Intratesticular Testosterone Concentrations Comparable With Serum Levels Are Not Sufficient to Maintain Normal Sperm Production in Men Receiving a Hormonal Contraceptive Regimen

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ABSTRACT: Intratesticular testosterone (ITT) is thought to play a key role in the control of spermatogenesis in man but is rarely measured. The purposes of this study were 1) to examine the relationship between intratesticular fluid and serum testosterone (T) at baseline and during treatment with a contraceptive regimen known to suppress spermatogenesis and 2) to measure intratesticular fluid androgenic bioactivity. Seven men received 6 months of T enanthate (TE) 100 mg weekly intramuscularly plus levonorgestrel (LNG) 62.5 or 31.25 μg orally daily. Testicular fluid was obtained by percutaneous aspiration at baseline and during month 6. Mean luteinizing hormone (LH) was suppressed 98% from 3.79 ± 0.80 IU/L at baseline to 0.08 ± 0.03 IU/L. Mean follicle stimulating hormone (FSH) was suppressed 97%, from 3.29 ± 0.67 IU/L to 0.10 ± 0.03 IU/L. Mean serum T levels were similar before (22.8 ± 1.9 nmol/L) and during treatment (28.7 ± 2.0 nmol/L) (P = .12). ITT (822 ± 136 nmol/L) was ~40× higher than serum T (P < .001) at baseline. ITT was suppressed 98% during treatment

to 13.1 ± 4.5 nmol/L, a level similar to baseline serum T (P = .08) but significantly lower than on-treatment serum T (P = .01). At baseline, intratesticular fluid androgenic bioactivity (583 ± 145 nmol/L) was 70% of the ITT concentration measured by radioimmunoassay. Intratesticular androgenic bioactivity was suppressed 93% to 40 ± 22 nmol/L (P < .01) during treatment, but was 3× higher than ITT (13.1 ± 4.5 nmol/L). Sperm counts declined from 65 ± 15 million/mL to 1.3 ± 1.3 million/mL. In summary, TE plus LNG dramatically suppressed ITT (98%) and intratesticular androgenic bioactivity (93%) to levels approximating those in serum. ITT levels comparable with serum T were insufficient to support normal spermatogenesis. Intratesticular androgenic bioactivity was higher than ITT during treatment, suggesting that other androgens may be prevalent in the low-ITT environment.

Key words: Intratesticular androgens, spermatogenesis, gonadotropins, progestogens.

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The hormonal control of spermatogenesis is based on the action of the pituitary gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH), on the testis. LH stimulates the Leydig cells in the testes to produce testosterone (T). Intratesticular T (ITT) mediates its effects within the testes through the androgen

receptor that is found on Leydig cells, Sertoli cells, and peritubular cells (McLachlan et al, 2002a). FSH activity is also important for quantitatively normal spermatogenesis (Matsumoto and Bremner, 1985; Matsumoto, 1990). Withdrawal of LH and FSH stimulation of the testis reduces sperm production. Suppression of LH levels results in a decrease in ITT (Morse et al, 1973; Huhtaniemi et al, 1987), which in turn suppresses sperm production in men (World Health Organization [WHO], 1990) and rats (Zirkin et al, 1989). ITT is believed to stimulate spermatogenesis directly in rats (Zirkin et al, 1989) and men (Matsumoto and Bremner, 1985) although not to quantitatively normal levels in men. The quantitative relationship between ITT and spermatogenesis in men has not been studied.

Studies in rats have shown that the ITT concentration is much higher, approximately 30-fold, than serum T

(Turner et al, 1984). Additionally, exogenous T administration has been shown to restore spermatogenesis in rats with gonadotropin suppression (Zirkin et al, 1989; Awoniyi et al, 1990, 1992). In the rat, reduction of ITT by approximately two thirds does not affect spermatogenesis quantitatively, but below this threshold, there is a direct relationship between ITT concentration and sperm number (Zirkin et al, 1989). The lower limit of ITT concentration necessary for quantitatively normal spermatogenesis is still 10-fold higher than normal serum T in the rat (Zirkin et al, 1989).

