHB are glycoproteins secreted by Sertoli cells. Antimüllerian hormone plays a key role in male embryos to trigger müllerian duct regression (21). Although AMH is present in high concentrations in adult men, its functional role is unclear (22). The glycoprotein INHB, also secreted by Sertoli cells, correlates strongly with sperm count, concentration, and testicular volume in adult men (23). Inhibin B largely functions to regulate the release of FSH (24–26). Both INHB and AMH have been reported to be lower in men with nonobstructive azoospermia (27), although attempts to use INHB and AMH as predictors for the success of infertility treatment in azoospermic men have not been successful to date (28, 29).

In addition to these testicular products, previous studies have suggested that serum concentrations of the T precursor 17-OHP reflect IT-T when normal concentrations of serum T are maintained (30). Approximately 70% of circulating 17-OHP is thought to be of testicular origin, and similar to T, production of 17-OHP is stimulated by LH and hCG (31, 32). Whether serum 17-OHP concentrations are a good biomarker for IT-T when IT-T concentrations are very low has not been investigated to date.

To investigate the relationship between serum markers of testicular function and IT-T, we evaluated serum INSL3, AMH, INHB, and 17-OHP in healthy men undergoing experimental gonadotropin suppression (3). In conjunction with the administration of a potent GnRH antagonist that effectively suppresses gonadotropins for 2 weeks, acyline (33), subjects were randomized to receive low doses of hCG to create a continuum of IT-T concentrations across the experimental groups (3). On the basis of their regulation by LH, we hypothesized that serum 17-OHP and INSL3 would be highly correlated with IT-T in the setting of low-dose LH (hCG) stimulation, whereas AMH and INHB concentrations, which are stimulated primarily by FSH, would not change.

MATERIALS AND METHODS

Subjects

The study design has been reported previously (3). In brief, we enrolled 40 healthy men, aged 18–50 years, with normal serum gonadotropins, serum T concentrations, and normal results on seminal fluid analyses. Men were excluded if they were in poor general health or had abnormal blood test results, active skin conditions that would prevent the use of T gel, active alcohol or drug abuse, history of testicular or scrotal surgery, infertility, chronic pain syndrome, use of steroids, T, or medications that might affect androgen metabolism, including ketoconazole, glucocorticoids, known bleeding disorder, or if they were using anticoagulant medications such as aspirin or warfarin. Thirty-seven men completed all study procedures. A blood sample for the assessment of serum hormones and proteins and a unilateral testicular fine-needle aspiration were performed on day 1 (2, 34). We then administered acyline (NeoMPS), a GnRH antagonist, 300 μg/kg SC to all subjects. On the same day, subjects then received their first dose of hCG (Pregnyl; Organon) or 1% T gel (Androgel; Solvay) based on treatment group randomization (groups 1–5): placebo hCG (normal saline), 15 IU hCG SC, 60 IU hCG SC, 125 IU hCG SC, or 1% T gel 7.5 g daily (resulting in 75 mg absorbed daily). Administration of hCG/placebo continued every other day for five doses in groups 1–4. Group 5 applied the T gel to the skin daily for 10 days. A blood sample was collected on day 7 to assess for effective suppression of gonadotropins as indicated by a serum LH concentration $<$1.2 IU/L. On day 10 subjects underwent a second testicular fine-needle