

Human Chorionic Gonadotropin and Testicular Function: Stimulation of Testosterone, Testosterone Precursors, and Sperm Production Despite High Estradiol Levels*

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ABSTRACT. Excessive gonadotropin stimulation of the testis induced by the administration of high doses of hCG or LH markedly decreases testicular function in experimental animals. The adverse effects of supraphysiological gonadotropin stimulation are thought to be mediated, in part, by the very high levels of estradiol produced. We administered a supraphysiological dosage of hCG together with exogenous testosterone (T) to normal men for several months. The combination of these agents produced very high serum estradiol (E_2) levels and (we assume) high intratesticular E_2 levels. In this setting of supraphysiological gonadotropin stimulation and high E_2 levels, we examined serum levels of T, the Δ^4 and Δ^5 steroid precursors of T, and sperm production. After a 3-month control period, five normal men received T enanthate (T; 200 mg, im, weekly) for 3-5 months. Then, while T was continued in the same dosage, all subjects were given hCG (5000 IU, im, three times weekly) for an additional 4-6 months. Serum E_2 levels during hCG plus T treatment increased to a mean (\pm SEM) of 158 ± 16 pg/ml.

Despite the very high E_2 levels generated by this prolonged administration of hCG and T, hCG stimulated a mean increase of 5.1 ng/ml in the total T level and 0.18 ng/ml in the free T level over those found during T administration alone. These

increments in T levels approximate normal blood T levels in man. Significant changes in serum levels of Δ^4 steroid precursors of T biosynthesis occurred during the study. Serum progesterone and 17-hydroxyprogesterone levels fell significantly with gonadotropic suppression induced by T administration alone and then increased significantly with hCG stimulation. In contrast to the changes seen in serum levels of Δ^4 precursors, there were no significant changes in levels of Δ^5 steroid precursors of T biosynthesis. An increased ratio of 17-hydroxyprogesterone to T during hCG administration was the only suggestion of an E_2 -induced block in steroid synthesis. hCG also significantly stimulated sperm production, as assessed by sperm concentration, motilities, and morphologies, in spite of the very high serum E_2 levels; the mean sperm concentration increased from 1.0 ± 1.0 million/cc during T administration alone to 46 ± 16 million/cc during hCG plus T treatment. We conclude that chronic administration of supraphysiological dosages of hCG can stimulate testicular function in man, despite very high E_2 levels, and that hCG in these dosages does not lead to severe testicular regression in man. Perhaps a higher dosage of hCG administered to men would replicate the severe testicular suppression reported in experimental animals. (*J Clin Endocrinol Metab* 56: 720, 1983)

hCG or LH, administered in high dosage, has been demonstrated to markedly suppress testicular function in experimental animals (1-7). In the rat, excessive stimulation by hCG or LH has been reported to cause marked loss (down-regulation) of testicular LH/hCG receptors (1, 2), postreceptor blockade of steroidogenesis (3, 5), decreases in testicular and accessory organ weights (4), and suppression of spermatogenesis (5). Studies in

the ram (6) as well as subhuman primates (7) have confirmed many of these adverse effects of high dosages of gonadotropins on testicular function.

The physiological significance of these observations in experimental animals, however, and the clinical relevance of these findings to humans remain unclear. Chronic administration of a high dosage of hCG to normal men (8) and observations in men with hCG-secreting neoplasms (9, 10) have suggested that exposure to supraphysiological levels of hCG may also lead to suppression of spermatogenesis in man.

These adverse effects of high dosages of hCG on testicular function have been thought to be mediated, in part, by the high levels of estradiol (E_2) produced as a result of gonadotropin stimulation. *In vitro* studies using rat and human Leydig cell cultures (11, 12) and *in vivo*

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studies in gonadotropin-replaced rats (13) have demonstrated that E_2 administration leads to a loss of testicular LH/hCG receptors as well as to a direct inhibition of testicular 17,20-desmolase and 17-hydroxylase activity. The same steps in testosterone (T) biosynthesis are blocked by high doses of hCG or LH. These steroidogenic blocks are manifested by relatively large accumulations of C-21 steroid precursors [progesterone (PROG), 17-hydroxyprogesterone (17OH-PROG), pregnenolone (PREG), and 17-hydroxypregnenolone (17OH-PREG)] with gonadotropin stimulation (3, 5, 14, 15). This has led to the suggestion by some investigators that the steroidogenic defect induced by high dosage gonadotropin exposure is a result of end-product inhibition by E_2 (3, 5, 14, 15). It has also been postulated that the inhibitory effects of E_2 on testicular function may be mediated by E_2 -induced elevation of PRL levels (16).

