# Dose-Dependent Increase in Intratesticular Testosterone by Very Low-Dose Human Chorionic Gonadotropin in Normal Men with Experimental Gonadotropin Deficiency

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**Context and Objective:** In men with infertility secondary to gonadotropin deficiency, treatment with relatively high dosages of human chorionic gonadotropin (hCG) stimulates intratesticular testosterone (IT-T) biosynthesis and spermatogenesis. Previously we found that lower dosages of hCG stimulated IT-T to normal. However, the minimal dose of hCG needed to stimulate IT-T and the dose-response relationship between very low doses of hCG and IT-T and serum testosterone in normal men is unknown.

**Design, Setting, Patients, and Intervention:** We induced experimental gonadotropin deficiency in 37 normal men with the GnRH antagonist acyline and randomized them to receive one of four low doses of hCG: 0, 15, 60, or 125 IU sc every other day or 7.5 g daily testosterone gel for 10 d. Testicular fluid was obtained by percutaneous aspiration for steroid measurements at baseline and after 10 d of treatment and correlated with contemporaneous serum hormone measurements.

**Results:** Median (25th, 75th percentile) baseline IT-T was 2508 nmol/liter (1753, 3502 nmol/liter). IT-T concentrations increased in a dose-dependent manner with very low-dosage hCG administration from 77 nmol/liter (40, 122 nmol/liter) to 923 nmol/liter (894, 1017 nmol/liter) in the 0- and 125-IU groups, respectively ($P < 0.001$). Moreover, serum hCG was significantly correlated with both IT-T and serum testosterone ($P < 0.01$).

**Conclusion:** Doses of hCG far lower than those used clinically increase IT-T concentrations in a dose-dependent manner in normal men with experimental gonadotropin deficiency. Assessment of IT-T provides a valuable tool to investigate the hormonal regulation of spermatogenesis in man. (***J Clin Endocrinol Metab*** **95: 3806–3813, 2010)**

Intratesticular testosterone (IT-T) is essential for spermatogenesis. In men with infertility secondary to hypogonadotrophic hypogonadism, injections of human chorionic gonadotropin (hCG), which mimics the activity of LH, stimulates the testicular biosynthesis of testosterone. Treatment with hCG (often in combination with injections of FSH) leads to spermatogenesis and fertility in approximately two thirds of men (1). In rodents, 75% reductions in IT-T are still compatible with normal spermatogenesis; however, sperm production falls off sharply below this threshold (2–4). However, the minimum concentration of IT-T necessary for spermatogenesis in man is unknown. This may be relevant in male hormonal contraceptive development because spermatogenesis is not

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Abbreviations: DHT, Dihydrotestosterone; hCG, human chorionic gonadotropin; IT-DHT, intratesticular dihydrotestosterone; IT-T, intratesticular testosterone.

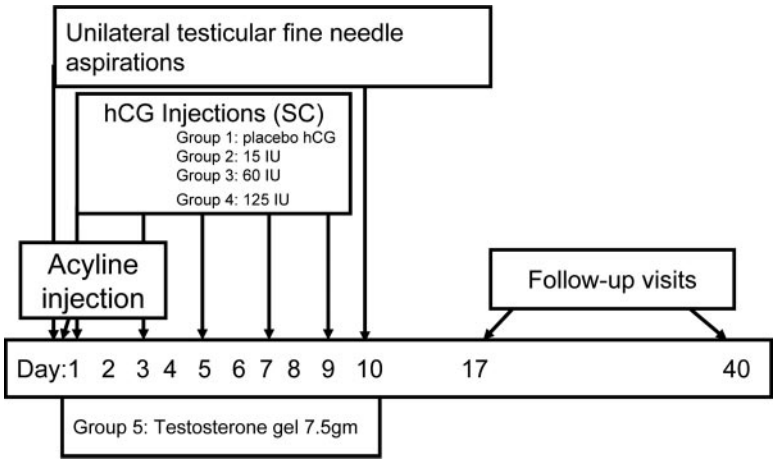


**FIG. 1.** Study design.

consistently suppressed in some men, despite marked suppression of gonadotropins. In these men, persistently elevated IT-T concentrations may allow for ongoing spermatogenesis despite gonadotropin suppression (5–8). A better understanding of the relationship between low concentrations of IT-T and spermatogenesis would be useful to optimize the treatment of male infertility and would inform efforts to develop a male hormonal contraceptive.

