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ORIGINAL ARTICLE

Serum Estradiol after Single Dose hCG Administration Correlates with Leydig Cell Reserve in Hypogonadal Men: Reassessment of the hCG Stimulation Test

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SUMMARY

The hCG stimulation test with assessment of serum testosterone (T) is used for evaluation of testicular function. This retrospective study was undertaken to estimate the diagnostic value of stimulated estradiol (E2) levels in the assessment of Leydig cell function. Serum T and E2 before and after repeated daily hCG injections in 23 adult men with clinically suspected or established primary hypogonadism were studied. After hCG administration serum T increased gradually with peak levels after 72 hours (Δ84%, p=0.003). In contrast, serum E2 concentrations reached their maximal levels 24 hours after the first injection (Δ168%, p=0.001). Serum T and E2 responses were more attenuated in men with LH ≥17 IU/L as compared to men with lower LH levels. Peak E2 levels after 24 hours correlated significantly with peak T levels after 3 days. We conclude that the increase in serum E2 levels 24 hours after a single hCG injection is an useful additional measure of Leydig cell function. Assessment of E2 increments would render the test procedure more practical, less time-consuming and more cost-effective than assessing peak T levels after 72 hours. (Clin. Lab. 2005;51:509-515)

KEY WORDS

hCG stimulation test, hypogonadism, estradiol, testosterone, testicular reserve

INTRODUCTION

In clinical practice the assessment of testicular function by basal routine hormone assay is straightforward. For a more comprehensive evaluation of Leydig cell function the human chorionic gonadotropin (hCG) stimulation test has been suggested [1]. Response of serum testosterone (T) to gonadotropin stimulation as a measure of Leydig cell reserve is commonly assessed in prepubertal boys to evaluate the presence or absence of testicular tissue and to elucidate defects of T biosynthesis or action [2-7]. In addition, in men with marginal gonadotropin elevations and/or low normal androgen levels a subnormal or delayed response of serum T levels after hCG stimulation has been equated with a decreased testicular reserve in hypo- and hypergonadotropic adult men [8-11].

Many protocols assessing the increase of total T levels after single or repeated intravenous or intramuscular hCG administrations have been proposed for testing testicular functional capacity. It has been shown that the maximal T response to a single-dose of hCG is equivalent to repeated daily injections with peak T levels after 72 hours [3, 10, 12, 13]. In addition to the assessment of serum T, some studies evaluated the stimulatory effect of hCG on serum estradiol (E2) concentrations in adult hypogonadal men [8, 9, 11]. The rise in serum E2 occurs since E2 is not only derived from peripheral conversion, but is also secreted by the testes themselves.

The aim of the present study was 1) to review the experience of the hCG stimulation test in a large series of adult men with different degrees of primary testicular

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Table 1: Baseline characteristics in all study participants and in subgroups according to serum LH levels. Significances between patients with serum LH levels above and below 17 IU/L (cohort's mean value) determined by unpaired *t* test or Mann-Whitney *U* test in nonparametric distribution, * p<0.05, ** p<0.0001. Data are mean ± SD (range).

	Total group	LH <17 IU/L	LH ≥17 IU/L
n	23	14	9
Age (yrs)	43.7 ± 12.3 (19 - 76)	42.9 ± 10.0	44.8 ± 15.9
BMI (kg/m^2)	25.3 ± 3.2 (18.8 - 31.6)	25.0 ± 2.7	25.9 ± 1.5
T (nmol/L)	12.0 ± 6.1 (2.2 - 30.7)	13.7 ± 6.4	9.4 ± 4.7
E2 (pmol/L)	84.4 ± 49.2 (25 - 217)	79.8 ± 49.0	91.6 ± 51.6
SHBG (nmol/L)	37.2 ± 11.1 (20.0 - 59.0)	35.4 ± 8.9	40.2 ± 14.5
LH (IU/L)	17.0 ± 9.6 (6.4 - 44.2)	10.9 ± 2.4	26.4 ± 8.8**
FSH (IU/L)	36.9 ± 19.9 (10.7 - 93.9)	27.4 ± 11.8	51.5 ± 21.6*

failure, and 2) to evaluate the predictive value of a single hCG dose on serum E2 levels. Specifically we aimed to assess whether T and E2 peaked simultaneously after hCG stimulation, and if their respective increases are related to each other. This is of major interest as maximal increments in serum E2 levels have been reported already after 24 hours, irrespective of single-dose or repeated-dose hCG regimens. Such a test procedure would be more practical and less time-consuming than measuring peak T after 72 hours.

