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Feedback Inhibition of Gonadotropins by Testosterone in Men With Hypogonadotropic Hypogonadism: Comparison to the Intact Pituitary-Testicular Axis in Primary Hypogonadism

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ABSTRACT: Men with hypogonadotropic hypogonadism (HH) due to hypothalamic-pituitary disease present with low serum testosterone levels combined with undetectable, low, or normal gonadotropin levels. Treatment consists of testosterone replacement to reverse the symptoms of androgen deficiency. The aim of this study was to examine the dynamics and feedback inhibition of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in relation to testosterone in 38 men with HH treated with testosterone. Findings were compared with 11 men with primary hypergonadism (PH). Testosterone replacement led to a suppression of FSH levels from 2.8 IU/L at baseline to 1.1 IU/L and to a suppression of LH levels from 2.3 to 0.8 IU/L. There was a linear correlation between levels of FSH and LH (after natural log transformation for both) and testosterone levels in both the HH and PH groups. However, the differences in intercepts and slopes between the groups were significant. To determine whether nonsuppressed FSH or LH during testosterone replacement reduces the probability of eugonadism, as reflected by normal testosterone levels, gonadotropin levels were measured and categorized as low (<0.5 IU/L), medium (0.5–2 IU/L), and high levels (>2 IU/L). The higher FSH or LH levels were found to significantly decrease the chance for achieving eugonadism. In conclusion, in men with HH due to hypothalamic-pituitary disease or injury, the pituitary-testicular hormonal axis maintains its physiological negative feedback between testosterone and gonadotropins. Thus, gonadotropin levels in men with HH might be useful, together with testosterone concentrations, for assessing the adequacy of androgen replacement.

Key words: FSH, LH, androgen replacement.
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Hypogonadotropic hypogonadism (HH) (secondary hypogonadism) may occur as an inherited and isolated deficiency (ie, Kallmann syndrome) or may be acquired as part of diseases of the hypothalamic-pituitary area (Hayes et al, 1998). Patients can have deficiencies of other pituitary hormones as well, including adrenocorticotropic hormone (ACTH), thyrotropin (TSH), and growth hormone. The diagnosis is based on the presence of low serum testosterone levels combined with undetectable, low, or normal gonadotropin levels. HH may be associated with hypothalamic damage affecting the secretion of gonadotropin-releasing hormone (GnRH) or with pituitary disease affecting the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Combined damage at both levels is also common. Symptoms include low libido, erectile dysfunction, hot flashes, gynecomastia, and weakness (Tenover, 1998), in addition to symptoms related to other pituitary-hormone deficiencies. The symptoms of secondary hypogonadism are not different from those of primary (testicular) hypogonadism (PH), and the 2 disorders can be distinguished only by gonadotropin levels. The treatment of hypogonadism consists of testosterone replacement to reverse the symptoms of androgen deficiency while achieving normal-range testosterone levels. Investigations of patients with PH have shown that gonadotropin levels are usually suppressed by effective testosterone replacement (Salehian et al, 1995; Swedloff et al, 2000).

The aim of the present study was to examine the dynamics and feedback inhibition of LH and FSH in relation to testosterone in treated patients with HH, compared with patients with PH, to assess the function of the pituitary-testicular hormone axis in pituitary disease. We hypothesized that, in patients with HH, a negative feedback control of gonadotropins proportional to testosterone level still exists, albeit at a new hormonal set point.

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Table 1. *Baseline characteristics of men with hypogonadotropic hypogonadism and primary hypogonadism*

Characteristic	Primary Hypogonadism (n = 11)	Hypogonadotrophic Hypogonadism (n = 38)
Age, mean y ± SD	47.8 ± 16.1	50.6 ± 15.3
Testosterone, mean ng/mL ± SD	2.1 ± 1.6	1.7 ± 1.0
FSH, mean IU/L ± SD	43.1 ± 31.0	2.8 ± 1.4
LH, mean IU/L ± SD	22.2 ± 20.2	2.3 ± 1.2
Isolated hypogonadism, no. of patients	11	13
Thyroid replacement, no. of patients	0	17
Glucocorticoids, no. of patients	0	16
Vasopressin treatment, no. of patients	0	4

Materials and Methods

Patients

The study sample included 38 men with HH and a mean age (±SD) of 50.6 ± 15.3 years (range, 19–79 years) at diagnosis. HH was defined as a serum total testosterone level of <3 ng/mL on 2 occasions before testosterone replacement, with low or normal gonadotropin levels (Darby and Anawalt, 2005). Subjects with HH and an undetectable gonadotropin level before testosterone replacement were excluded because the dynamics and feedback inhibition of the gonadotropins could not be studied.