Understanding the intratesticular steroid environment in man is challenging. Until recently methods for measuring intratesticular hormone concentrations in men required testicular biopsy (9–11); therefore, prior studies were performed mainly in infertile men requiring testicular biopsy and general anesthesia for the evaluation and treatment of their condition. More recently the technique of fine-needle tissue aspiration has been used to obtain intratesticular fluid in normal men (5, 12–14). This technique can be safely performed in the outpatient setting using local anesthesia without serious adverse effects. We previously used this technique to examine the dose-response relationship between hCG as a proxy for LH and IT-T in normal men. However, although the doses of hCG in our previous work were lower than those used to treat patients with hypogonadotropic hypogonadism, IT-T concentrations were similar to those in untreated normal men (15). In addition, our previous work relied on exogenous testosterone to suppress the hypothalamic-pituitary-gonadal axis, and there was concern that the exogenous testosterone could potentially increase IT-T concentrations. Therefore, in this study, we experimentally induced low levels of IT-T in normal men using the GnRH antagonist, acyline, and subsequently stimulated testicular testosterone biosynthesis with very low doses of hCG, lower than we used previously. In addition, we included a group of men treated with exogenous testosterone to determine whether treatment with testosterone would affect intratesticular steroid concentrations. In this way, we sought to ascertain the dose-response relationship between very low doses of LH-like stimulation and IT-T in man.

## Subjects and Methods

### Subjects

Healthy men, aged 18–50 yr, were recruited for this study using rosters from prior research studies and newspaper and online advertisements. Informed consent was obtained from all subjects before the screening evaluation. Subjects had to have a normal history and physical examination (body mass index 19–32 kg/m$^2$), including a normal andrological history, normal testicular volume as measured by a Prader orchidometer, a normal prostate examination, normal serum gonadotropins and testosterone levels, and normal seminal fluid analysis based on the 1999 World Health Organization criteria with sperm concentration greater than 20 million/ml, greater than 50% motility, and greater than 15% normal morphology (16). Exclusion criteria included poor general health; abnormal blood test results; active skin conditions that would prevent the use of testosterone gel; active alcohol or drug abuse; history of testicular or scrotal surgery; infertility; chronic pain syndrome; use of steroids, testosterone, or medications that might affect androgen metabolism including ketoconazole, glucocorticoids; known bleeding disorder; or use of medications that may affect bleeding time (such as ongoing aspirin or warfarin use). All subjects had to agree to use a reliable form of contraception during the study.

The study design is illustrated in Fig. 1. Briefly, after enrollment, subjects were randomized to one of five treatment groups and also randomized to the side of the unilateral testicular fine-needle aspirations (right *vs.* left on d 1 *vs.* d 10) by two random number sequences. Previous studies have shown a very high correlation between testes in a given man (12, 13). All subjects had a baseline testicular fine-needle aspiration on d 1. Local anesthesia was provided using 1% buffered lidocaine injected into the spermatic cord. A blood sample was obtained for quantification of serum hormones immediately after lidocaine administration and within 2–10 min of the aspiration. For the testicular aspiration, a 19-gauge needle was used as previously described (12, 13, 15). After the procedure, all subjects received a sc injection of the GnRH antagonist acyline (NeoMPS, San Diego, CA) 300 $\mu$g/kg into the abdominal skin. Subjects then received the first dose of medication based on treatment group randomization: group 1 received placebo hCG (normal saline) sc every other day for five doses, group 2 received 15 IU hCG (Pregnyl; Organon, Roseland, NJ) sc every other day for five doses, group 3 received 60 IU hCG sc every other day for five doses, group 4 received 125 IU hCG sc every other day for five doses, and group 5 received the 1% testosterone gel, Androgel (Solvay, Marietta, GA) 7.5 g daily for 10 d. hCG administration was performed by study personnel at scheduled visits.

On d 10, all subjects returned to the clinic for vital signs assessment, documentation of adverse events, and concomitant medications and a physical examination. All subjects then had a testicular

fine-needle aspiration of the testis not aspirated on d 1, with contemporaneous serum hormone assessment after lidocaine administration. Subjects returned on d 17 and 40 for a physical examination, blood draw, and seminal fluid analysis to ensure that their physical examinations, hormone, and other laboratory measurements and seminal fluid parameters had returned to normal. Subjects whose blood or semen parameters had not returned to normal returned monthly until all values had normalized. The Institutional Review Board of the University of Washington approved this study protocol before study initiation. In addition, this trial was registered in advance (www.clinicaltrials.gov, National Clinical Trials no. 00839319).