In contrast to these reported suppressive effects of hCG, the stimulatory effects of hCG on testicular function have been the basis for its use in the therapy of various hypogonadotropic states in men. hCG alone, in various dosages (usually between 2000–5000 IU three times weekly), has been shown by several investigators to maintain normal T levels in hypogonadotropic men (17). In addition, hCG treatment alone or in combination with human menopausal gonadotropin (hMG) treatment has been shown to initiate and maintain spermatogenesis in gonadotropin-deficient men (17). There has been very little documentation of what serum E_2 levels are achieved with chronic administration of these therapeutic dosages of hCG to hypogonadotropic men. However, urinary estrogen excretion has been demonstrated to be markedly elevated (18), suggesting that these dosages of hCG may also produce very high serum E_2 levels. Very recently, D'Agata *et al.* (19) reported the steroidogenic responsiveness of eight men with hypogonadotropic hypogonadism treated with hCG (1500 IU three times a week for 23 months). During hCG treatment, these investigators found that T levels rose toward the upper end of the normal range (~ 6 – 9 ng/ml), while E_2 levels rose to levels above the normal range (~ 100 – 160 pg/ml).

In the present study, we determined the effect on testicular function (T and sperm production) of high serum E_2 levels and, presumably, high intratesticular E_2 levels produced by simultaneous administration of hCG and T to normal men. Normal men were treated first with T enanthate (T). Then, while T was continued, a moderately high dose of hCG was administered simultaneously. The dosage of hCG used in this study has been used clinically by some investigators to stimulate normal T levels in hypogonadotropic hypogonadal men (17). Monthly E_2 levels were measured, and the effects of these hormonal treatments on testicular function were determined by monitoring total and free T levels, levels

of steroid precursors of T biosynthesis, and seminal fluid analyses.

Materials and Methods

Experimental subjects

Five normal healthy men, aged 25–40 yr, volunteered to participate in a study designed to investigate the role of FSH in human spermatogenesis. The gonadotropin and seminal fluid data from this study have been reported previously (20). The study protocol was reviewed and approved by the Human Subjects Review Committee of the University of Washington, and informed consent was obtained from each subject.

Subjects were normal, as defined by a normal medical history, physical examination, complete blood count, routine chemistries and urinalysis, and at least six normal seminal fluid analyses obtained over a 3-month period (*i.e.* sperm concentration greater than 20 million/cc, motility greater than 50%, and greater than 60% oval forms). In addition, all subjects had normal basal T, LH, and FSH levels, normal LH and FSH secretory patterns on blood sampling every 20 min for 6 h, and normal LH and FSH responses to a 4-h continuous iv infusion of 50 μ g LHRH (20).

Study protocol

Control period. Each subject underwent a 3-month period of control observation during which no hormones were administered. Hormonal measurements and seminal fluid analyses were performed at regular intervals, as described below.

T alone period. After the control period, each subject received T (Delatestryl, E. R. Squibb and Sons, Princeton, NJ; 200 mg, im, weekly) for a period of 3–5 months to suppress endogenous gonadotropins and testicular function. T was administered until three successive sperm counts were less than 5 million/cc.

hCG plus T period. After the T alone period, while continuing T at the same dosage, hCG (Profasi, Serono Laboratories, Inc., Braintree, MA; 5000 IU, im, three times weekly) was administered simultaneously in all subjects for an additional 4–6 months to replace LH activity. hCG plus T was administered until three successive sperm counts returned to the individual subject's control range or for a maximum of 6 months.

hCG alone period. After the hCG plus T period, one subject continued to receive hCG alone at the same dosage (5000 IU, im, three times weekly) for an additional 6 months after discontinuation of T.

The entire study was performed at the V.A. Medical Center and the USPHS Hospital at Seattle, WA. All injections were administered by the investigators or their assistants, and careful records were kept to assess compliance with the study protocol.

At monthly intervals, subjects were interviewed by one of the investigators concerning general health and sexual functioning; a physical examination was performed, and blood samples were obtained for determination of serum T and E_2 levels. During the treatment periods, these monthly blood samples

were obtained immediately before scheduled injections of T and/or hCG.

Near the end of each study period, blood samples were obtained by pooling the first and the last 2 h of samples from 6-h, every 20 min, blood-sampling studies. These pooled blood samples were drawn between 0800–1000 and 1400–1600 h and were obtained 1 day before the next scheduled T injection and 1 day before the next scheduled hCG injection. These samples were used to measure serum PREG, 17OH-PREG, PROG, 17OH-PROG, dehydroepiandrosterone (DHEA), DHEA sulfate (DHEA-S), Δ^4 -androstenedione (Δ^4 -A), cortisol, PRL, sex steroid-binding globulin (SSBG), and free T levels.

Twice monthly, seminal fluid analyses were performed on specimens that each subject obtained by masturbation after at least 2 days of abstinence from ejaculation.