Twenty-five of the 38 men with HH had adult-onset hypothalamic-pituitary disease with hypopituitarism affecting several pituitary hormonal axes, including gonadotropins. Nonfunctional pituitary adenoma was the most common pituitary pathologic finding, noted in 13 patients, followed by prolactinoma in 5, acromegaly in 2, pituitary apoplexy in 2, and pituitary metastasis, histiocytosis X, and thalamic tumor in 3 (1 of each of the 3 different pathologies). Twenty-two patients had undergone pituitary surgery, either transsphenoidal or transcranial, and 14 had also received sellar radiotherapy. Drug therapy included thyroid hormones in 17 patients, glucocorticoids in 16, growth hormone in 10, and vasopressin replacement in 4.

The remaining 13 men with HH had adult-onset disease of unknown etiology. All had a normal sella or empty sella on magnetic resonance imaging. They had never had pituitary surgery or pituitary hormone replacement therapy. The diagnosis in these cases was made during evaluation of complaints of erectile dysfunction or decreased libido.

The control group consisted of 11 men with PH and a mean age (±SD) of 47.8 ± 16 years (range, 18–68 years) at diagnosis. PH was defined as a low serum testosterone level with elevated levels of serum FSH (>11 IU/L in all 11 men) and LH (>8.4 IU/L in 9 men). PH was caused by undescended testes in 2 men and Klinefelter syndrome in 1 man; 2 patients had a history of bilateral testicular surgery. In 6 men, the etiology was unknown.

The study patients and control patients were evaluated retrospectively, according to our local institutional guidelines for research involving human subjects. The baseline characteristics of the 2 groups are shown in Table 1.

All patients with HH and PH were treated with injections of testosterone enanthate (250 mg; Schering AG, Berlin, Germany) every 2–4 weeks. In individual patients, a constant injection interval was usually used, after initial adjustment. Serum levels of total testosterone, FSH, LH, prolactin, and prostate-specific antigen (PSA) were measured (usually twice) before onset of hormone replacement and several times during treatment, 3–36 months after onset, at the midinjection intervals. Hormone levels were also assessed if the men temporarily stopped treatment for more than 2 months. Testosterone replacement was discontinued if the PSA level increased more than 4 ng/mL during treatment.

Testosterone and Gonadotropin Assays

Testosterone—Total testosterone level was determined by a radioimmunoassay (Coat-A-Count; DPC, Los Angeles, Calif), with a sensitivity of 0.1 ng/mL and intra- and interassay coefficients of variation (CVs) of 4% and 10%, respectively. Reference levels for men aged 20–50 years are 3–10 ng/mL, and reference levels for men aged older than 50 years are 1.8–8 ng/mL. An alternative fluoroimmunoassay for testosterone (AutoDELFIA; Wallac Oy, Turku, Finland) was used as well, with a sensitivity of 0.1 ng/mL and intra- and interassay CVs of 3% and 7%, respectively. The reference range for men is 2.7–9.6 ng/mL.

LH and FSH—LH and FSH levels were determined by chemiluminescent immunometric assays (Immulite 2000; DPC). The LH assay has a sensitivity of 0.05 IU/L and an interassay CV of 6%, and the FSH assay has a sensitivity of 0.1 IU/L and an interassay CV of 6%. Reference levels for LH in men are 1–7 IU/L, and reference levels for FSH are 0.1–8 IU/L. The alternative fluoroimmunometric assay for LH (hLH, AutoDELFIA) has a sensitivity of 0.05 IU/L and intra- and interassay CVs of 9% and 3%, respectively; for FSH (hFSH, AutoDELFIA), the sensitivity is 0.05 IU/L and the intra- and interassay CVs are 1.5% and 3%, respectively. Reference levels in men are 1–8.4 IU/L for LH and 1–10.5 IU/L for FSH.

