Serum 17-hydroxyprogesterone strongly correlates with intratesticular testosterone in gonadotropin-suppressed normal men receiving various dosages of human chorionic gonadotropin

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Objective: To determine if serum concentrations of testosterone precursors would correlate with intratesticular testosterone (ITT) concentration measured directly by testicular aspiration and allow for a less invasive means of inferring ITT.
Design: Controlled clinical study.
Setting: Healthy volunteers in an academic research environment.
Patient(s): Twenty-nine normal men.
Intervention(s): We determined ITT concentration by testicular aspiration before and after treatment in men receiving exogenous T to block endogenous gonadotropin production and randomly assigned to one of four doses of hCG (0, 125 IU, 250 IU, or 500 IU every other day) for 3 weeks.
Main Outcome Measure(s): The association between serum 17-hydroxyprogesterone (17OH-P), androstenedione, and DHEA and ITT.
Result(s): With T administration alone, serum 17OH-P decreased significantly and increased significantly when 500 IU hCG was administered. End-of-treatment ITT strongly correlated with serum 17OH-P. Moreover, serum 17OH-P, but not androstenedione or DHEA, was independently associated with end-of-treatment ITT by multivariate linear regression.
Conclusion(s): Serum 17OH-P is highly correlated with ITT in gonadotropin-suppressed normal men receiving T and stimulated with hCG. Serum 17OH-P is a surrogate biomarker of ITT and may be useful in research and in men receiving gonadotropin therapy for infertility. (Fertil Steril® 2008;89:380–6. ©2008 by American Society for Reproductive Medicine.)

Key Words: Intratesticular testosterone, 17-hydroxyprogesterone, male infertility, male contraception

Testosterone is essential for the initiation and maintainence of spermatogenesis within the testes (1–4). Testosterone is synthesized and secreted from the Leydig cells of the testicular interstitium when stimulated by LH (5). Intratesticular testosterone (ITT) mediates its effects by binding to the androgen receptor, which is found in Leydig cells, Sertoli cells, and peritubular cells but is not present in developing germ cells (6). Intratesticular testosterone concentrations in normal men are approximately 100 times those in serum (7–11). Experimental suppression of LH secretion from the pituitary by the administration of exogenous T leads to pronounced decreases in ITT and dramatic reductions in sperm production (7, 9, 10, 12). In contrast, the administration of the LH-receptor agonist hCG increases ITT and stimulates spermatogenesis, both in men with hypogonadotropic hypogonadism from pituitary disease (13) and in men with experimentally induced gonadotropin deficiency (14).

Despite the central role of ITT in male reproduction, the quantitative relationship between LH signal, ITT and spermatogenesis is unknown. This deficit in knowledge stems largely from the difficulty in evaluating the intratesticular milieu over time within an individual during hormonal manipulation. Current options for measuring ITT include repeat surgical testicular biopsies, which can lead to testicular injury (15), and testicular aspirations, which can be painful and have the potential to cause bleeding or other complications.

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Therefore, the identification of a serum surrogate or biomarker of ITT would be of great value, because it might allow for serial monitoring of ITT during hormonal manipulation without the need for more invasive procedures. Such a surrogate marker could be useful clinically in conjunction with measurements of serum T to determine the optimal dosage of hCG for gonadotropin-deficient men being treated for infertility. In addition, a serum marker of ITT could be useful in monitoring men on treatments aiming to suppress endogenous T biosynthesis, such as therapy for hormone-responsive prostate cancer or experimental hormonal male contraceptives.

Previous work investigating the response of the testis to hCG has demonstrated that serum concentrations of T precursors such as 17-hydroxyprogesterone (17OH-P), androstenedione (A), and DHEA are increased by hCG administration (16). Therefore, we hypothesized that serum concentrations of these T precursors might correlate with ITT and potentially provide a serum biomarker of ITT and Leydig cell function.

Therefore, we measured serum 17OH-P, A, and DHEA in a group of normal men receiving testosterone enanthate (TE) to suppress endogenous gonadotropins and various doses of hCG. We correlated ITT with serum measurements of these T precursor hormones to ascertain the relationship between them and ITT during various degrees of Leydig cell stimulation with hCG.