SUBJECTS AND METHODS

Subjects

Twenty-three men (mean age 43.7±2.5 years) in whom a hCG stimulation test was performed for endocrine evaluation of suspected or definite primary hypogonadism were studied retrospectively. Patients were included in the analysis in case of a history of primary hypogonadism and/or elevated serum levels for luteinizing hormone (LH). We excluded subjects with hypogonadotropic hypogonadism and patients on T replacement therapy. The underlying testicular disorders leading to hypergonadotropic hypogonadism were Klinefelter's syndrome (n=6), unilateral or bilateral cryptorchidism (n=4), gonadal failure after oncological treatment (chemotherapy and/or total body irradiation; n=5), orchitis (n=1), partial androgen insensitivity syndrome (n=2), and idiopathic primary gonadal failure (n=5). All patients gave their written informed consent to participate in the study.

hCG test

Basal blood samples were drawn on days 1, 2, 3 and 4 at 08:00 h in each patient, followed by an intramuscular injection of hCG (5000 IU; Pregnyl Organon, Pfäffikon, Switzerland) on days 1, 2 and 3. Serum samples were collected in the fasting state, immediately put on ice, and processed within 30 minutes. Thereafter, they were kept frozen at –20 °C until analysis. An 1.5 to 2.7-fold increment in serum T (ΔT), and a 2.3 to 2.9-fold increase in serum E2 (ΔE2) after hCG administration were considered to reflect normal gonadal function [8].

Hormonal analysis

All laboratory analyses were conducted at the Department of Central Laboratories at the University Hospital Basel. Serum levels for LH (reference range, 1.3-5.8 mU/l), FSH (reference range, 1.5-12.4 mU/l), total testosterone (T; reference range, 9.9-28.0 nmol/l), and estradiol (E2; reference range, 40.0-161.0 pmol/l) were measured using an electrochemiluminescence immunoassay (Elecsys, Roche Diagnostics, Rotkreuz, Switzerland). Serum sex hormone-binding globulin (SHBG) concentrations were determined by a chemiluminescence immunoassay (Immulite, DPC Bühlmann Laboratories, Allschwil, Switzerland; reference range, 13.0-71.0 nmol/l).

Statistical analysis

All data are expressed as mean±SD unless stated otherwise. Unpaired *t* test (two-sided), or Mann-Whitney *U* test in the case of non-parametric distributions, were used to identify differences among patient groups. Changes in T and E2 after hCG administration and their differences in subsets of patients according their baseline LH level were estimated in a repeated measures ANOVA model. Spearman rank correlation coefficients were calculated between basal T, basal E2, basal LH levels and ΔT and ΔE2, respectively. Significance was defined as $p \leq 0.05$. Data were analysed using Statistica for Windows (version 6.0, StatSoft, Inc., Tulsa, OK).