Hormone assays

T and E₂ RIA. The RIA for T and E₂ used reagents provided by the WHO Matched Reagent Programme (21). The antisera were raised in a rabbit against BSA conjugates of T and 17 β -estradiol. Anti-T antiserum exhibited cross-reactivity of 14% with 5 α -dihydrotestosterone, 6% with 5 α -androstenediol, and less than 2% with other steroids tested. Anti-E₂ antiserum exhibited 17% cross-reactivity with estrone. The T assay was preceded by ether extraction, and the E₂ assay was preceded by ether extraction and Celite chromatography using 40% ethyl acetate eluant. In both assays, separation of bound from free hormone was accomplished using dextran-coated charcoal separation. The assay sensitivity was 10 pg/tube (0.1 ng/ml) for T and 6 pg/tube (12 pg/ml) for E₂. The intraassay and interassay variabilities were 5.1% and 9.8%, respectively, for T and 8.2% and 8.8%, respectively, for E₂.

SSBG and free T determination. SSBG determinations were performed by Dr. Stephen R. Plymate, Madigan Army Medical Center, Tacoma, WA, using a modification of procedures used to measure androgen-binding protein described by Musto and Bardin (22). Serum samples were incubated with charcoal for 16 h at 4 C before dihydrotestosterone saturation analysis so as to remove circulating steroids (22). The methodology for this assay has been described previously (23). Free T levels were calculated from the formula of Pearlman (24) using the equilibrium association constant of T for SSBG reported by Moll *et al.* (25).

Steroid precursor RIA. The RIAs for serum PREG, 17OH-PREG, PROG, 17OH-PROG, DHEA, DHEA-S, Δ^4 -A, and cortisol were performed using methodology described previously (26).

PRL RIA. Serum PRL was measured by RIA, courtesy of Dr. Alan P. Petersen, Department of Laboratory Medicine, University of Washington, using the method of Sinha *et al.* (27).

Seminal fluid analysis. Sperm concentrations in seminal fluid samples were determined by a Coulter counter (Coulter Electronics, Hialeah, FL), and concentrations less than 15 million/cc were confirmed by direct determination using a hemocytometer. These methods have been described previously (28). Sperm morphology and motility were assessed as described by

MacLeod (29).

Statistical analysis. Mean hormone levels were determined for monthly blood samples during each study period, as well as for pooled blood samples at the end of each study period for each subject. These data were then compared using Student's paired *t* test.

As sperm counts are not normally distributed, log transformation of sperm counts was employed to normalize these data before statistical analysis. Mean sperm counts during the control period, during the last 6 weeks of the T alone period, and during the 10th to 30th weeks of the hCG plus T period were calculated for each subject. These data were then compared using Student's paired *t* test. Sperm counts after 6 weeks of T and after 10 weeks of hCG were chosen to eliminate the transition effects of gradually falling sperm counts in the first 5 weeks of T treatment and the gradually rising sperm counts during the first 9 weeks after starting hCG.

Results

Serum E₂ levels (Fig. 1, upper panel)

After a 3-month control period, administration of T (200 mg, im, weekly) for 3–5 months led to an increase of approximately 3-fold in E₂ levels, from a mean (\pm SEM) of 25 ± 5 pg/ml to 74 ± 16 pg/ml ($P < 0.02$). The normal range of serum E₂ in our laboratory is 13–55 pg/ml. As we have reported previously, T administration alone also led to a marked suppression of endogenous gonadotropin secretion (20). With the addition of hCG (5000 IU, im, three times weekly) to T, the mean E₂ levels increased further to 158 ± 16 pg/ml ($P < 0.001$ compared to T alone), a level approximately 6 times the mean control value. As we have reported previously, with this dosage of hCG, a moderately supraphysiological serum level of LH bioactivity was achieved (a level about 6 times greater than the control value), and mean serum FSH levels remained undetectable by RIA during simultaneous hCG plus T administration (20).

Serum T levels (Fig. 1, lower panel)

With T administration alone, mean T levels rose from 6.0 ± 1.1 to 13.3 ± 0.7 ng/ml ($P < 0.001$ compared to control). During hCG plus T treatment, T levels increased even further to 18.4 ± 0.8 ng/ml ($P < 0.001$ compared to T alone). Thus, T levels increased by a mean of 5.1 ng/ml from the T alone period to the hCG plus T period; this increment in the T level, stimulated by hCG, approximates the normal blood T level in man.