MATERIALS AND METHODS

Subjects

The Institutional Review Board at the University of Washington approved all procedures involving human subjects. All subjects were participants in a previously published study to determine the dose-response of ITT to hCG stimulation conducted at the University of Washington (10). Complete information on study design and results can be found in the original publication (10). In brief, 29 normal healthy men between 18 and 45 years of age (mean 24 ± 6.5 years) were recruited for the study. Subjects were healthy, as determined by medical history and physical examination, and had normal clinical laboratory tests, normal testicular volume, normal serum T, LH, and FSH, and sperm concentration of greater than 20 million/mL after 48 hours of ejaculatory abstinence. Exclusion criteria included chronic medical or mental illness, previous or current ethanol abuse, anabolic steroid use, abnormal screening labs, single testicle, abnormal reproductive physiology, infertility, or a family history of congenital adrenal hyperplasia.

Study Procedures

All subjects were treated with 200 mg IM TE on days 0, 7, and 14 to suppress endogenous gonadotropin secretion from the pituitary. After their enrollment, the hospital pharmacist randomly assigned subjects to one of four hCG treatment groups: hCG 0 (saline placebo), 125, 250, or 500 IU, administered SC every other day for 3 weeks (11 total doses). The first dose of hCG was administered immediately after the first percutaneous fine needle aspirate of testicular fluid on day 0, and the last dose of hCG was administered on day 20 of the study. Study personnel administered all injections. On day 21, a second percutaneous testicular fine needle aspiration was performed.

Blood was drawn immediately before the aspirations at baseline and on day 21. Serum was separated by centrifugation and stored at −70°C until assayed for hormone concentrations after the completion of the study.

Testicular Fluid Aspiration

Testicular fluid was sampled by fine needle aspiration at baseline and after 3 weeks of treatment with TE plus placebo or hCG (10). With the subject lying on an examination table in the supine position, the skin over the spermatic cord at the external ring was cleansed with alcohol. Then, a bilateral spermatic cord block was performed with 10 mL 1% buffered lidocaine. The skin over the anterior-superior aspect of the testes was then cleansed with alcohol, and a 19-gauge butterfly needle was inserted into the testicular parenchyma. Negative pressure was generated with an attached syringe until testicular fluid was obtained. The testicular fluid sample was immediately placed on ice and centrifuged at 300*g*. The aspirate supernatant was stored at −70°C. Fluid from the right and left testes was pooled for the measurement of T.

Measurements

Serum 17OH-P was measured by immunofluorometric assay (Diagnostic Products, Los Angeles, CA). The sensitivity of the assay was 0.6 nmol/L. The intra- and interassay coefficients of variation were, respectively, 6.7% and 11% for low pools, and 3.5% and 8.5% for high pools. The normal range in men aged 20–59 years is 1.8–10.3 nmol/L. Serum A was measured by RIA (Diagnostic Products). The sensitivity of the assay was 0.45 nmol/L. The intra- and interassay coefficients of variation were, respectively, 5.6% and 9.8% for low pools, and 2.8% and 9.0% for high pools. The normal range in men aged 20–59 years is 1.0–9.2 nmol/L. Serum DHEA was measured by RIA (Diagnostic Products). The sensitivity of the assay was 0.7 nmol/L. The intra- and interassay coefficients of variation were, respectively, 5.6% and 10.2% for low pools, and 7.3% and 7.0% for high pools. The normal range in men aged 20–59 years is 4.9–43.3 nmol/L. The cross-reactivities of these assays with related hormonal compounds is less than 1%. The measurement of T in testicular aspirates has been described in detail previously (9–12). The sensitivity of the intratesticular fluid T RIA was 10 pg/tube with inter- and intra-assay coefficients of variation of 11.2% and 9.6%, respectively.

Statistical Analyses

Because of non-normal distributions within groups, hormone concentrations are expressed as median with 25th and 75th

percentiles included in parentheses. Change in serum hormone concentrations within a group between baseline and day 21 was compared with a Wilcoxon signed rank test. Serum hormone concentrations between dose groups of hCG were compared by Kruskal-Wallis analysis of variance with a Wilcoxon rank sum post hoc test and a Bonferroni correction for multiple comparisons. Correlations between ITT and hormone concentrations were performed using Spearman technique. The association between ITT and hormone concentrations, age, and weight was analyzed by univariate and multivariate linear regression using robust standard errors on log-transformed data and back-transformed for presentation. For all comparisons, a two-sided alpha of $<.05$ was considered to be significant. Statistical analyses were performed using Stata version 8.0 (College Park, TX).