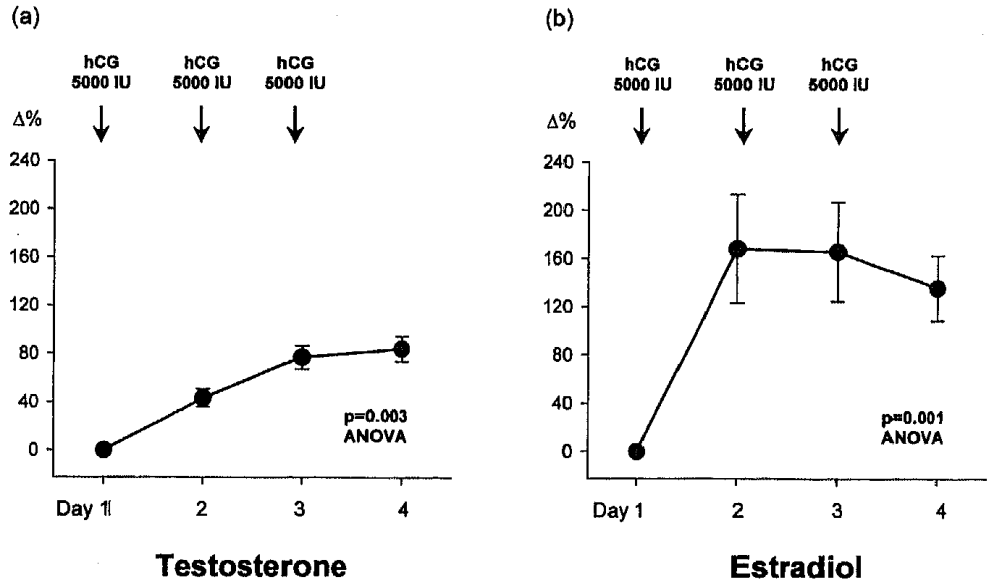

Figure 1: Serum T and E2 response following repeated doses of hCG in 23 men with primary testicular failure (mean % change; 5000 IU on day 1, 2, and 3). Data are mean ± SEM.

RESULTS

Baseline characteristics

Table 1 summarizes the baseline characteristics of all 23 patients with clinically suspected or established primary testicular failure. Serum LH levels were elevated in all patients, as were FSH concentrations, except for two subjects in which FSH levels were within the upper reference range (10.7 IU/L and 11.3 IU/L, respectively). Despite a marked increase in serum LH, circulating T levels were heterogeneously distributed with low (n=11), normal (n=11) or elevated (n=1) T concentrations.

To compare different degrees of testicular failure, patients were divided according to their mean serum LH concentrations (LH <17 IU/l, n=14; LH ≥17 IU/l, n=9). Even though T levels tended to be lower in men with LH ≥17 IU/L, T levels were not significantly different compared to their levels in men with LH<17 IU/L (p=0.11) despite significant differences in LH and FSH concentrations. Similarly, E2 (p=0.57) and SHBG (p=0.42) levels were comparable between the two groups. There was no difference in age and BMI between the two groups.

Changes in serum testosterone and estradiol during hCG stimulation

During repeated hCG injections serum T increased gradually from 12.0±6.1 nmol/L at baseline to a peak level of 22.3±2.7 nmol/L after 72 hours (p=0.003, ANOVA; Figure 1). The major increment was observed during the first 48 hours with a mean increase of 43.3% between days 1 and 2 (p=0.06, ANOVA, posthoc with LSD), and a lesser increase of 33.8% between days 2 and 3 (p=0.20). Thereafter, T levels rose only marginally (6.9% between days 3 and 4, p=0.76; Figure 2). In all study participants maximal stimulated T levels were below the lower cut-off level given for physiological T response after HCG (increment of 150% to 270%).

In contrast to the progressive increase of T, serum E2 levels showed a rapid and maximal increase 24 hours after the first hCG administration (Figure 1). Serum E2 increased from 84.4±49.2 pmol/L at baseline to a maximal level of 207.4±155.6 pmol/L (Δ 168%, p<0.001) at day 2. Thereafter, E2 levels tended to decrease (p=ns; Figure 2). In all but four subjects maximal stimulated E2 levels were below the lower cut-off level given for physiological E2 response after HCG (increment of 230% to 290%). Men with normal E2 stimulation were characterized by only mildly increased LH levels (12.2 ± 1.5 pmol/L; range, 11.3-14.4).

Changes in T and E2 levels during hCG administration were further analyzed in subsets according to baseline LH levels (Figure 3). Despite comparable T levels at baseline, the magnitude of T stimulation was significantly different between both groups (repeated measures ANOVA, p=0.007). In patients with incipient testicular failure (LH <17 IU/L) T increased gradually to a peak level of 27.0±3.1 nmol/L after 72 hours (Δ 101%, p<0.05 compared to baseline), whereas patients with