Serum SSBG and free T levels (Table 1)

There were no significant differences in mean serum SSBG levels during control, T alone, and hCG plus T periods. Thus, the calculated serum free T level increased

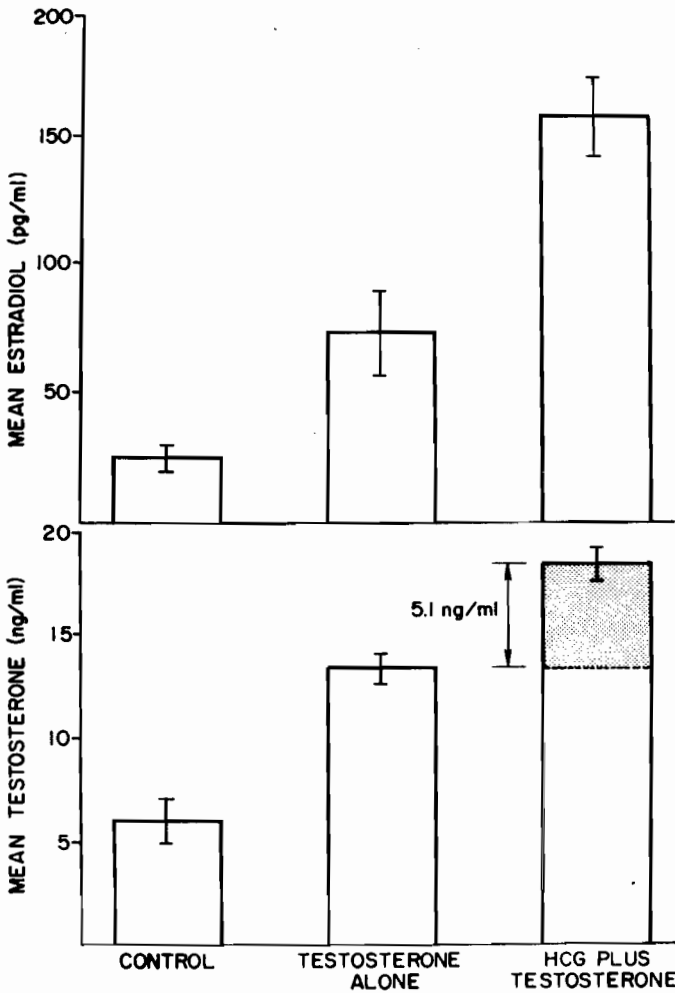


FIG. 1. Mean monthly serum E_2 levels (upper panel) and serum total T levels (lower panel) in five normal men during the control period, during T administration alone, and during hCG plus T administration (mean \pm SEM). The mean rise of 5.1 ng/ml in the T level from the T alone period to the hCG plus T period is depicted by the shaded area.

TABLE 1. Serum SSBG and T levels

	Control	T alone	hCG plus T
Serum SSBG (ng DHT/ml)	8.5 \pm 1.7	8.6 \pm 1.7	6.3 \pm 0.6
Serum total T (ng/ml)	6.7 \pm 0.7	13.1 \pm 1.1 ^a	17.0 \pm 1.2 ^{b,c}
Serum free T (ng/ml)	0.23 \pm 0.01	0.59 \pm 0.05 ^b	0.77 \pm 0.05 ^{b,c}

Values are the mean \pm SEM pooled blood samples at the end of each period.

^a $P < 0.01$ compared to control.
^b $P < 0.001$ compared to control.
^c $P < 0.01$ compared to T alone.

from a mean of 0.23 \pm 0.01 ng/ml during control period to 0.59 \pm 0.05 ng/ml during T alone. With the addition of hCG to T, the mean free T level rose to an even higher level of 0.77 \pm 0.05 ng/ml. Therefore, hCG stimulated a mean increase of 0.18 ng/ml in the free T level above

that during T alone. This increment in the free T level approximates the normal free T level in man.

Steroid precursors of T biosynthesis (Fig. 2)

There were marked changes in serum levels of Δ^4 steroid precursors of T biosynthesis during the T alone as well as in the hCG plus T periods. The mean serum PROG level during the control period was 0.13 \pm 0.01 ng/ml. With T administration alone, the PROG level was suppressed to a mean of 0.09 \pm 0.01 ng/ml ($P < 0.01$ compared to control). With the addition of hCG to T, the mean PROG level rose to 0.14 \pm 0.002 ng/ml; higher than that with T alone ($P < 0.001$), but not significantly different from the mean control level. The mean serum 17 OH-PROG level was also markedly suppressed during T alone (0.22 \pm 0.04 ng/ml compared to 0.87 \pm 0.16 ng/