RESULTS

Subjects

All 29 participants completed the study. All T injections were administered on schedule, and 316 of 319 hCG or placebo injections were administered per protocol. Of the three missed injections, one was in a placebo subject and one occurred in two different subjects in the 250 IU hCG group, both on study day 5. The aspiration procedure was well tolerated by all participants without complications or significant discomfort. Unfortunately, in one subject in the 125 IU hCG group and one in the 250 IU hCG group, insufficient testicular fluid was obtained during the end-of-treatment aspiration procedure for measurement of ITT. Therefore, analyses were performed on data from the 27 subjects in whom both baseline and end-of-treatment ITT measurements were available. After 3 weeks of treatment with exogenous TE, the mean serum LH was suppressed from 4.11 ± 0.37 IU/L to 0.21 ± 0.03 IU/L (5% of baseline) on day 21, and the mean serum FSH was suppressed from 2.83 ± 0.37 IU/L to 0.09 ± 0.01 IU/L on day 21 (both $P<.0001$).

Serum Testosterone Precursors

At end-of-treatment, serum 17OH-P was significantly reduced in the 0 hCG group [median (25%, 75%)]: 5.1 (3.8, 6.2) nmol/L at baseline versus 2.1 (1.5, 2.5) nmol/L at end of treatment; $P=.02$. In contrast, serum 17OH-P was significantly increased in the 500 IU hCG group: 4.6 (4.1, 5.4) nmol/L at baseline versus 7.8 (5.5, 9.4) nmol/L at end of treatment; $P=.02$. In addition, serum A was significantly increased in the 250 IU and 500 IU hCG groups ($P=.03$ and $P=.02$, respectively) but not in the 0 or 125 IU hCG groups compared with baseline.

At baseline between hCG dose groups, there were no significant differences in the serum concentrations of 17OH-P, A, or DHEA (Table 1). At the end of treatment, however, serum 17OH-P was significantly elevated in 250 IU and 500 IU hCG groups compared with the 0 and 125 IU groups ($P<.05$ for all comparisons) (Table 1). The dose-dependency between hCG dose groups and serum 17OH-P was highly statistically significant ($P<.001$). In contrast, neither serum A nor serum DHEA differed significantly between dose groups of hCG at the end of treatment.

Intratesticular Testosterone and Testosterone Precursors

At baseline, ITT did not correlate with serum 17OH-P or DHEA and was inversely correlated with A ($r = -0.47$; $P=.01$). In contrast, during treatment with hCG, ITT strongly correlated with 17OH-P ($r = 0.78$; $P<.0001$; Fig. 1A), correlated less strongly with A ($r = 0.52$; $P=.006$) (Fig. 1B), and did not correlate with DHEA ($r = 0.08$; $P=.68$) (Fig. 1C). Similarly, change in ITT from baseline to end of treatment was significantly correlated with change in serum 17OH-P ($r = 0.64$; $P<.001$) (Fig. 2A) but not with change in serum A or DHEA (Fig. 2B and 2C).

Modeling of factors associated with ITT with univariate linear regression revealed a statistically significant association between ITT and end-of-treatment 17OH-P ($\beta = 199$ [95% CI 127–271]; $P<.001$) (Table 2). This association remained significant with adjustment for end-of-treatment values of the other T precursors, serum T, age, and weight ($\beta = 168$ [95% CI 102–235]; $P<.001$). End-of-treatment A was significantly associated with ITT in univariate regression ($\beta = 177$ [95% CI 67–286]; $P=.003$); however, with adjustment for confounders this association was no longer significant ($P<.79$).