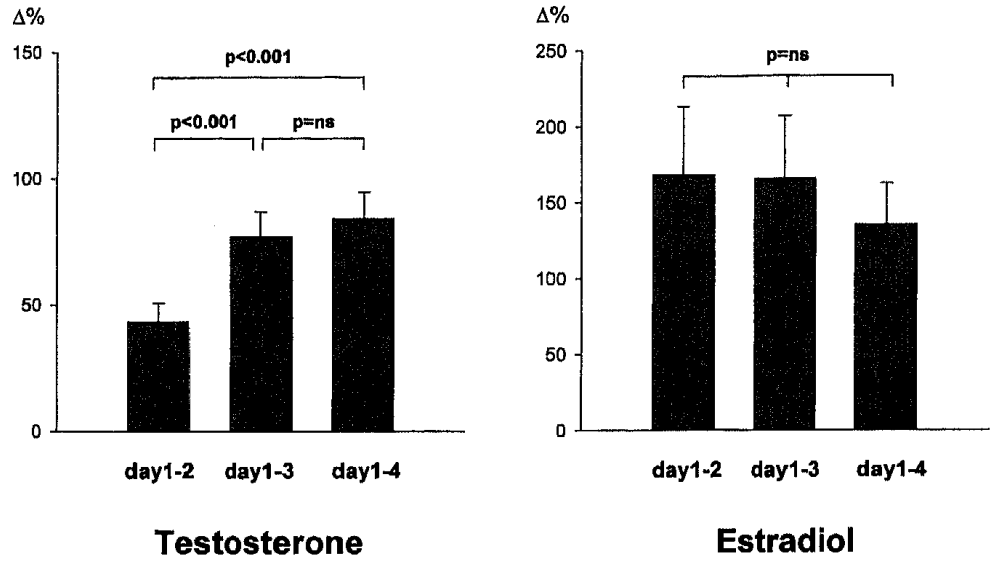

Figure 2: Mean (±SEM) percent changes in T and E2 after hCG stimulation during days 1 and 4. A normal response after hCG administration is defined as an increase in T levels by 150 to 270%, and an increment in E2 levels by 230 to 290% from baseline.

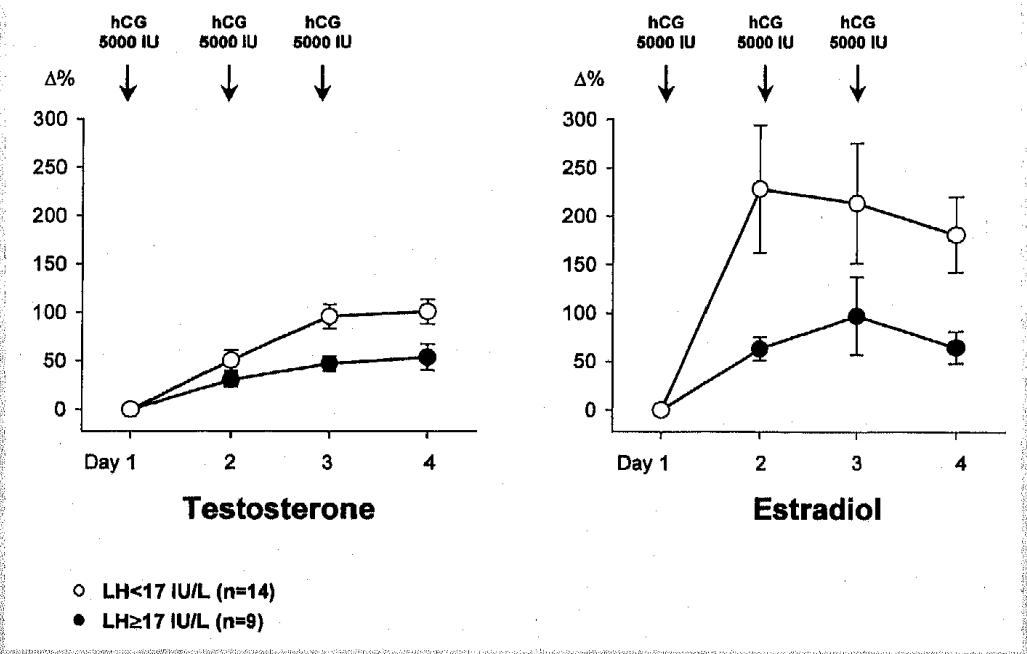

Figure 3: Serum T and E2 response after hCG in subsets of patients with LH levels <17 IU/L (n=14, open circles) and LH levels ≥17 IU/L (n=9, filled circles). Data are mean ± SEM.