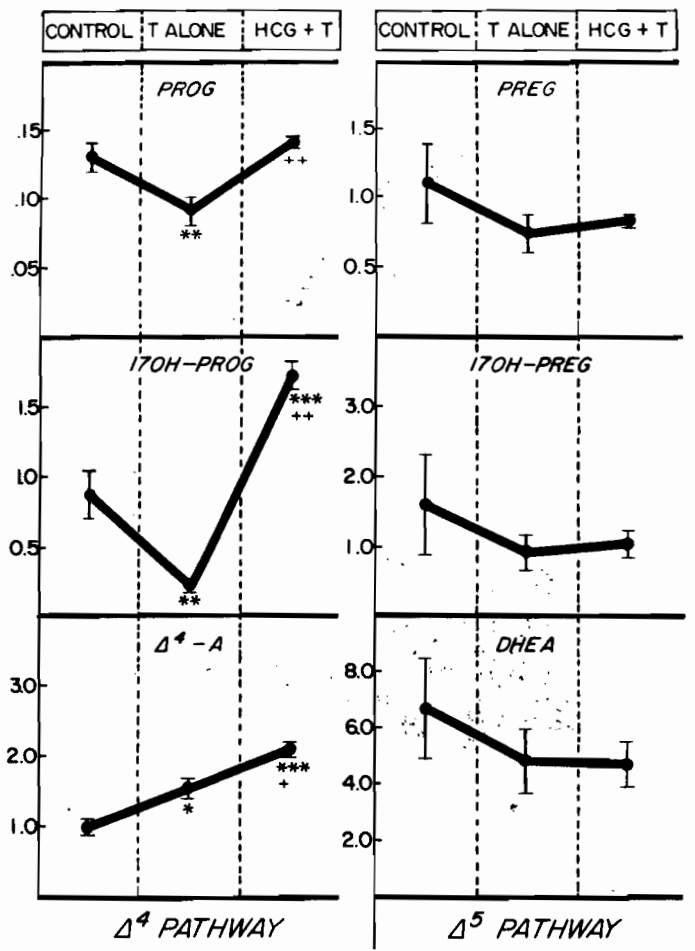


FIG. 2. Mean serum levels of Δ^4 steroid precursors of T biosynthesis [serum PROG, 17OH-PROG, and Δ^4 -A levels (upper, middle, and lower panels, on left)] and Δ^5 steroid precursors [serum PREG, 17OH-PREG, and DHEA levels (upper, middle, and lower panels, on right)] during the control, T alone, and hCG plus T periods in five normal men (mean \pm SEM). All hormone values are expressed as nanograms per ml. *, $P < 0.03$; **, $P < 0.01$; ***, $P < 0.001$ (compared to control). †, $P < 0.01$; ††, $P < 0.001$ (compared to T alone).

ml during the control period; $P < 0.01$). During the hCG plus T period, the mean 17 OH-PROG level (1.72 ± 0.10 ng/ml) was higher than during the T alone as well as the control periods ($P < 0.001$). Presumably because of conversion of T to Δ^4 -A, the mean serum Δ^4 -A level increased from 1.00 ± 0.11 ng/ml during the control period to 1.55 ± 0.15 ng/ml during T alone despite the gonadotropin suppression induced by T administration. During the hCG plus T treatment, the mean Δ^4 -A level rose even further to 2.10 ± 0.11 ng/ml.

In contrast to Δ^4 steroid precursors, there were no significant differences in the mean serum levels of Δ^5 steroid precursors of T biosynthesis. The mean serum PREG level was 1.09 ± 0.29 ng/ml during the control period, 0.73 ± 0.13 ng/ml during T alone, and 0.82 ± 0.04 ng/ml during hCG plus T treatment. The mean serum 17OH-PREG level was 1.58 ± 0.71 ng/ml during the control period, 0.91 ± 0.25 ng/ml during T alone, and 1.04 ± 0.18 ng/ml during hCG plus T treatment. The serum DHEA level was 6.62 ± 1.80 ng/ml during the control period, 4.78 ± 1.09 ng/ml during T alone, and 4.72 ± 0.76 ng/ml during hCG plus T treatment. Although there was some tendency for all levels of Δ^5 steroid precursors to fall with T alone, the differences between control and T alone did not achieve statistical significance. Levels during hCG plus T treatment were not statistically significantly different from those during T alone.

Seminal fluid analyses

Seminal fluid data from this study was reported previously (20) and are included here for comparison with serum steroid levels. The mean sperm concentration was severely suppressed with exogenous T administration, from 106 ± 28 million/cc during the control period to 1 ± 1 million/cc during T alone. Three subjects became azoospermic on T alone, while the other two subjects became severely oligospermic, with sperm concentrations less than 2 million/cc. With the addition of hCG to T, sperm concentrations rose significantly above those found during T alone, reaching a mean of 46 ± 16 million/cc after 10 weeks of hCG plus T treatment ($P < 0.001$ compared to T alone). Sperm concentrations during hCG plus T treatment in two subjects returned to their own control range. The other three men did not consistently achieve sperm concentrations during hCG plus T treatment in their control range; the mean sperm concentrations in these men were 12, 13, and 94 million/cc. Seminal fluid volume did not change significantly throughout the entire study, so the sperm concentration accurately reflected sperm production in the ejaculate. Sperm motility was greater than 50%, and morphological examination revealed greater than 60% oval forms consistently

during the control period as well as at the end of the hCG and T period in all subjects.

hCG alone (Fig. 3)

In the one subject who continued to receive hCG alone in the same dosage (5000 IU, im, three times weekly) after the hCG plus T period, mean monthly serum E_2 levels were 134 ± 13 pg/ml during the hCG alone compared to 17 ± 3 pg/ml in his control period (Fig. 3, lower panel). Thus, in this subject, the mean E_2 level during hCG alone was approximately 8 times that during his control period. Despite this very high level of E_2 , this man's mean sperm concentration during hCG alone (96 ± 10 million/cc) was no different from that during the control period (113 ± 21 million/cc; Fig. 3, upper panel). Furthermore, this subject's mean T level during hCG alone (8 ± 0.5 ng/ml) was higher than during his control period (4.7 ± 0.5 ng/ml; Fig. 3, middle panel).