All samples from each individual were analyzed in the same hormone assay.

Statistical Analysis

Data were analyzed by S-PLUS 6.0 (Insightful Inc, Seattle, Wash) and SAS V8 software (SAS Institute Inc, Cary, NC). For the variables LH and FSH, the natural log transformation was used after adding the respective means. Separate models were formulated to study the relationship between FSH and LH with testosterone before and during hormone replacement. The patients were initially divided into 3 groups: HH with (n =

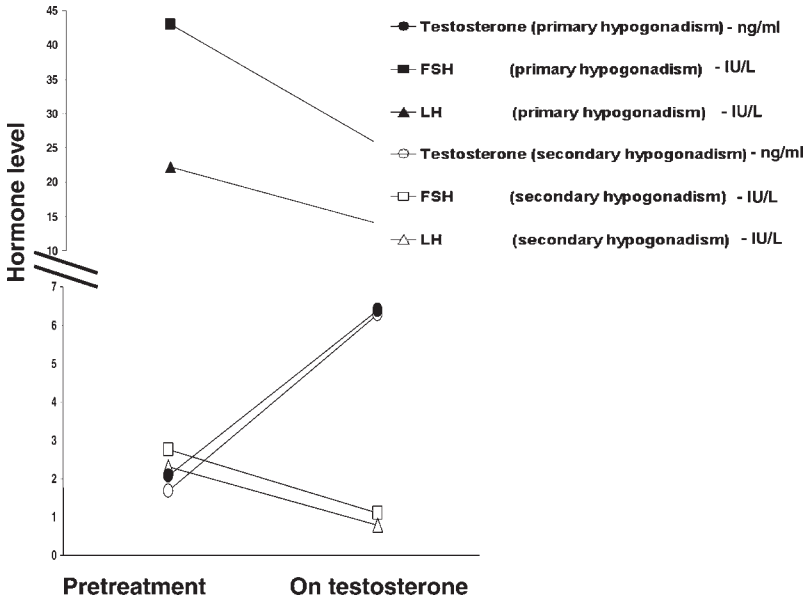

Figure 1. Mean levels (±SD) of total testosterone (circles), FSH (squares), and LH (triangles) before treatment and during treatment with testosterone enanthate injections. Findings for the HH group appear as open symbols, and findings for PH appear as black symbols.

25) and without (n = 13) an established pituitary etiology and PH (n = 11). To detect significant differences in the intercepts and slopes among the 3 groups, we used mixed-effect models, which account for within-subject dependency. First we checked for curvature in the mean function by fitting a quadratic rather than a linear effect. The regression coefficients (results not shown) showed that a second-degree term was not necessary for each group in either model (FSH and LH). The linear-effect analysis of the intercepts and slopes yielded no significant differences in either the LH or FSH model between the 2 HH groups (results not shown); therefore, they were united and studied as a single group.

To test whether a high FSH or LH level significantly decreases the probability of having normal testosterone levels, logistic regression mixed-effect models were used. Three gonadotropin levels (low, medium, and high) served as independent variables. It should be noted that, for a variable with 3 categories, 2 dummy variables are required in the model. If at least 1 of the dummy variables is significant, the categorical variable is expected to be significant as well.

A P value of less than .05 (2-tailed) was considered to be statistically significant, unless otherwise specified.

Results

Figure 1 shows the hormone levels before and after initiation of treatment with testosterone enanthate injections. In the patients with HH, the mean total testosterone level (±SD) increased from 1.7 ± 1.0 ng/mL at baseline (pretreatment) to 6.3 ± 3.8 ng/mL during treatment, and in the PH group, values increased from 2.1 ± 1.6 ng/mL to 6.4 ± 3.8 ng/mL. The mean FSH level (±SD) in the HH group decreased from 2.8 ± 1.4 IU/L at baseline to 1.1 ± 1.1 during treatment, and the mean LH level decreased from 2.3 ± 1.2 to 0.8 ± 1.2 IU/L. Corresponding values in the PH group were as follows: the FSH level decreased from 43.1 ± 31.0 to 25.4 ± 30.8 IU/L, and the LH level decreased from 22.2 ± 20.2 to 13.9 ± 21.5 IU/L.