DISCUSSION

In the present work, we demonstrated a significant association between serum concentrations of 17OH-P and ITT in men receiving TE and various dosages of hCG. Serum 17OH-P decreased by roughly 60% in men receiving placebo, whereas it increased by 70% in men at the highest dose (500 IU) of hCG. Although 17OH-P did not correlate with ITT at baseline, it was very strongly correlated with ITT on treatment, both when absolute concentration and when change from baseline were taken into account. Moreover, end-of-treatment concentrations of serum 17OH-P were significantly associated with ITT after correction for other variables using linear regression. Although the overall correlation between ITT and serum 17OH-P was strong, in the lowest-dose hCG group the ITT increased much more than the serum 17OH-P. This implies that serum 17OH-P might not be as sensitive to changes in ITT mediated by lower levels of hCG stimulation as it is to the larger increases in ITT stimulated by higher doses of hCG.

The finding that 17OH-P correlates with ITT is the first description of a serum biomarker of ITT during treatment with T plus hCG, and may be of great benefit in clinical research studies aimed at understanding the quantitative relationship between LH signal, ITT, and spermatogenesis. In addition, this observation might have utility in determining the optimal treatment dose of recombinant LH or hCG in men with infertility due to hypogonadotropic hypogonadism, although how much information it could add to measurement of serum T

TABLE 1

Serum and intratesticular hormone concentrations at baseline and after 3 weeks of treatment with one of four doses of hCG. All values are median (25%, 75% quartiles).

	hCG treatment group				
	Group 1: 0 IU (n = 7)	Group 2: 125 IU (n = 7)	Group 3: 250 IU (n = 6)	Group 4: 500 IU (n = 7)	*P* value (trend)
Baseline					
Intratesticular testosterone (nmol/L)	1111 (982, 1630)	1063 (848, 1188)	1239 (1105, 1535)	1227 (918, 1338)	.48
17-hydroxyprogesterone (nmol/L)	5.1 (3.8, 6.2)	5.3 (3.5, 6.3)	4.9 (3.8, 6.8)	4.6 (4.1, 5.4)	.98
Androstenedione (nmol/L)	6.6 (5.6, 7.3)	6.6 (6.1, 9.3)	6.4 (4.8, 6.8)	6.5 (6.0, 8.2)	.73
DHEA (nmol/L)	35 (24, 48)	30 (22, 53)	32 (24, 44)	34 (22, 39)	.99
End of treatment					
Intratesticular testosterone (nmol/L)	58 (57, 110)[a]	780 (409, 1146)[b]	1037 (913, 1291)[b]	1470 (738, 1967)[b]	.001
17-hydroxyprogesterone (nmol/L)	2.1 (1.5, 2.5)[a]	2.1 (1.9, 3.7)	6.3 (3.2, 8.8)[b,c]	7.8 (5.5, 9.4)[a,d]	<.001
Androstenedione (nmol/L)	6.8 (6.2, 7.6)	5.6 (5.2, 9.2)	8.4 (7, 12)[a]	9.5 (8.3, 11)[a]	.10
DHEA (nmol/L)	28 (23, 31)	23 (15, 30)	24 (20, 31)	27 (24, 41)	.44

[a] $P<.05$ compared with baseline.
[b] $P<.01$ compared with group 1.
[c] $P<.05$ compared with group 2.
[d] $P<.01$ compared with groups 1 and 2.

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remains to be determined. Moreover, it might provide a useful marker of testicular function as part of an "hCG stimulation test" for men already being treated with replacement doses of T for hypogonadism. For example, it has recently been shown that measurement of serum E_2 can be useful as a marker of testicular reserve, because serum E_2 concentrations are more quickly and dramatically elevated after a single dose of hCG than those of serum T (17). Finally, measurement of serum 17OH-P in men enrolled in trials of experimental male hormonal contraceptives might provide insight into the relationship between ITT, endogenous T production, and suppression of spermatogenesis. Obviously, because men in these studies are already receiving exogenous T as part of the contraceptive regimen, serum T cannot be used for this purpose (18).

Most of the circulating 17OH-P in men is likely of testicular and not adrenal origin. Orchiectomy reduces circulating levels of 17OH-P by approximately 70% (19, 20), a reduction very similar to that seen in our group of subjects receiving exogenous T and placebo (and therefore having little circulating LH activity). Therefore, roughly 30% of circulating 17OH-P is likely of nontesticular, presumably adrenal, origin. The testicular secretion of 17OH-P is known to be second only to that of T, with T accounting for 70% of steroid output and 17OH-P accounting for 20% (21).