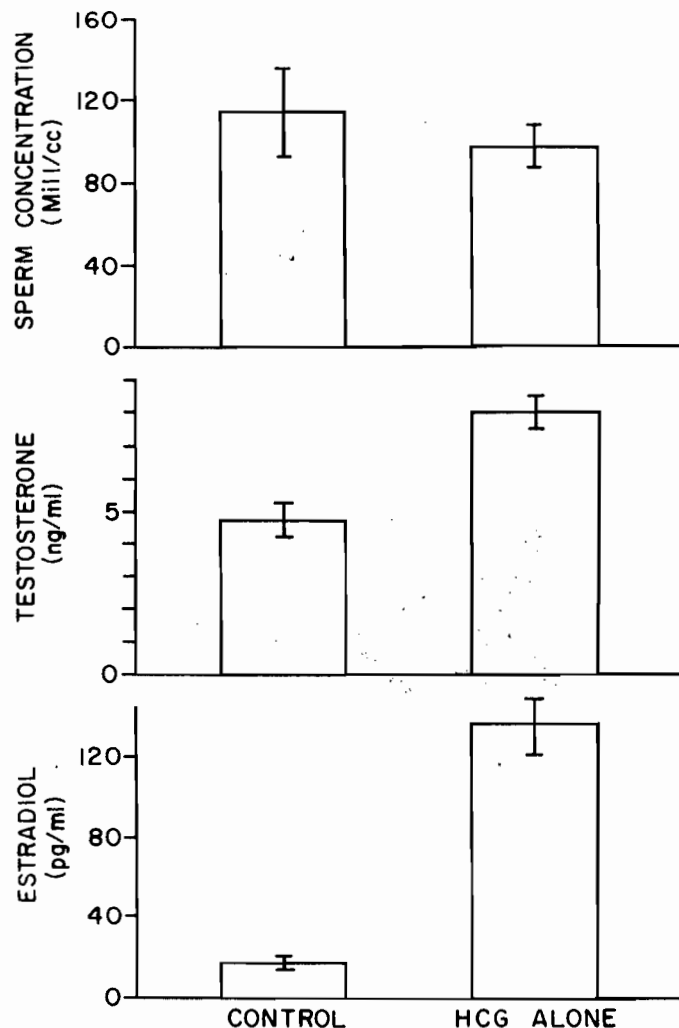


FIG. 3. Mean monthly sperm concentrations (upper panel), serum T levels (middle panel), and serum E_2 levels (lower panel) during the control and hCG alone periods in one normal man (mean \pm SEM).

Serum cortisol, DHEA-S, and PRL levels (Table 2)

There were no significant differences in mean serum cortisol and serum DHEA-S levels in any of the study periods.

The mean serum PRL level was 16.7 ± 4.4 ng/ml during the control period. This increased to a mean of 21.8 ± 6.1 ng/ml during the period of T administration alone ($P < 0.05$ compared to control) and rose further to 32.6 ± 9.6 ng/ml during the hCG plus T period ($P < 0.05$ compared to control and T alone values).

Clinical findings

All subjects remained in good health throughout the course of the study. No adverse side effects of T or hCG were noted, except for the development of mild acne during T administration in three subjects. There were no significant changes in the amount of palpable breast tissue (within 1 cm of control measurement) or testicular size (within 1 cm of control, measured by calipers) during any of the hormonal treatments.

Discussion

Our results in the T alone phase of the study confirm earlier findings that the administration of exogenous T to normal men leads to marked increases in blood T and E_2 levels and to the suppression of gonadotropin and sperm production (20, 30). In this setting of endogenous hypogonadotropism and high E_2 levels induced by T, our subjects were given hCG in a moderately high dosage. The dosage of hCG used in this study was chosen because it represented one of the higher dosages used in clinical practice and was shown to maintain normal T levels in hypogonadotropic men (17). We have shown previously that this dosage is clearly supraphysiological in terms of LH bioactivity (20). In the present study, we documented that the mean serum E_2 level achieved during simultaneous administration of hCG with T was also clearly supraphysiological. Testicular function was assessed in this setting of supraphysiological gonadotropin stimulation and high serum (and presumably intratesticular) E_2 levels produced by simultaneous hCG and T administration.