Studies of testicular biopsy specimens in men have also found a steep testicular-to-serum T gradient, with ITT levels approximately 100-fold higher than serum levels (Heller et al, 1969; Morse et al, 1973; McLachlan et al, 2002c). Withdrawal of LH and FSH is known to result in decreased sperm production, and restoration of LH-like activity with human chorionic gonadotropin (hCG) partially restores spermatogenesis. Azoospermic or severely oligospermic normal men with gonadotropin suppression induced by treatment with exogenous T show partial recovery of spermatogenesis in response to high-dose hCG treatment with sperm counts in the 20–40 million/mL range (Matsumoto and Bremner, 1985). While manipulation of T levels through hCG administration has been shown to correlate with shifts in quantitative sperm production, the levels of ITT produced and the dose-response relationship of ITT to human spermatogenesis are unknown.

Lack of knowledge of the hormonal milieu inside the testes needed to support spermatogenesis stems from the difficulty in evaluating the intratesticular microenvironment. It is particularly difficult to repeatedly sample the intratesticular environment over time within individuals during hormonal manipulation. Repeat surgical testicular biopsies are problematic. Alternative procedures include percutaneous biopsy of small amounts of testicular tissue or aspiration of testicular fluid. Blind percutaneous fine-needle aspiration of fluid yields a mixture of interstitial and seminiferous tubule fluid as demonstrated by microscopic examination to confirm the presence of sperm (Jarow et al, 2001). Testicular fluid aspiration allows for repeated measurements of intratesticular hormones with a minimally invasive procedure that is safe and effective.

The reduction of sperm production induced by gonadotropin suppression is the basis for hormonal strategies for male contraception. One effective model of gonadotropin suppression and impaired spermatogenesis has been the administration of exogenous T in quantities that have been shown to reduce sperm production to azoospermic levels in approximately 70% of men (Matsumoto, 1990; WHO, 1990, 1996). The reason for failure to achieve uniform azoospermia is unknown. There were no demonstrable differences in serum T, LH, or FSH between those men who did achieve azoospermia with exogenous T and those who did not (Amory et al, 2001). However, the addition of an exogenous progestogen to T has been shown to result in a greater proportion of men achieving azoospermia than with T alone (Bebb et al, 1996; Meriggiola et al, 1996; Meriggiola and Bremner, 1997; Meriggiola et al, 1997, 1998; Anawalt et al, 1999; Wu et al, 1999; McLachlan et al, 2002c). Progestogens potentially improve rates of azoospermia through greater suppression of FSH, LH, and therefore ITT, as well as through a possible direct effect within the testis (Meriggiola and Bremner, 1997; Amory and Bremner, 2000). However, despite improved rates of azoospermia with the addition of a progestin, nonuniform suppression of spermatogenesis remains a problem. Another possibility for failure to uniformly achieve azoospermia may be differences in the resulting ITT concentration and/or androgenic bioactivity within the testes in men on hormonal contraceptive regimens. Greater insight into the intratesticular hormonal environment necessary and sufficient for quantitatively and qualitatively normal spermatogenesis in men may increase our ability to develop a safe, effective, reversible hormonal contraceptive for men as well as to treat some types of infertility.

This study was done with the aim of expanding our knowledge of the intratesticular hormonal microenvironment relevant to spermatogenesis. We hypothesized that intratesticular testosterone (ITT) and androgenic bioactivity are suppressed by hormonal contraceptive regimens. In order to reduce interindividual variability and bias, we chose to examine the intratesticular microenvironment by measuring T and androgenic bioactivity in repeated testicular fluid samples obtained by aspiration after hormonal manipulation with a hormonal regimen known to suppress spermatogenesis. The objectives of this study were to 1) examine the relationship between serum and intratesticular T in men treated with a hormonal contraceptive regimen and 2) determine intratesticular androgenic bioactivity in relation to ITT in men before and after treatment with a hormonal contraceptive regimen.

Materials and Methods

Subjects

This study was part of a larger study examining testosterone and levonorgestrel as a male hormonal contraceptive (Bradley Anawalt, unpublished data). Healthy men between the ages of 18 and 45 years with normal reproductive physiology were recruited for the contraceptive trial. Normal reproductive physiology was defined as normal physical exam, including testicular exam with Prader orchidometer, serum testosterone, LH, FSH, and 3 normal seminal fluid analyses on specimens collected 2 weeks apart after 48 hours of ejaculatory abstinence (sperm count >20 million/mL and normal motility). Subjects had to be free from significant