## Measurements

Testicular fluid samples were immediately placed on ice and then centrifuged at 300 × *g*, and the supernatant fluid was stored at −70 C. All testicular fluid and serum samples were assayed simultaneously for IT-T and intratesticular dihydrotestosterone (IT-DHT), by liquid chromatography-tandem mass spectrometry on a Waters Aquity UPLC coupled with a Micromass Premiere-XE tandem quadrupole mass spectrometer (Waters Corp., Milford, MA) using a modification of our previously described method (12, 17). The intra- and interassay coefficients of variation were 3.5 and 7.7% for testosterone and 3.5 and 6.3% for dihydrotestosterone (DHT). The assay sensitivities for IT-T and IT-DHT were less than 0.1 pmol/liter.

Serum LH and FSH were quantified by immunofluorometric assay (6). The sensitivity of the LH assay was 0.019 IU/liter and the intra- and interassay coefficients of variation for a midrange pooled value of 1.2 IU/liter was 3.2 and 12.5%, respectively. The sensitivity of the FSH assay was 0.016 IU/liter and the intra- and interassay coefficients of variation were 2.9 and 6.1% for a midrange pooled value of 0.96 IU/liter. Serum hCG was measured by immunofluorometric assay (Delfia; Wallac, Inc., Turku, Finland). The hCG assay used in this study is specific for the intact heterodimer and was calibrated against the 4th International Standard for Chorionic Gonadotropin (75/589) (18). The intra- and interassay coefficients of variation for hCG were 3.4 and 3.7%, respectively, and the lower limit of detection was less than 1 IU/liter. Serum 17-hydroxyprogesterone was measured by RIA (Siemens Healthcare Diagnostics, Deerfield, IL) with an intra- and interassay coefficient of variation of 5.6 and 6.4%, respectively, and a lower limit of detection of less than 0.3 nmol/liter. All samples for all subjects were batched and measured in one assay.

## Statistical analysis

Due to nonnormality, the data were expressed as medians and 25th and 75th percentiles. Analysis of both baseline and end-of-treatment hormone concentrations was performed on the 31 subjects who completed all study procedures and who suppressed serum LH below the lower limit of the normal range by the end of treatment. Six subjects had serum LH values above 1.2 IU/liter on d 7. These subjects were therefore excluded from analysis because their IT-T was affected by normal concentrations of LH. Due to nonnormality, comparisons of hormone concentrations between groups were performed in a nonparametric fashion using Kruskal-Wallis ANOVA with a Wilcoxon rank-sum *post hoc* test. Correlations between serum hormone levels and intratesticular hormones, and between intratesticular hormones were performed on the 23 subjects in the four groups

**TABLE 1.** Median baseline characteristics, serum, and intratesticular hormones of 31 participants by treatment group (25th, 75th interquartile range)