TABLE 2. Serum cortisol, DHEA-S, and PRL levels

	Control	T alone	hCG plus T
Serum cortisol (ng/ml)	107 ± 20	92 ± 10	83 ± 8
Serum DHEA-S (ng/ml)	3.6 ± 0.5	3.4 ± 0.3	4.5 ± 0.5
Serum PRL (ng/ml)	16.7 ± 4.4	21.8 ± 6.1^a	$32.6 \pm 9.6^{a,b}$

Values are the mean \pm SEM pooled blood samples at the end of each period.

^a $P < 0.05$ compared to control.

^b $P < 0.05$ compared to T alone.

Our results demonstrate that hCG can stimulate an increment in blood T levels that approximates the normal serum T level in man, despite a mean serum E_2 level during hCG stimulation approximately 6 times greater than the control value. Because endogenous testicular T production is markedly reduced as a result of exogenous T-induced gonadotropin suppression, serum T levels during the T alone period reflected, for the most part, the amount of exogenous T administered. With the addition of hCG to T, endogenous T production by the testes is stimulated, and serum T levels would be expected to rise. Thus, the incremental rise in T levels from the T alone period to the hCG plus T period is a reflection of hCG-stimulated T production by the testis. In our subjects, hCG stimulated a mean increase of 5.1 ng/ml in the total T level and an increase of 0.18 ng/ml in the free T level over those found during T administration alone. Both of these increases, stimulated by hCG, approximate normal blood T levels in man.

Measurement of the major Δ^4 and Δ^5 steroid precursors of T biosynthesis in this study demonstrated that major changes in serum levels occurred only in Δ^4 steroid precursors (PROG, 17OH-PROG, and Δ^4 -A). Serum levels of precursors in the Δ^5 pathway (PREG, 17OH-PREG, and DHEA) were not significantly affected by either the gonadotropin suppression induced by exogenous T administration or the gonadotropic stimulation of hCG. The lack of significant changes in serum cortisol and DHEA-S in any study period suggests that the changes seen in serum Δ^4 steroid precursor levels are not related to changes in adrenal steroid output.

High dose hCG exposure has been shown to induce a partial block of testicular steroid synthesis at the 17,20-desmolase and 17-hydroxylase steps of T biosynthesis (3, 5, 7, 14, 15). These blocks, manifested by relatively large accumulations of C-21 steroid precursors (PREG, 17OH-PREG, PROG, and 17OH-PROG) compared to T, have been attributed to end-product inhibition by the very high E_2 levels stimulated by hCG or to rate-limiting activities of the enzymes involved. The presence of E_2 receptors on Leydig cells (31, 32), the finding that E_2 blocks the same steps of T biosynthesis as hCG or LH (12, 13), and the demonstration that hCG- or LH-induced steroidogenic blocks are abolished by the estrogen antagonist tamoxifen (33, 34) strongly suggest that E_2 may modulate Leydig cell function and may mediate the steroidogenic blocks induced by excessive gonadotropin stimulation.

In human studies, several groups have used the larger increase in serum the 17OH-PROG level relative to the T level (*i.e.* increased 17OH-PROG to T ratio) during hCG administration as indirect evidence for a block in testicular steroidogenesis (14, 15, 34). Although measurement of intratesticular steroid levels would provide

more direct evidence of inhibition of testicular steroidogenesis, previous studies have demonstrated a good correlation of intratesticular and serum steroid levels during gonadotropin stimulation (35). Also, a recent study in nonhuman primates demonstrated that hCG induced a rapid and disproportionate accumulation of both serum and testicular 17OH-PROG relative to T (7).

The relatively smaller increase in the serum PROG level compared to the large increase in the serum 17OH-PROG level with hCG stimulation in our study suggests that no significant block in steroid synthesis exists at the 17-hydroxylase step. It is possible that the relatively larger increase in the serum 17OH-PROG level than in serum Δ^4 -A or T level (*i.e.* increased 17OH-PROG to Δ^4 -A and 17OH-PROG to T ratios) during hCG stimulation could be evidence for a partial block of 17,20-desmolase activity. However, interpretation of the Δ^4 -A and T levels is complicated by the fact that levels of Δ^4 -A and T are higher during the T alone period compared to control levels as a result of exogenous T administration and its conversion to Δ^4 -A. No accumulation of serum levels of Δ^5 pathway precursors was demonstrable during chronic, high dosage hCG administration.

This lack of significant change in serum levels of Δ^5 steroid precursors during hCG administration could be due to rapid conversion to T within the testes. *In vitro* studies from several laboratories have demonstrated that the Δ^5 pathway is the predominant pathway for T biosynthesis in man (36).