disease as determined by medical history, physical examination, and clinical laboratory test results within normal limits. Exclusion criteria included chronic medical or mental illness, previous vasectomy or unilateral testis, previous or current ethanol abuse, anabolic steroid use, and participation in a contraceptive study within the past 6 months. A subset of these men were concurrently recruited to participate in this study examining intratesticular androgen levels before and after being treated with the hormonal contraceptive regimen consisting of testosterone and levonorgestrel. Ten healthy male subjects with normal reproductive function as defined above underwent percutaneous fine-needle aspiration of testicular fluid at baseline. They were then randomized to receive a hormonal contraceptive regimen consisting of intramuscular testosterone enanthate (TE), 100 mg intramuscularly every week (Delatestryl, Bristol-Myers Squibb, Princeton, NJ), and oral levonorgestrel (LNG), 31.25 or 62.5 µg per day (Wyeth-Ayerst Laboratories, Philadelphia, Pa) for 6 months. All subjects gave informed consent prior to study participation. The Human Subjects Review Committees at the University of Washington and Johns Hopkins University approved this study protocol prior to initiation of the study.

Study Design

Ten men underwent baseline percutaneous testicular fluid aspiration and were randomized to 1 of 2 treatment groups: TE, 100 mg intramuscular weekly, plus oral LNG 31.25 or 62.5 µg daily for 6 months. Weekly intramuscular TE injections were given by a study investigator or study nurse. Participants documented self-administration of daily oral LNG with a medication log to monitor compliance during the study. During the final (sixth) month of treatment with TE plus LNG, they underwent a second aspiration of testicular fluid. Seven of the original 10 subjects had sufficient testicular fluid samples at baseline and month 6 for testicular fluid analysis. There were no differences in baseline serum T, LH, FSH, sperm count, age for the original 10 men, and the 7 who had testicular fluid at both time points. Results are reported for the 7 participants for whom we had sufficient intratesticular fluid T and androgenic bioactivity data. Blood samples were drawn at baseline and then monthly. Serum was stored in a freezer at −70°C prior to pooled analysis at the end of the study. Semen samples were collected at baseline and then every 2 weeks. Semen samples were collected at least 2 days after ejaculatory abstinence for measurement of total sperm count and sperm concentration. After completion of the 6-month treatment phase, the participants entered a recovery phase, during which semen samples were collected every 2 weeks. Completion of the recovery phase was defined as 2 consecutive sperm concentrations within the range of their baseline sperm concentrations. All subjects completed the study.

Testicular Fluid Aspiration

Testicular fluid was aspirated percutaneously by previously described methods (Jarow et al, 2001). Samples were obtained at baseline and month 6 of treatment with TE plus LNG. Subjects were placed in the supine position and draped with sterile cloth. The skin over the sperm cord was cleansed with alcohol on both sides. A spermatic cord block was then performed bilaterally with 1% buffered lidocaine. After adequate anesthesia was es-

tablished, the skin overlying the anterior-superior portion of the testes was then cleansed with alcohol. A 19-gauge butterfly needle with tubing was attached to a 5-mL syringe via a 3-way stopcock and inserted percutaneously into the superior anterior portion of the testicle. Negative pressure was created in the syringe with the 3-way stopcock. The needle was held in place and gentle pressure applied to the testis until an adequate amount of testicular fluid was withdrawn into the tubing. The tubing was then clamped with a hemostat and the needle withdrawn to eliminate reflux of tunica fluid. Bleeding was minimal and superficial at the skin surface of the testis without contamination of the testicular fluid sample. The tubing with testicular fluid sample was immediately placed on ice. Testicular fluid samples were withdrawn from the butterfly tubing and centrifuged. Both supernatant and pellet were stored at −70°C. Right and left testicular fluid samples were pooled for ITT measurement. Percutaneous testicular fluid aspirations were performed at baseline and month 6 of treatment. There were no complications from the percutaneous testicular fluid aspiration procedure in this study. Percutaneous fine-needle aspiration of testicular fluid has proven to be an effective, safe, and well-tolerated procedure.

Measurements

Semen samples were assessed for volume and then analyzed for total sperm count, sperm concentration and motility at screening with sperm count and concentration at follow-up. Sperm counts were determined by a Coulter counter (Coulter Electronics, Inc, Hialeah, Fla). Sperm counts less than 20 million/mL were confirmed through direct determination with a hemocytometer (Gordon et al, 1965; Bremner et al, 1981). Sperm motility was assessed according to the World Health Organization (WHO) laboratory manual for semen analysis (WHO, 1992). Azoospermia was defined as a sperm count of zero. Oligo-azoospermia was defined as a sperm count of less than 3 million/mL.