| | 0 IU hCG (n = 6) | 15 IU hCG (n = 7) | 60 IU hCG (n = 5) | 125 IU hCG (n = 5) | Testosterone gel (n = 8) | All subjects (n = 31) |
|---|---|---|---|---|---|---|
| Age (yr) | 21 (20, 26) | 25 (20, 29) | 22 (20, 24) | 22 (21, 26) | 22 (20, 24) | 22 (20, 26) |
| BMI (kg/m$^2$) | 24.8 (23.6, 26.3) | 24.1 (23.2, 26.7) | 24.9 (21.2, 26.3) | 25.8 (22.9, 26) | 23.7 (21.1, 25.4) | 24.1 (22.9, 26.3) |
| Serum hormones | | | | | | |
| LH (IU/liter) | 3.5 (3.1, 4.8) | 3.0 (2.6, 4.9) | 3.4 (3.4, 4.9) | 2.9 (2.3, 3.7) | 3.8 (2.6, 4.6) | 3.4 (2.6, 4.9) |
| FSH (IU/liter) | 2.7 (1.2, 3.4) | 2.4 (2, 2.8) | 2.6 (2.2, 3.2) | 2.2 (1.9, 2.5) | 1.9 (1.3, 2.8) | 2.4 (1.5, 3.0) |
| Testosterone (nmol/liter) | 13.0 (11.3, 16.9) | 15.0 (11.4, 20.9) | 14.2 (12.7, 14.9) | 16.8 (14.4, 18.6) | 15.0 (13.7, 18.3) | 14.6 (12, 17.3) |
| DHT (nmol/liter) | 1.0 (0.7, 1.5) | 1.3 (0.9, 1.7) | 1.2 (1, 1.3) | 0.9 (0.7, 1.5) | 1.1 (1.1, 1.2) | 1.1 (0.9, 1.4) |
| Estradiol (pmol/liter) | 89 (47, 150) | 65 (34, 125) | 62 (48, 78) | 77 (44, 95) | 86 (54, 95) | 77 (46, 104) |
| 17-Hydroxyprogesterone (nmol/liter) | 4.7 (3.8, 7.8) | 4.9 (4.2,6.5) | 5.9 (5.1, 7.6) | 4.3 (4, 4.5) | 4.6 (3.7, 5.4) | 4.9 (3.9, 6.5) |
| Intratesticular hormones | | | | | | |
| Testosterone (nmol/liter) | 3467 (2508, 3839) | 2425 (1700, 3380) | 1821 (1753, 2412) | 3502 (2305, 3959) | 2933 (1527, 3390) | 2508 (1753, 3502) |
| DHT (nmol/liter) | 18.0 (11.9, 24.5) | 5.0 (4.5, 11) | 7.6 (7.1, 21.3) | 18.8 (11.1, 22.2) | 12.9 (7.9, 15.5) | 11.9 (7.3, 21.3) |

BMI, Body mass index.

receiving hCG using the Spearman technique. No corrections were made for multiple comparisons. All statistical analyses were performed using STATA version 10.0 (College Station, TX). For all comparisons, an alpha of less than 0.05 was considered significant.

## Results

### Subjects

Sixty-one men were screened for the study and 40 met all inclusion criteria. Thirty-seven subjects completed all study procedures. Of the three subjects who withdrew from the study, one subject withdrew consent after randomization but before undergoing any procedures on d 1, another subject did not return for subsequent visits after the d 1 visit, and a third subject was dismissed from the study by the investigator after having a syncopal reaction due to the lidocaine injection on d 1. This subject had no further complications on subsequent follow-up.

Of the 21 men who failed to meet the screening criteria for the study, 10 subjects had abnormal seminal fluid analyses, four subjects had clinically evident varicoceles on physical examination, two subjects exceeded the body mass index criteria, two subjects developed medical problems before study initiation, two subjects did not complete the screening procedures, and one subject had repeatedly low serum total testosterone. Of the enrolled subjects, 10 subjects had abnormal seminal fluid, thirty-two were Caucasian, three were African-American, three were Asian Pacific Islander, and two were of Asian descent.

There were no serious adverse events during the study. Seventeen subjects reported 22 nonserious adverse events. Eight subjects had bruising at the site of the lidocaine injection, three had upper respiratory infections, two had hot flashes (one requiring a rescue dose of testosterone after the d 10 visit), and two subjects had itching from the acyline injection requiring treatment with oral diphenhydramine. Median testicular aspirate volume was 10 $\mu$l both at baseline and after treatment ($P > 0.2$).

### Serum and intratesticular hormones

Baseline subjects' characteristics, serum, and intratesticular hormones are reported in Table 1. There were no statistically significant differences in any of the measurements between the treatment groups for baseline measurements. At baseline, median IT-T was 170 times higher than serum testosterone, whereas IT-DHT was 11 times higher than serum DHT. Of note, IT-T did not correlate with IT-DHT at baseline. In addition, IT-T and IT-DHT did not significantly correlate with serum LH, FSH, testosterone, DHT, estradiol, or 17-hydroxyprogesterone. Baseline intratesticular hormones did not correlate with age, race, or body mass index.