On statistical analysis, there was a linear correlation between FSH and LH (after natural log transformation for both) and testosterone levels in the HH and PH groups. However, the between-group differences in the intercepts and slopes were significant for both FSH and LH. For FSH, the intercept of the HH group was 16.01, and the intercept of the PH group was 23.87 (t = 3.20; df = 50; P = .0024); the respective slopes were −3.41 and −7.82 (t = 4.40; df = 113; P < .0001). For LH, the intercept of the HH group was 10.70, and the intercept of the PH group was 13.72 (t = 1.98; df = 50; P = .053); the respective slopes were −2.22 and −4.68 (t = 3.37; df = 113; P = .001).

Figure 2a (for FSH) and b (for LH) show the correlation between gonadotropin and testosterone levels in patients with HH and PH. It is noteworthy that the figures represent the original patient data after natural log transformation, which are slightly different from the numbers calculated by the statistical model, and do not take into account the within-observation dependency.

Figure 3 shows the individual regression results for 6 patients with HH. The curves for these subjects had different slopes when measurements of LH were plotted

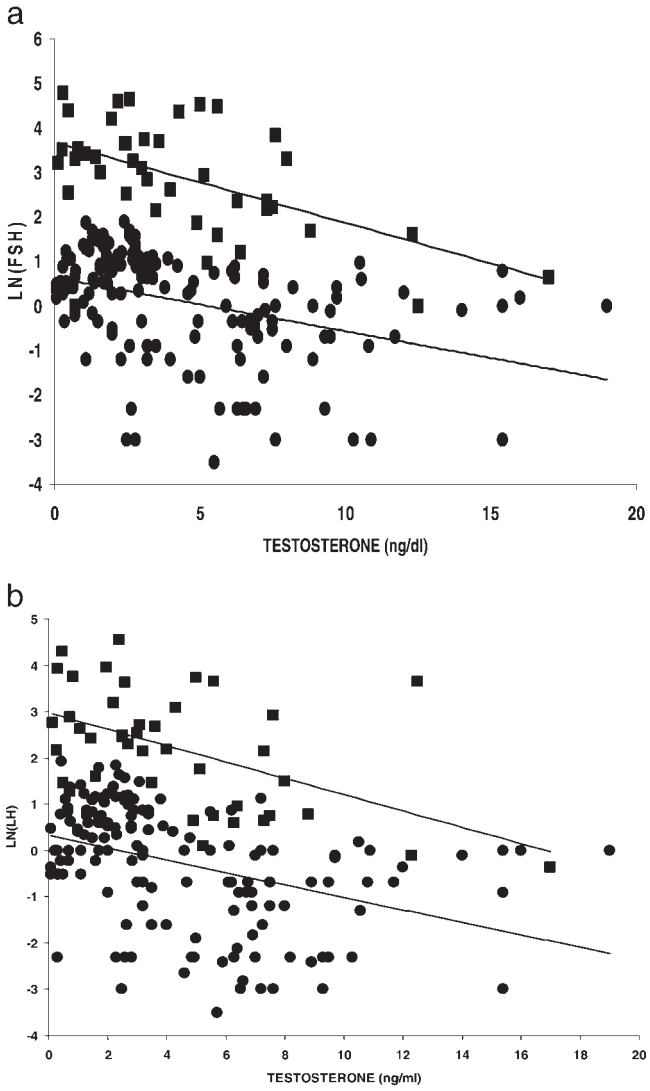

Figure 2. Linear correlation between natural log transformed **(a)** FSH and **(b)** LH curves plotted against the total testosterone curve for patients with HH (black circles) and PH (black squares). Hormone levels were measured several times during testosterone replacement therapy. The figures represent original data for the patients, which are slightly different from the numbers calculated by the statistical model (see "Results").

against testosterone before and during androgen replacement. The findings indicate that, with increasing doses of testosterone and normalization of total testosterone levels, LH will eventually be suppressed to a level representing eugonadism. The rate of suppression of LH (as well as FSH; data not shown) may be different in individuals with secondary hypogonadism who have different LH/testosterone slopes.