Serum T, LH, and FSH were measured by immunoflourometric assay (Delfia, Wallace, Inc, Turku, Finland). Samples were run in duplicate. All samples from a single individual were run in the same assay to reduce interassay variability. The T assay sensitivity was 0.5 nmol/L with intra- and interassay coefficients of variation of 4.4% and 7.3% for a midrange pooled value of 11.4 nmol/L. The sensitivity of the LH assay was 0.019 IU/L and the intra- and interassay coefficients of variation for a midrange pooled value of 1.2 IU/L were 3.2% and 12.5%, respectively. The sensitivity of the FSH assay was 0.016 IU/L with intra- and interassay coefficients of variation of 2.9% and 6.1% for a midrange pooled value of 0.96 IU/L. LNG levels were determined by radioimmunoassay (RIA) at the California Regional Primate Center (courtesy of Dr Lisa Laughlin, University of California, Davis) (Ahsan et al, 1988).

An RIA procedure was used to determine testosterone concentration in duplicate samples of serum and testicular fluid at baseline and month 6 (Chen et al, 1994; Jarow et al, 2001). The assay sensitivity was 10 pg/tube. The intra-assay coefficient of variation was 11.2% and the interassay coefficient of variation was 9.6%.

The mammalian 2-hybrid method (Raivio et al, 2001) was used to assay bioavailable androgen concentration in testicular fluids obtained by percutaneous aspiration. The assay utilizes

COS-7 cells transfected with plasmids containing the N-terminus of the androgen receptor (AR) fused to the VP16 activation domain, the ligand binding domain of AR fused to the Gal4 DNA-binding domain, the AR coactivator ARIP3, and a firefly luciferase reporter under the regulation of 5 Gal4 binding sites (pG5-LUC). In brief, binding of androgen promotes the interaction of the AR ligand-binding domain fusion protein to the AR N-terminal domain fusion protein. The AR ligand-binding domain-GAL4 DNA-binding domain fusion protein binds to 1 or more of the GAL4-binding sites, which activates the luciferase reporter gene. The sensitivity of the assay is enhanced by the coexpression of the AR coactivator, ARIP3, which binds to and stabilizes the AR N- and C-terminal protein interaction. A constitutively active Renilla luciferase plasmid is used to control for transfection efficiency. The results are quantified based on the ratio of firefly : Renilla luciferase activity. Based on a standard curve of relative luciferase activity to known testosterone concentration, the biologically active androgen in each test sample was determined from the relative luciferase activity. The results were linear with dilution, implying the absence of interfering substances within serum or human testicular fluid. Androgenic bioactivity is expressed in nanomoles per liter. The intra-assay coefficient of variation was 10%, the interassay coefficient of variation, 23%. The assay sensitivity was 0.069 nmol/L. There was no cross-reactivity with estradiol or progesterone, but LNG was found to have androgenic bioactivity (up to 60% cross-reactivity, depending on LNG concentration). Dihydrotestosterone (DHT) and high concentrations of androstenedione register androgenic bioactivity but dehydroepiandrosterone (DHEA) does not. DHT is a potent androgen with 0.78 nmol/L yielding luciferase activity equal to 10 nmol/L of T while 100 nmol/L of androstenedione yielded luciferase activity equal to 1.3 nmol/L of T (Raivio et al, 2001).

Statistical Analysis

All results are presented as mean ± SEM. The lower limit of assay detection was entered into the database when sample hormone concentrations were undetectable for the purposes of statistical analysis. Comparisons of serum and intratesticular hormones as well as androgenic bioactivity before and after hormonal therapy were done with paired *t* tests. Comparisons of serum and intratesticular testosterone concentrations were done with 2 sample *t* tests. Comparisons between LNG dose groups were done with the nonparametric Mann-Whitney ***U*** test. Alpha was set at the .05 level for the purpose of determining statistical significance. Stata 6.0 was used for the statistical analysis (StataCorp LP, College Station, Tex).