After 10 d of treatment, median serum LH decreased from 3.4 (2.6, 4.9) IU/liter to 0.19 (0.1, 0.5) IU/liter, and median serum FSH decreased from 2.4 (1.5, 3.0) IU/liter to 0.27 (0.2, 0.4) IU/liter ($P < 0.0001$ for both comparisons). In addition, all treatment groups had a statistically significant decrease in IT-T ($P < 0.05$) from baseline. There was no significant correlation between posttreatment serum LH and IT-T (r = 0.01, $P = 0.97$). Intratesticular hormones showed a strong dose-response relationship to hCG. Subjects who received acyline plus 0 IU hCG,

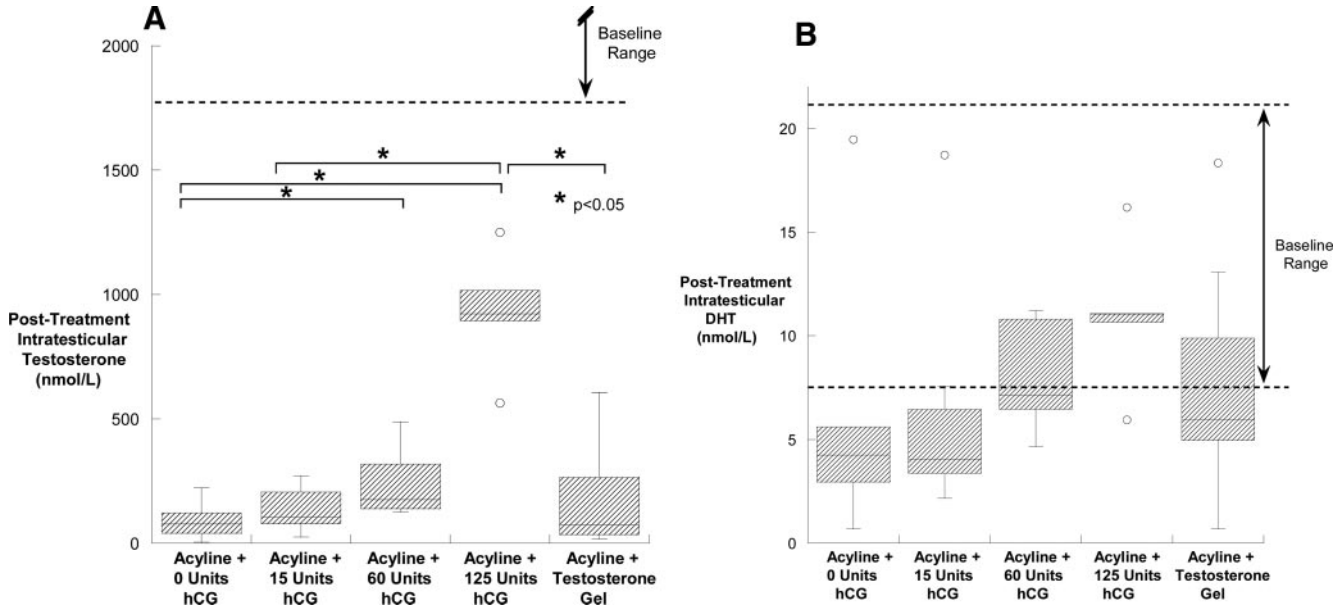


**FIG. 2.** A and B, Box plots of IT-T (A) and IT-DHT (B) in gonadotropin-suppressed subjects on d 10 by treatment group (n = 6 for the 0 IU hCG, n = 7 for the 15 IU hCG group, n = 5 for the 60 IU hCG group, and 125 IU hCG group and n = 8 for the testosterone group). *Dotted lines* represent the upper and lower limits of the baseline range.

had a median IT-T of 77 nmol/liter (40, 223 nmol/liter), which was statistically indistinguishable from those in the 15 IU hCG group [median IT-T 136 nmol/liter (79, 258 nmol/liter)] and from subjects in the testosterone gel group [median IT-T 73 nmol/liter (34, 264 nmol/liter)]. Subjects in the 60 IU hCG group had a median IT-T of 319 nmol/liter (139, 2455 nmol/liter) that was significantly greater than that of the placebo and testosterone gel groups ($P < 0.05$). Subjects in the 125 IU hCG group had the highest IT-T with a median of 987 nmol/liter (895, 1250 nmol/), which was significantly greater than that in the placebo, the 15 IU hCG group ($P < 0.05$ for both comparisons), and the testosterone gel group ($P < 0.01$) (Fig. 2A).

In general, IT-DHT was lower after treatment compared with baseline, but only in the 0 IU hCG group, the 125 IU hCG group, and the testosterone gel groups was this reduction statistically significant. There were no significant differences in IT-DHT between the treatment groups at the end of treatment (Fig. 2B).