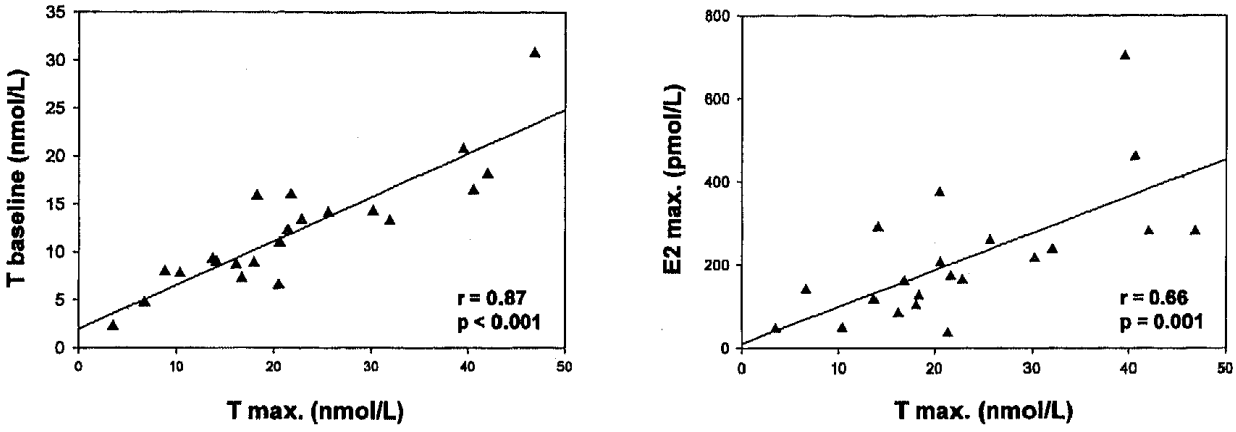

Figure 4: Relationship between peak T after repeated hCG stimulation (day 4) and (a) baseline T and (b) peak E2 concentrations (day 2) in 23 men with primary testicular failure.

more marked hypogonadism (LH ≥17 IU/l) showed only minimal stimulation (mean peak testosterone 14.1 ± 2.2 nmol/L; Δ 54%, p=ns compared to baseline). The difference in the maximal T response to hCG between both groups was highly significant (between-group comparison, p<0.005).

Similarly, peak E2 levels were higher in men with mild testicular failure (LH <17 IU/L) compared to subjects with marked hypogonadism (LH ≥17 IU/L). In men with LH levels <17 IU/L stimulated E2 levels were highest after 24h with a mean increase of 228% (p<0.001 compared to baseline). Subjects with LH ≥17 IU/L had a delayed and less pronounced E2 peak (Δ97%, p<0.01 compared to baseline; Figure 3). The difference in the maximal E2 response to hCG between both groups was significant (between-group comparison day 2 *vs.* day 3, p=0.03), whereas the difference in E2 response at day 2 tended to be higher in men with LH <17 IU/L (p=0.07).

Correlation analysis

Whereas no significant correlations were found between LH and baseline T and E2 levels, serum LH correlated negatively with the mean change in T levels (as assessed as difference between baseline levels and peak levels after 4 days; r=-0.46, p=0.03). In contrast, LH did not correlate with the mean change in E2 levels. Of note, baseline T was correlated with baseline E2 (r=0.54, p<0.01), peak E2 after 2 days (r=0.55, p<0.01), peak T after 4 days (r=0.87, p<0.001; Figure 4), and mean maximal change in T levels (r=0.63, p<0.005). Furthermore, a significant positive correlation was observed between peak E2 levels after 2 days and baseline E2 concentrations (r=0.63, p<0.005), as well as between peak E2 levels after 2 days and maximally stimulated T levels after 4 days (r=0.66, p=0.001; Figure 4).

DISCUSSION

The results of the present study show that the increase in serum E2 levels 24 hours after a single hCG injection is a useful additional measure of Leydig cell function in hypogonadal men. As most hCG stimulation test protocols are based on repeated measurements of serum T levels after single or repeated doses of intramuscular hCG with an expected T peak after 72 hours, evaluation of early changes in E2 levels would render the test procedure more practical, less time-consuming and more cost-effective.