No differences in serum 17OH-P have been observed in the baseline state in large cross-sectional studies of men with infertility (22). However, two studies of men with idiopathic infertility have revealed an increased ratio of 17OH-P to T after stimulation with hCG in infertile men compared with normal control subjects (23). This effect is especially pronounced in men with baseline elevations in serum FSH (24) and may imply a subtle impairment in the activity of the 17,20-lyase or 17-beta-hydroxysteroid dehydrogenase in these patients. An increased ratio of 17OH-P to T has been observed also in a population of men with varicocele-related infertility; however, it is uncertain if the elevated 17OH-P seen in this series was testicular or adrenal in origin, because subjects also had significantly elevated serum concentrations of 11-beta-hydroxyandrostenedione, an exclusively adrenal androgen (25).

The present study is limited in that it involves only normal men recruited and treated at one site. As a result, ethnic,

FIGURE 1

Association between the end-of-treatment intratesticular testosterone and (**A**) 17-hydroxyprogesterone, (**B**) androstenedione, and (**C**) DHEA (n = 27). Note the differences in the x-axes for each plot.

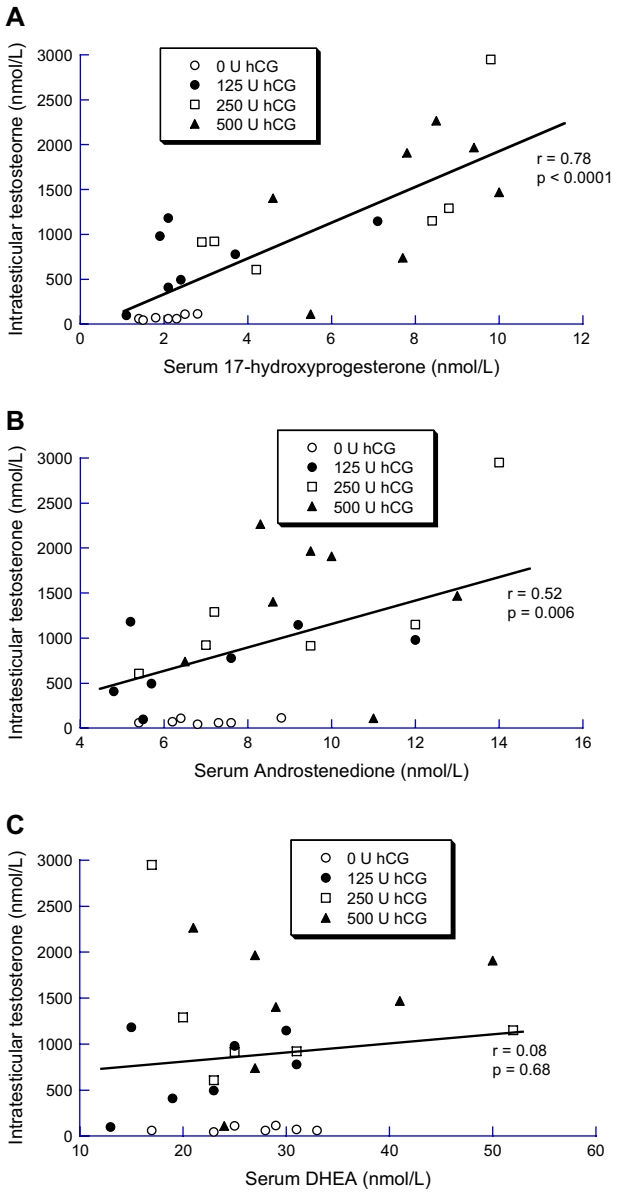

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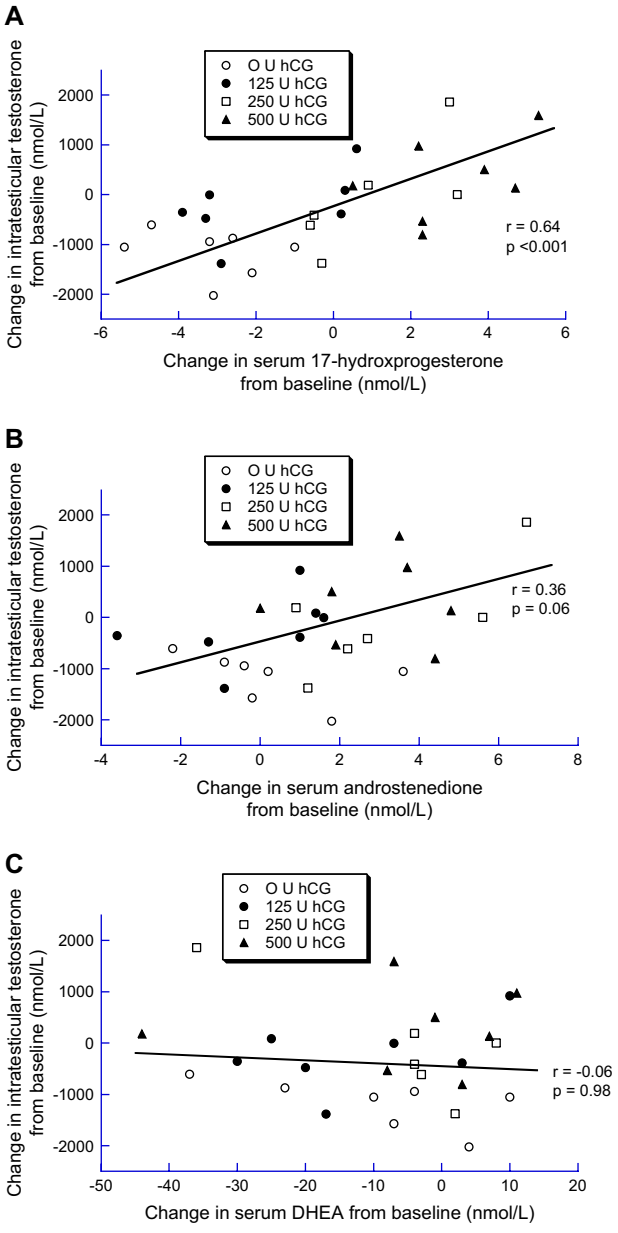
FIGURE 2

Association between the change in intratesticular testosterone and the change in (**A**) 17-hydroxyprogesterone, (**B**) androstenedione, and (**C**) DHEA between baseline and end of treatment (n = 27). Note the differences in the x-axes for each plot.