After 10 d of treatment, in the 23 subjects receiving hCG, serum testosterone correlated highly with IT-T and serum DHT correlated highly with IT-DHT (Fig. 3, A and B). Interestingly, at the end of treatment, four subjects had normal serum testosterone concentrations with IT-T concentrations that were significantly reduced from baseline (Fig. 3A). Moreover, both serum testosterone and IT-T correlated strongly with posttreatment serum hCG concentration (Fig. 4, A and B).

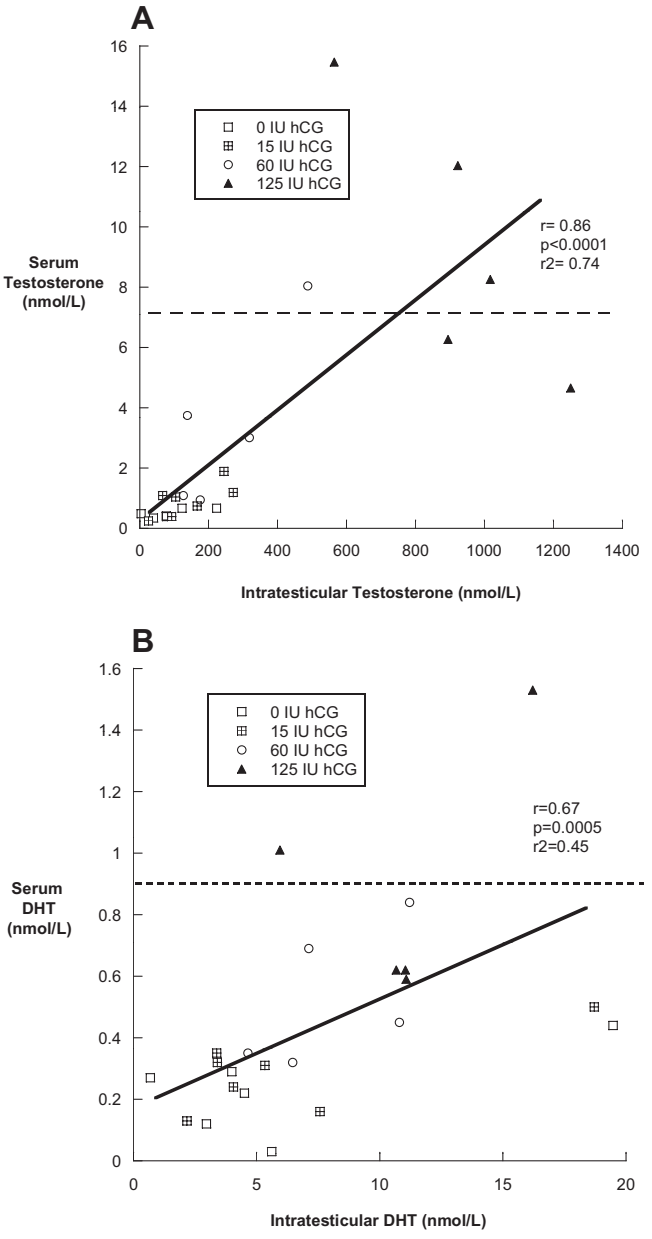


**FIG. 3.** A and B, Correlations between posttreatment serum and intratesticular testosterone (A) and DHT (B) for all subjects receiving hCG (n = 23). The *dotted line* represents the lower limit of the normal range for testosterone and serum DHT.

## Discussion

In the study, we used testicular aspiration, coupled with gonadotropin suppression, and graded, low doses of hCG to determine the dose-response relationship between intratesticular androgens and hCG in man. This study is the first to examine the relationship of such low doses of hCG with intratesticular androgens and to correlate the concentrations of intratesticular androgens with contemporaneously measured serum hormones. Interestingly, we have shown that IT-T concentrations remain much higher than serum testosterone concentrations despite marked LH suppression. Furthermore, we have demonstrated that very low level LH-like stimulation of the testes with hCG increases IT-T in a dose-dependent manner. Importantly, our results suggest that the threshold dose for stimulating IT-T in humans is likely to lie between 15 and 60 IU of hCG. The measurement of IT-T, coupled with sensitive and specific liquid chromatography-tandem mass spectrometry hormone measurements, and longer-term low-dose gonadotropin administration in this experimental gonadotropin-deficient human model will permit more detailed investigation of the hormonal regulation of spermatogenesis in man than previously possible.