In a further analysis, the probability for achieving eugonadism in patients having HH treated with testosterone, as reflected by normal testosterone level ($\geq$3 ng/mL), was assessed among the different gonadotropin (LH and FSH) categories as follows: low, less

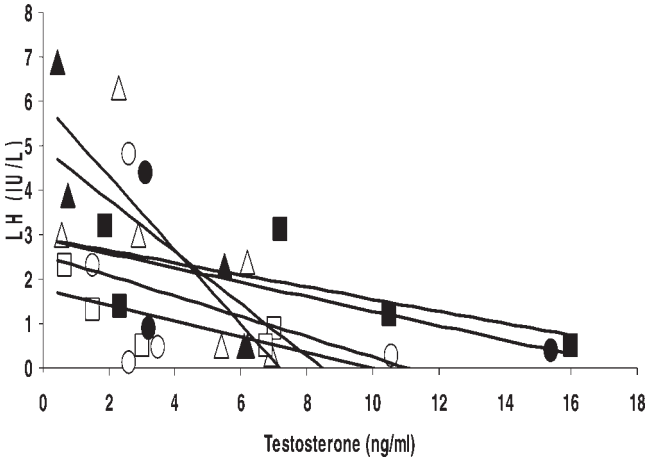

Figure 3. Slopes of LH plotted against total testosterone for 6 male subjects with HH before and during testosterone replacement. Each patient is represented by different symbol.

than 0.5 IU/L; medium, 0.5–2 IU/L; and high, greater than 2 IU/L. As shown in Figure 4, the higher gonadotropin levels were associated with testosterone levels within the normal range in only 17%–19% of cases, compared with 84% when LH and FSH were suppressed below 0.5 IU/L. We then applied a logistic-regression mixed-effect model to test the association of a higher LH or FSH level with the likelihood of a normal testosterone level, wherein the 3 gonadotropin levels (low, medium, and high) served as the independent variables, and the low gonadotropin level as the reference level. The results showed negative values of the coefficient estimates, indicating that higher FSH or LH levels significantly decreased the chances of the

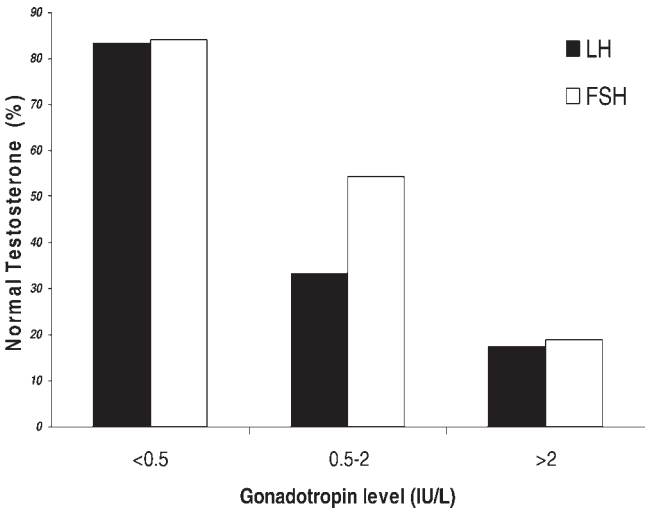

Figure 4. Percentage of total testosterone levels within the normal range (3–10 ng/mL) according to LH and FSH category in treated patients with HH.