Results

Study Population

Mean age was 36 ± 2 years with the range 25 to 42 years. There were no statistical differences in baseline or month 6 age, sperm count, serum T, FSH, LH, intratesticular T, or intratesticular fluid androgenic bioactivity between the group receiving 62.5 μg and the group receiving 31.25 μg of LNG. Therefore, results are reported for the group

Characteristics and biochemical parameters of 7 men at baseline and month 6; values are mean ± SEM*

	Baseline, n = 7†	Month 6, n = 7
Age	36 ± 2	
Sperm Count (millions/ml)	65 ± 15	1.3 ± 1.3‡
Serum T (nmol/L)	22.8 ± 2	28.7 ± 2.0
Intratesticular T (nmol/L)	822 ± 136	13.1 ± 4.5‡
Intratesticular Androgenic Bioactivity (nmol/L)	583 ± 145	40 ± 22‡
Serum LH (IU/L)	3.79 ± 0.80	0.08 ± 0.03‡
Serum FSH (IU/L)	3.29 ± 0.67	0.10 ± 0.04‡

* T indicates testosterone; LH, luteinizing hormone; and FSH, follicle stimulating hormone.
† Three of the initial 10 subjects had insufficient testicular fluid for analyses.
‡ Statistically significantly different from baseline value (α = .05).

of 7 as a whole, irrespective of LNG dose group (the Table).

Serum Hormones

Baseline serum T was 22.8 ± 2.0 nmol/L. Trough (pre-injection) serum T after treatment with TE plus LNG was within the normal range (8–32 nmol/L) at 28.7 ± 2.0 nmol/L. The slight increase in serum T after 6 months treatment with TE 100 mg weekly plus LNG was not statistically different from baseline. LH was suppressed by 98% from 3.79 ± 0.80 IU/L at baseline to 0.08 ± 0.03 IU/L at month 6. FSH was suppressed by 97% from 3.29 ± 0.67 IU/L at baseline to 0.10 ± 0.03 IU/L at month 6 of therapy with TE plus LNG (the Table).

The mean serum LNG 1 hour after the daily oral dose at month 5 was 0.27 ± 0.07 nmol/L for the 3 men taking 31.25 μg LNG daily and 0.79 ± 0.13 nmol/L for the 4 men taking 62.5 μg LNG daily. The overall mean serum LNG level for all 7 men was 0.57 ± 0.13 nmol/L at month 6.

Intratesticular Testosterone

ITT was measured in the original 10 men at baseline and in 7 at month 6. The mean ITT concentration for the 10 men at baseline was 793 ± 96 nmol/L, approximately 40-fold higher than the serum T concentration, 20.0 ± 2.3 nmol/L. The mean baseline ITT for the 7 of the 10 men who had sufficient fluid volume for analysis at both time points was 822 ± 136 nmol/L. The mean ITT decreased 98% to 13.1 ± 4.5 nmol/L during month 6 of TE plus LNG (Figure 1).

Androgenic Bioactivity

Baseline intratesticular androgenic bioactivity was 583 ± 145 nmol/L, approximately 70% of the ITT concentration (822 ± 136 nmol/L). After 6 months of treatment with TE plus LNG, intratesticular bioactivity was suppressed
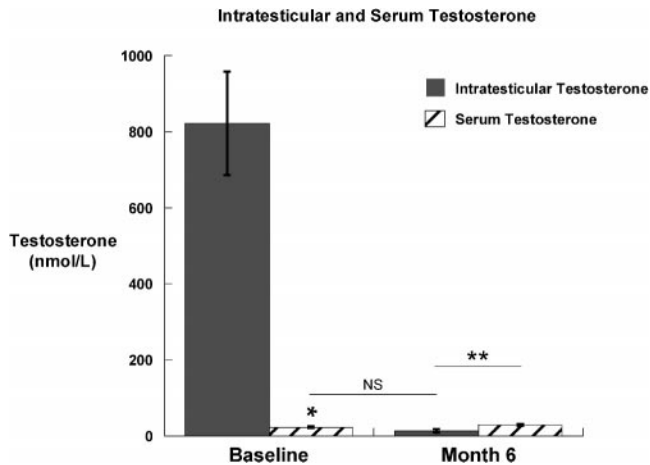
Figure 1. Intratesticular fluid testosterone (ITT, solid black bars) and serum testosterone (striped bars) at baseline and after 6 months of treatment with testosterone enanthate plus levonorgestrel (n = 7). Values are mean ± SEM. * indicates ITT is significantly different from serum T at baseline ($P < .001$). ITT was significantly suppressed from baseline to month 6 ($P < .001$). There was no significant difference between serum T at baseline and month 6 ($P = .12$); **, ITT was significantly lower than serum T at month 6 ($P = .01$), but was not significantly different from baseline serum T ($P = .08$); NS, not significant.