Normal men appear to be more sensitive to hCG than infertile men with hypogonadotropic hypogonadism. This difference in sensitivity is likely due to the fact that steroidogenesis in men with long-term gonadotropin deficiency is impaired, possibly secondary to Leydig cell immaturity. A similar phenomenon has been observed in the *hpg* mouse, in which larger doses of gonadotropins are required to initiate spermatogenesis than to maintain it once established (19). Our previous work in this area, which used doses of hCG closer to those used in hypogo-

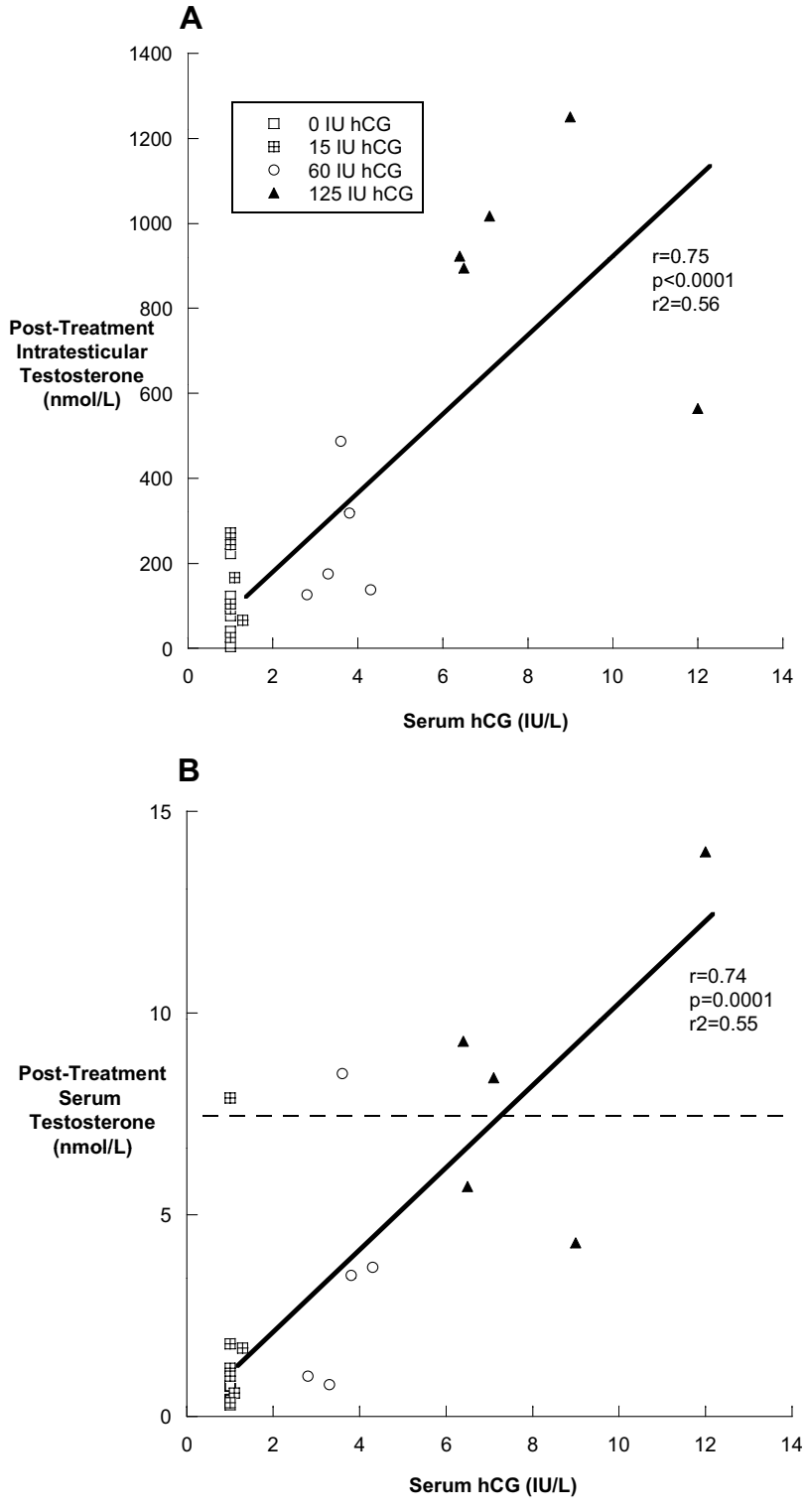


**FIG. 4.** A and B, Correlations between posttreatment serum hCG and IT-T (A) and serum testosterone (B) for all subjects receiving hCG (n = 23). The *dotted line* represents the lower limit of the normal range for serum testosterone.