Our results also demonstrate that despite very high serum E_2 levels, hCG can reinitiate and maintain sperm production, as assessed by sperm concentrations, motilities, and morphologies. In two of our five subjects, sperm production returned to their individual control ranges on hCG plus T treatment despite a mean E_2 level of 159 pg/ml. The other three men achieved sperm concentrations in the normal adult range with normal motilities and morphologies during hCG plus T treatment, but sperm concentrations were not consistently in their own control ranges. The mean E_2 level during hCG plus T treatment in these three men was 133 pg/ml, slightly lower than the mean level of E_2 in the two subjects who achieved sperm concentrations in their control ranges. Thus, differences in E_2 levels cannot explain the differences in sperm production observed during hCG plus T treatment in these two groups.

In two of the three subjects who failed to achieve sperm counts in their control ranges, irregular administration of hCG was documented on review of medication records and may have accounted for the failure of sperm counts to return to control levels. Furthermore, it is possible that the administration of hCG for a longer period of time might have allowed for more return of spermatogenesis in these subjects. However, it is also conceivable

that in some individuals, high dose hCG and/or high E_2 levels may impair maximum spermatogenic capacity, whereas sperm production in other individuals is not affected.

One subject continued to receive hCG alone for 6 months after the hCG plus T period. Despite serum E_2 levels approximately 8-fold higher than control levels, normal sperm production and greater than normal T levels were maintained throughout the entire 6 months of hCG administration alone.

There was a rise in the PRL level associated with increasing estrogen levels in our subjects, such that during hCG plus T administration, PRL levels were about twice the control values. This elevated PRL level had no detectable adverse effect on testicular function.

Despite 3- to 5-fold higher E_2 levels during the T alone and hCG plus T periods, none of our subjects developed gynecomastia or had a significant change in testicular size during the entire study. The reason for this somewhat surprising absence of gynecomastia may be the high levels of T occurring concomitantly with the high E_2 levels. Concomitantly high T and E_2 levels may also be the reason that significant changes in SSBG levels were not found in our subjects.

From the results presented, we conclude that hCG can stimulate normal T and sperm production in some men despite the very high levels of E_2 generated. Prolonged exposure to a moderately suprphysiological dosage of hCG, a dosage equal to or above those used in treating hypogonadotropic men, did not cause severe testicular regression. Our results are similar to those reported by Sherins (37), who administered high dosage hCG (1000 IU every other day for 2-12 months) to 14 men with idiopathic oligospermia. This treatment resulted in a 2-fold increase in plasma T levels and unchanged sperm concentration, in spite of a 4-fold rise in plasma E_2 levels.

Our results do not necessarily contradict the reports of marked suppression of testicular function with exogenous administration of high dosage hCG or excessive endogenous LH stimulation induced by LHRH or LHRH agonists. It is possible that higher dosages of hCG, which presumably would generate higher levels of E_2 could adversely effect spermatogenesis and/or testicular steroid synthesis. However, men with hCG-secreting testicular neoplasms generally have T levels that are normal to slightly elevated and elevated E_2 levels (38, 39). Serum E_2 levels in some of these patients are comparable to those obtained in the present study, yet histologically, there has been evidence of seminiferous tubular degeneration in the contralateral testis. Therefore, in some of these patients it is possible that some effect of high levels of hCG other than excessive E_2 production causes seminiferous tubular damage or perhaps that these patients have testicular dysfunction associated with, but not

caused by, elevated hCG levels. Finally, LHRH and LHRH agonists have been demonstrated to exert a direct suppressive effect on testicular function (40). In addition to stimulating excessive amounts of endogenous LH, this direct effect of LHRH and LHRH agonists may contribute to their adverse effect on the testis.

Finally, while we have demonstrated that hCG in this experimental setting can stimulate a significant increase in T and sperm production despite very high E₂ levels, we do not conclude that E₂ was having no inhibitory effect on T biosynthesis or spermatogenesis. It is possible that even higher T levels or sperm production would have been found if E₂ levels were not so elevated. Perhaps an experimentally produced block of T to E₂ aromatization or a block of estrogen action induced by an estrogen antagonist would allow the demonstration of lower steroid precursor levels (e.g. PROG, 17OH-PROG, and Δ⁴-A), higher T levels, and higher sperm counts.

Addendum

Non-SSBG-bound T and E₂ levels were determined by differential ammonium sulfate precipitation of pooled blood samples at the end of each study period. A technique similar to procedures used by O'Connor *et al.* (41) was used. The following results were obtained:

	Control	T alone	hCG plus T
Non-SSBG-bound T (ng/ml)	3.0 ± 0.4 ^a	10.3 ± 1.8 ^a	16.0 ± 0.9 ^a
Non-SSBG-bound E ₂ (pg/ml)	18 ± 4	51 ± 15	81 ± 13

^a Mean ± SEM; pooled blood sample at end of each period.

These data confirm the free T findings in our study. Despite a 4-fold elevation of non-SSBG-bound E₂ compared to the control value, hCG stimulated a normal increment of 5.7 ng/ml in non-SSBG-bound T.

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