The stimulatory effect of hCG on testicular T production is well known and has been widely used in the assessment of Leydig cell reserve in normal and pathological conditions [2, 4, 5, 8, 11, 14-18]. Several protocols assessing the increase of total T levels after intravenous or intramuscular hCG administrations have been proposed for testing testicular functional capacity. They differ mainly in the number of injections and variations in the hCG doses. The most widely used test has been daily intramuscular injection of 1500 to 5000 IU hCG for 3 or 4 days with an observed maximal T increment 72 hours after the first hCG injection. Based on these regimens a 1.5 to 2.7-fold increase in serum T levels has been found to represent normal Leydig cell function [8]. Confirming earlier reports [10], we found a delayed maximal T response after 72 hours in hypogonadal men compared to the reported response in eugonadal men, although the increase in serum T is largely dependent

on the degree of Leydig cell insufficiency. In all study participants maximal stimulated T levels were below the lower cut-off level given for a physiological T response, mirroring impaired testicular function. As expected, men with incipient testicular failure based on a normal or mildly elevated LH level (LH<17 IU/L) presented with a significantly higher T peak compared to men with more marked hypogonadism (LH ≥17 IU/L). It is of interest, however, that the prestimulatory T levels were comparable between the two groups. This finding confirms that the hCG stimulation test is a useful tool for early detection of Leydig cell insufficiency in patients with clinically assumed primary hypogonadism.
In contrast to the delayed T response, we observed a maximal increase in serum E2 already 24 hours after the first hCG injection. Thereafter serum E2 levels were gradually decreasing even in the presence of continued daily hCG injection. The maximal observed rise in E2 concentrations was even higher than the increase seen for serum T levels (Δ 168% *vs.* 84%). This is in agreement with earlier studies in normal and hypogonadal men showing an early rise in serum E2 after single [11, 13] or repeated hCG injections [8].

Approximately 60% of serum E2 is derived from peripheral aromatization of circulating testosterone in men. A further 20% is derived from peripheral conversion of estrone to E2, and the remaining 15-20% is directly secreted by the testes, although the latter proportion increases with circulating blood LH levels. The rise in serum E2 is based on the fact that E2 is not only derived from peripheral conversion, but is also secreted by the testes themselves. In contrast to the biphasic T response, serum E2 follows a monophasic pattern after hCG administration with a peak level after 24 hours and a gradual decrease thereafter [11, 13].
The percent increase in E2 was more pronounced in men with mild (LH <17 IU/L) *vs.* advanced (LH ≥17 IU/L) testicular failure (E2 max., p=0.03). The comparison between both groups at day 2 did not reach the level of significance (p=0.07) which is most likely due to limited number of patients evaluated. However, as peak E2 levels after 24 hours showed a good correlation with peak T levels after 72 hours (r=0.66, p=0.001), the maximal response in serum E2 seems to reflect accurately the degree of Leydig cell function.

The maximal T response to a single-dose of hCG is equivalent to repeated daily injections with peak T levels after 72 hours [3, 8, 10, 13]. Based on the long half-life of hCG after i.m injection and due to the fact that plasma hCG levels remain high enough to stimulate steroidogenesis for at least 3 to 4 days, the use of a single-dose protocol with measurement of T at baseline and after 72 hours has been advocated [3, 13]. In fact, repeated daily injections of hCG are of little practical benefit. The single hCG injection protocol has shown favorable diagnostic responses of the Leydig cells in delayed puberty, as well as in hypogonadal males [12, 19]. The results from this study have important clinical applications in diagnosis. There is evidence that the evaluation of testicular function using the hCG stimulation test can be further simplified by assessing the changes in serum E2. Specifically, we advocate to base the response of hCG on measurements in serum E2 with E2 concentrations analyzed before and 24 hours after a single injection of 5000 IU hCG.
In conclusion, in most clinical situations the diagnosis of primary hypogonadism is straight-forward and can be based on measurements of serum T and LH levels. By analyzing peak E2 levels after a single hCG administration, however, the hCG stimulation test would become a more practical and less cost-intensive tool for the assessment of Leydig cell function in adult men. Further studies with larger cohorts of healthy and hypogonadal men are needed to further evaluate whether serum E2 after hCG stimulation can be recommended as an additional tool for the investigation of Leydig cell function.

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