nadotrophic infertile men, resulted in IT-T concentrations that were not significantly lower than normal (15). Therefore, in this study we chose very low doses of hCG to better understand the full dose-response relationship. Notably, in this study we found that having a normal serum testosterone while receiving hCG does not correspond to an IT-T concentration similar to those observed at baseline. The implications of this for the induction of spermatogenesis in men with hypogonadotropic hypogonadism are unknown. However, it is possible that the observation that serum hCG is highly correlated with IT-T may prove useful in the treatment of men with infertility from gonadotropin deficiency. As a result, clinicians may consider measuring both serum testosterone and serum hCG to ensure the adequacy of treatment; however, future studies of the relationship between serum hCG and IT-T in men with hypogonadotropic hypogonadism will be required to determine the utility of this measurement.

Prior studies have shown that serum 17-hydroxyprogesterone, which is secreted by the testes in high concentrations, is a good correlate of IT-T (20, 21). However, in our study 17-hydroxyprogesterone was not significantly correlated with IT-T. This difference is likely due to the significantly lower IT-T concentrations induced in this study by the lower doses of hCG used. Our study confirmed prior work demonstrating the relatively low concentrations of DHT in the testes (12). Whereas the concentrations of testosterone were greater than 100-fold higher in the testes than in the serum, the DHT concentrations were only 11-fold higher in the testes compared with the serum. Apparently the majority of DHT is produced from conversion by 5$\alpha$-reductase in peripheral tissues rather than by conversion within the testes. Whether the relatively low concentrations of IT-DHT may play a role in supporting spermatogenesis in some settings is unknown. More likely, the relatively significant persisting concentrations of IT-T observed in the absence of LH bioactivity play a role in maintaining spermatogenesis. If this is the case, hormonal contraceptive approaches based on gonadotropin suppression may not be able to fully suppress spermatogenesis.

Strengths of this study include the quantitation of intratesticular and serum androgen concentrations using mass spectrometry. Some prior studies using RIAs to measure IT-T may have underestimated the true concentration of IT-T, and some lacked the sensitivity to detect IT-DHT (7, 8, 12–15). One weakness of the study was the number of subjects who did not suppress their LH secretion below the normal range after the acyline injection. When comparing these subjects with subjects who responded to acyline, we saw no baseline differences in age, body mass index, or baseline hormones to account for this differential response to acyline. Prior studies have not shown a similar 15% rate of failure to suppress with acyline (22). Because of this unanticipated failure of acyline, the number of subjects in each group was less than we had planned. Nevertheless, the dose-response relationship was still clearly evident from the data. An additional weakness of our study is that despite the knowledge that serum hormone concentrations fluctuate with circadian rhythms (23), aspirations were not performed at a standardized interval after identified LH pulses, which may have increased the variance of intratesticular androgen measurements. Future studies exploring the temporal relationship between LH pulsatility and intratesticular androgens will be necessary to better understand this feature of intratesticular androgen biosynthesis.

The knowledge gained from this study will be useful in future studies aimed at determining the relationship between IT-T and other androgens and spermatogenesis in man. It is noteworthy that the testosterone concentrations in the testes in the absence of LH or hCG were still 4–5 times higher than normal serum testosterone concentrations. Such IT-T concentrations are still high enough to support spermatogenesis in some men, *i.e.* in studies of male hormonal contraception (5, 6). Future studies designed to determine the necessary threshold for spermatogenesis in man will have to further suppress IT-T concentrations by attempting to block both gonadotropin-mediated and constitutive intratesticular testosterone production. Addition of testosterone biosynthesis inhibitors, such as ketoconazole, may be required to further lower IT-T concentrations.

In conclusion, this study demonstrates the strong dose-response relationship between IT-T and very low-dose hCG administration in gonadotropin-suppressed men. This work provides crucial information for future studies determining the role of intratesticular androgens on spermatogenesis in man and may improve the treatment of men with infertility and inform efforts to develop male hormonal contraceptives.

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