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nutritional, and geographic differences in the ability to respond to stimulation with hCG were not assessed. In addition, there is a fairly large variance in the measurement of ITT that could bias the conclusions. Moreover, a single measurement after 21 days may not accurately reflect changes in ITT over longer periods, perhaps due to atrophy of Leydig cells, which could be of relevance to the treatment of male infertility. Clearly, larger studies of the relationship between ITT and serum 17OH-P in fertile and infertile men with and without hCG stimulation should be performed to corroborate and extend these findings. In addition, longer-term studies of hCG stimulation will need to be conducted to better understand the relationship between ITT, 17OH-P, and spermatogenesis.

TABLE 2

Simple univariate and multivariate linear regression of end-of-treatment intratesticular testosterone with end-of-treatment serum concentrations of testosterone precursors. Regression was performed using robust standard errors. The multivariate model controlled for age, weight, and the other testosterone precursor concentrations.

	Univariate model			Multivariate model[a]		
Testosterone precursor	β	95% CI	*P* value	β	95% CI	*P* value
17-hydroxyprogesterone	199	(127–271)	<.001	168	(102–235)	<.001
Androstenedione	177	(67–286)	.003	22	(−147–191)	.79
DHEA	12	(−20–44)	.45	−19	(−48–10)	.19

[a] Controlled for age, weight, serum testosterone, and serum concentrations of other two testosterone precursors.

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In conclusion, we demonstrated an association between serum 17OH-P and ITT in the setting of stimulation of the testes with hCG in gonadotropin-suppressed normal men. Because ITT is central to spermatogenesis, this finding may aid future research designed to understand the relationship between ITT and spermatogenesis as well as to optimize the treatment of men requiring injections of hCG or recombinant LH for the treatment of male infertility.

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