Table 2. *Coefficient estimates to assess dependency of testosterone normalization by FSH category*

Parameter	Estimate	Standard Error	Degrees of Freedom	*t*	*P*	95% Confidence Interval
Intercept	1.371	0.455	36	3.01	.0047	0.448 to 2.295
Coefficient of medium level	−2.343	0.732	36	−3.20	.0029	−3.828 to −0.858
Coefficient of high level	−0.912	0.528	36	−1.73	.0925	−1.981 to −0.158

Table 3. *Coefficient estimates to assess dependency of testosterone normalization by LH category*

Parameter	Estimate	Standard Error	Degrees of Freedom	*t*	*P*	95% Confidence Interval
Intercept	1.533	0.401	36	3.82	.0005	0.719 to 2.347
Coefficient of medium level	−2.769	0.714	36	−3.88	.0004	−4.216 to −1.322
Coefficient of high level	−2.145	0.645	36	−3.32	.0020	−3.453 to −0.836

patient to have a normal testosterone level (Tables 2 and 3). On the basis of this model, the estimated probability of achieving eugonadism was calculated for each measurement. The mean value was 0.806 in the low-FSH group, 0.554 in the medium-FSH group, and 0.337 in the high-FSH group. For LH, the corresponding means were 0.819, 0.358, and 0.229.

In 7 men with HH, testosterone treatment was temporarily stopped. The mean testosterone levels (±SD) in this subgroup were 1.7 ± 1.0 ng/mL at baseline and 7.3 ± 2.2 ng/mL during treatment; the FSH level was suppressed from 3.1 ± 1.4 to 1.2 ± 1.0 IU/L, and the LH level was suppressed from 3.2 ± 2.0 to 0.7 ± 1.0 IU/L (Figure 5). After cessation of treatment, the mean testosterone level (±SD) decreased to 1.9 ± 1.4 ng/mL, whereas the FSH level increased to 2.3 ± 1.3 IU/L and the LH level increased to 1.8 ± 1.1 IU/L, indicating the presence of an active feedback inhibitory mechanism between gonadotropins and testosterone.

Discussion

Our data show that, in patients with HH due to acquired destructive processes of the anterior pituitary and a significant decrease in gonadotroph cell number and gonadotropin release, the negative feedback between testosterone and both FSH and LH is maintained. A low or normal gonadotropin (FSH and LH) level is the rule for men with HH, and as shown by others (Salehian et al, 1995; Sheckter et al, 1989; Swerdloff et al, 2000) and in the present study, testosterone replacement suppresses it even further (Figure 1). This finding indicates the presence of a negative feedback mechanism between testosterone and gonadotropins in men with HH, similar to that in healthy men in whom FSH and LH are suppressed in response to exogenous testosterone in supraphysiologic doses (Matsumoto, 1990), as well as in men with PH (Salehian et al, 1995; Swerdloff et al, 2000), albeit at a shifted set point. The linear regression lines of natural log transformed curves for LH and FSH plotted against the testosterone levels in the patients with HH in our study (Figure 2), as well as the inverse correlation between testosterone normalization and gonadotropin level (low, medium, and high) (Figure 4), further emphasize the ability of testosterone to modulate gonadotropin release by the few gonadotroph cells left in the injured pituitary.

The effectiveness of the pituitary-testicular axis feedback, however, is reduced in patients with HH, in both directions. The ostensibly "normal" LH and FSH

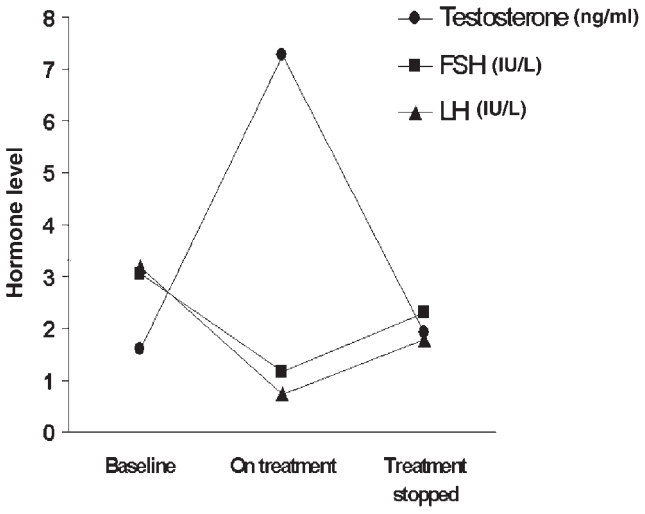

Figure 5. Mean pretreatment (baseline) total testosterone (circles), FSH (squares), and LH (triangles) levels in 7 men with HH during replacement therapy and after testosterone enanthate injections were temporarily stopped.