Figure 2. Intratesticular fluid **(A)** testosterone and **(B)** androgenic bioactivity at baseline (solid black bars) and month 6 of treatment (striped bars) with testosterone enanthate plus levonorgestrel (n = 7). Values are mean + SEM. * indicates both intratesticular T ($P < .001$) and androgenic bioactivity ($P < .01$) were significantly suppressed from baseline at month 6.

93% from 583 ± 145 nmol/L to 40 ± 22 nmol/L, but was threefold higher than the month-6 ITT concentration (13.1 ± 4.5 nmol/L) (Figure 2).

Spermatogenesis

The mean baseline sperm count was 65 ± 15 million/mL. Mean sperm count was suppressed by 98% to 1.3 ± 1.3 million/mL in month 6. Six of the 7 men achieved a sperm count of 0 at month 6 of the treatment phase. One participant's sperm count declined to only 9 million/mL at month 6 of treatment followed by a nadir sperm count of 3.4 million/mL after 6 weeks in the recovery phase. This subject, who failed to suppress to azoospermia, received 62.5 μg of LNG daily plus TE. He was one of the older subjects at 40 years. Although this individual's baseline gonadotropins were below the overall group mean (LH = 2.7 IU/L and FSH 1.1 IU/L vs mean LH = 3.79 IU/L and mean FSH = 3.29 IU/L), he had both the highest baseline serum T (31.4 nmol/L) and ITT (1607 nmol/L). His month-6 FSH was 0.08 IU/L, similar to the group mean FSH of 0.10 IU/L. His month-6 LH was approximately twice the group mean (LH = 0.17 IU/L compared with group mean LH = 0.08 IU/L). He also had one of the highest ITT levels, 29 nmol/L, while receiving TE plus LNG. One other participant who did become azoospermic had a similar ITT of 30 nmol/L. All men returned to their baseline sperm concentrations in the recovery phase within 4.5 months.
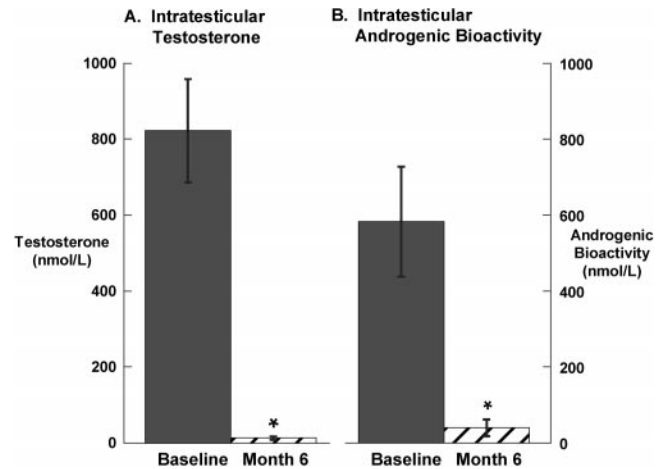
Discussion

Treatment for 6 months with exogenous T plus low-dose oral LNG resulted in a dramatic decrease in ITT by 98% to levels that approximate normal serum T (Figure 1) in young men as well as a 93% decrease in androgenic bioactivity (Figure 2). The baseline intratesticular fluid T (822 ± 136 nmol/L) was lower than the T concentration measured in testicular homogenates of controls (2231 ± 403 nmol/L) in a previous contraceptive trial using exogenous T and a different progestogen, depot-medroxyprogesterone (McLachlan et al, 2002c). However, both contraceptive regimens suppressed ITT approximately 98%: T and LNG suppressed ITT to 1.6% of baseline (13 ± 4.5 nmol/L) after 6 months in this study, while T and DMPA suppressed ITT to 2.2% of baseline at 2 weeks in the McLachlan study (McLachlan et al, 2002c). The higher T found in testicular homogenate may be due to release of intracellular T from crushed Leydig cells, while testicular fluid represents T released by Leydig cells. Our results show that percutaneous fine-needle aspiration of testicular fluid is a safe and reliable alternative method to testicular biopsy for sampling the intratesticular environment. Additionally, for the first time, we have shown that repeated percutaneous testicular aspiration can be accomplished in normal men. This facilitates a reduction in the inter-individual variation inherent in the cross-sectional design of studies using testicular biopsy by allowing for repeated sampling in the same individual over time.

The presence of T in the intratesticular environment is necessary for normal spermatogenesis in men (McLachlan et al, 2002b). The recognition of the high ITT concentrations relative to serum T concentrations has led

to the speculation that intratesticular T concentrations must be relatively high in order to support quantitatively and qualitatively normal spermatogenesis in men. Treatment for 6 months with TE plus LNG suppressed ITT by 98% to levels that approximate normal serum T in normal young men (Figure 1). This degree of ITT suppression is similar to that seen in previous studies of gonadotropin withdrawal either through exogenous T administration (Morse et al, 1973) or through gonadotropin releasing hormone agonist treatment in prostate cancer patients (Huhtaniemi et al, 1987). Intratesticular T concentrations approximating normal serum T levels were coincident, with a dramatic decrease in sperm count from a mean of 65 to 1.3 million/mL. In fact, 6 of the 7 men achieved azoospermia on this contraceptive regimen. Only one individual was a nonresponder, with a sperm count of 9 million/mL at month 6. These results suggest that the presence of a testis-to-serum T gradient, with ITT concentrations higher than those in serum, is necessary for normal spermatogenesis in men. However, while levels of T comparable with those in the serum within testicular fluid are insufficient to support normal spermatogenesis, the actual threshold T concentration necessary for normal sperm production in men remains unknown as does the dose-response relationship between ITT and spermatogenesis.

This study confirms a large testis-to-serum testosterone gradient in normal young men as previously observed in cross-sectional studies of testicular biopsy tissue (Morse et al, 1973; McLachlan et al, 2002c) and a previous study of aspirated testicular fluid (Jarow et al, 2001). T levels were approximately 40-fold higher in the testes than in the serum in healthy men with normal reproductive physiology. It is unknown whether this very large gradient has physiological purpose or only reflects the reservoir of T produced inside the testes by Leydig cells. A similar testis-to-serum T gradient has been observed in rats (Turner et al, 1984; Zirkin et al, 1989).

Androgenic bioactivity was 70% of the intratesticular T concentration measured by RIA at baseline. Protein-bound serum T is not measured in the androgenic bioassay (Raivio et al, 2001). Intratesticular T is partially bound to binding proteins such as sex hormone-binding globulin (SHBG)/androgen binding protein (ABP) although in very small (<1%) amounts (Jarow et al, 2001). Therefore, lower androgenic bioactivity in testicular fluid than the actual measurable T concentration is likely partially due to protein binding to SHBG/ABP but this is not the likely explanation for the 30% difference between androgenic bioactivity and ITT. The reduced androgenic bioactivity may also be due to binding to other unidentified proteins within the testes or the presence of currently unknown substances that interfered with the bioassay but not the immunoassay.

From a contraceptive development perspective, it is interesting to note that azoospermia was not uniformly achieved despite gonadotropin suppression and dramatically reduced ITT. The reason some men fail to suppress spermatogenesis with gonadotropin withdrawal is poorly understood. One possibility is that the low ITT environment may trigger compensatory mechanisms within the testes that preserve spermatogenesis and fertility. One possible compensation may be an increase in 5α-reductase activity within the testes resulting in relatively higher intratesticular DHT despite severe gonadotropin suppression (McLachlan et al, 2002c). Even after suppression of ITT by 98% and reduction of androgenic bioactivity by 93%, androgenic bioactivity in testicular fluid was threefold higher than the ITT concentration. The relatively higher androgenic bioactivity may relate to a relative increase in intratesticular androgens other than T, such as DHT. McLachlan did a comparison of intratesticular androgens in biopsy specimens from men treated with TE alone vs TE plus depot medroxyprogesterone acetate (DMPA) (McLachlan et al, 2002c). They reported a 98% reduction of ITT in men treated with TE plus DMPA after 12 weeks of therapy but found that intratesticular DHT as well as androstanediol levels were preserved at pretreatment levels. DHT is a more potent androgen than T and even a small increase in DHT concentration within the testes may help preserve low levels of spermatogenesis.