levels are not enough to induce normal testosterone release. Furthermore, as indicated by the difference in the slopes (natural log transformed LH and FSH vs testosterone; Figure 2) in the HH and PH groups, in addition to the decrease in gonadotroph number in the pituitary, the functioning gonadotrophs may lose some of their sensitivity to the effect of circulating testosterone. Nevertheless, some men with acquired HH treated with testosterone may have persistent spermatogenesis (Drincic et al, 2003), probably because of the low levels of gonadotropins still present.

We have previously reported parallel findings for patients with central hypothyroidism due to anterior pituitary hypofunction (Shimon et al, 2002). As in primary hypothyroidism, thyroid hormone replacement further decreased the baseline TSH levels to below the normal range in patients with central hypothyroidism, and a linear regression was demonstrated between natural log transformed TSH and free thyroxine (FT4) levels in central hypothyroidism. These observations support the notion that, in patients with hypopituitarism and central hypothyroidism, the hypothalamus-pituitary-thyroid axis is still active. In the present study, this was found to be true also for the pituitary-testicular hormonal axis in men with central/pituitary hypogonadism. However, unlike patients with central hypothyroidism in whom thyroid hormone replacement usually continuously maintained physiologic thyroid hormone levels, in patients with HH, the administration of testosterone injections achieved physiologic levels of testosterone only part of the time, usually at the midinjection intervals.

Our study shows that the GnRH-gonadotropin-testosterone axis functions in most subjects with central hypogonadism. Testosterone suppressed LH and FSH, and LH and FSH levels were inversely correlated with normalization of the testosterone level in individual patients. The different slopes for subjects who received replacement may reflect different sensitivities of the hypothalamic-pituitary axis to testosterone. The appropriateness of testosterone replacement therapy for patients with HH is usually reflected by normal testosterone concentrations. However, whether replacement therapy needs to achieve mid-normal or upper-normal values for optimal outcome remains unclear. Ideally, testosterone levels should be in the mid-normal range at the midinjection interval and above the lower limit of normal before the next injection (Matsumoto, 1994). As such, our finding of the suppression of gonadotropins by testosterone in patients with central hypogonadism may have clinical importance. In our sample, LH and FSH levels greater than 2 IU/L were associated with inadequate testosterone replacement in most of the men with HH, and gonadotropin suppression to a level less than 0.5 IU/L usually reflected normalization of the testosterone level (Figure 4). Thus, in the absence of reliable clinical signs of adequate hormone replacement in central hypogonadism, especially when other pituitary hormone deficits exist, monitoring LH and FSH levels during testosterone treatment, together with testosterone, may be important for a comprehensive hormonal follow-up regimen. The comparison of gonadotropin levels before and after testosterone replacement therapy may serve as a useful index, complementary to testosterone levels, for assessing the adequacy of androgen replacement in the individual patient with pituitary hypogonadism (Figure 3). However, suppression of gonadotropin to undetectable levels may reflect androgen overreplacement that is unfavorable and even dangerous, especially in elderly patients (Morales, 2002; Rhoden & Morgentaler, 2004). Moreover, central hypogonadism is commonly associated with deficiencies of other pituitary hormones, including TSH, ACTH, and growth hormone. Thus, glucocorticoids and thyroid hormones should be replaced as early as possible in patients with hypopituitarism, before androgen replacement is initiated.

In summary, in men with HH, the pituitary-testicular hormone axis is usually intact and maintains the physiologic negative feedback between androgens and gonadotropins. This is reflected by the suppression of gonadotropins to lower levels when testosterone is adequately replaced. Together with testosterone serum levels, gonadotropin levels may be important for assessing the adequacy of hormone replacement in the individual patient with hypogonadism.

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