LNG also registered androgenic bioactivity and may partially account for the elevation in intratesticular androgenic bioactivity after 6 months of treatment of TE plus LNG. However, testicular fluid LNG levels were not measured in this study. The concentration of LNG within the testes after prolonged therapy is unknown. The mean serum LNG level at month 6 was 0.57 ± 0.13 nmol/L which is 2 orders of magnitude lower than the androgenic bioactivity (40 ± 22 nmol/L) that registered in testicular fluid at month 6. Therefore, it is difficult to conclude that the higher androgenic bioactivity in testicular fluid relative to the intratesticular T concentration after 6 months of therapy is due to passive diffusion of LNG from the blood into the testes even with some sequestration by binding proteins. Binding proteins are present in small amounts in aspirated testicular fluid and may represent SHBG and/or ABP in men (Jarow et al, 2001). However, the presence of SHBG correlates with lower androgenic bioactivity in testicular fluid (Raivio et al, 2001). Thus, the threefold higher androgenic bioactivity relative to testosterone concentration observed after 6 months of treatment is not likely due predominantly to the presence of free or protein-bound LNG in testicular fluid.

FSH also plays an important role in controlling spermatogenesis in men through its action on the Sertoli cell, although its exact role is unclear (Bremner et al, 1981;

Matsumoto et al, 1986; McLachlan et al, 1988, 2002a,b). Both FSH and T are critical for development of normal spermatogenesis during puberty. FSH is used in addition to hCG to stimulate spermatogenesis in the treatment of hypogonadotropic hypogonadal men and has been shown experimentally to restore quantitatively normal sperm production in men with induced gonadotropin suppression (Matsumoto and Bremner, 1985; Matsumoto et al, 1986). It is possible that low but detectable levels of FSH may allow for the persistence of low-level sperm production in men. In particular, FSH withdrawal appears to cause a failure of early spermatogonial development and spermiation (McLachlan et al, 2002a,b). Hormonal contraceptive regimens profoundly suppress FSH levels but FSH is generally still detectable (Meriggiola et al, 1996; McLachlan et al, 2002c). Persistent low-level FSH activity may maintain persistent spermatogenesis in the low-T intratesticular environment.

The characteristics of the nonresponder in this study may also point to other potential important areas of hormonal control of spermatogenesis. This individual suppressed his sperm count from 55.3 million/mL at baseline to 9 million/mL during month 6 of treatment. His sperm production continued to decrease to a nadir of 3.4 million/mL after 6 weeks in the recovery phase, suggesting that he may have become azoospermic with longer suppression. He was treated with the higher dose of LNG (62.5 μg), although this is a relatively low dose of LNG compared with those used in previous male contraceptive studies (Anawalt et al, 1999). While all men suppressed gonadotropin levels to less than 0.5 IU/L, the 1 man who failed to suppress to azoospermia had the lowest basal and highest treatment gonadotropin levels of the 7 men studied. He also had the highest baseline ITT (1607 nmol/L) and had one of the highest on-treatment ITT levels (29 nmol/L), although his percent decrease was similar to the other men at 98%. One other individual in the 62.5-μg group had a similar on-treatment ITT of 30 nmol/L but did suppress to azoospermia, suggesting that either the failure of the nonresponder to suppress to azoospermia may not be related to ITT or that there is not an absolute ITT threshold for spermatogenesis but variation in individual ITT concentrations necessary for normal sperm production.

In summary, this is the first report of ITT and androgenic bioactivity measured in intratesticular fluid in normal men receiving an experimental hormonal contraceptive regimen. Intratesticular fluid T is much higher than serum T levels in normal young men. Intratesticular androgenic bioactivity is approximately 70% of ITT. Treatment with testosterone (TE) plus a progestogen (LNG) profoundly suppressed ITT (98%) as well as androgenic bioactivity (93%). Suppression of ITT to levels comparable with those in serum was associated with near-complete suppression of spermatogenesis with 6 out of 7 subjects (86%) achieving azoospermia. Androgens other than T may be important in the low-ITT environment because intratesticular androgenic bioactivity was higher than ITT levels measured by immunoassay.

In conclusion, percutaneous aspiration of testicular fluid is an effective method for repeated sampling of the intratesticular environment and measurement of intratesticular steroid hormones. This technique is clearly useful for future experiments designed to assess the concentration of substances in intratesticular fluid in men. It may also prove useful in clinical diagnosis as well. ITT suppressed by 98% with a contraceptive regimen to levels approximating serum T are insufficient for normal spermatogenesis. Further investigation of intratesticular hormones will be needed to define the testicular hormonal environment needed to achieve azoospermia in male contraceptive regimens. Future studies will include the characterization of the dose-response relationship of intratesticular testosterone and spermatogenesis as well as the effects of FSH coadministration on the relationship between intratesticular androgens and spermatogenesis.

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