

In Men, Peripheral Estradiol Levels Directly Reflect the Action of Estrogens at the Hypothalamo-Pituitary Level to Inhibit Gonadotropin Secretion

Garrett Raven, Frank H. de Jong, Jean-Marc Kaufman, and Willem de Ronde

Department of Endocrinology (G.R., W.d.R.), Vrije Universiteit Medical Center, 1007 MB Amsterdam, The Netherlands; Department of Internal Medicine (F.H.d.J.), Erasmus Medical Center, 3000 DR Rotterdam, The Netherlands; and Department of Internal Medicine (J.-M.K.), Ghent University Hospital, B-9000 Ghent, Belgium

Context: Estradiol inhibits gonadotropin release in men by an action at the hypothalamus and pituitary. Because of the tissue-specific regulation of aromatase, peripheral estradiol levels may not reflect brain estradiol concentrations.

Objective: We evaluated whether local aromatization of testosterone in the hypothalamus or pituitary is important for gonadotropin release and to what extent circulating estrogens affect gonadotropin levels and peripheral testosterone levels.

Design, Subjects, and Interventions: We suppressed aromatase activity in 10 young healthy men with letrozole 2.5 mg once daily, restored plasma estradiol levels with estradiol patches (100 $\mu\text{g}/\text{d}$ for the first week, 50 $\mu\text{g}/\text{d}$ the second week, 25 $\mu\text{g}/\text{d}$ the third week, and no estradiol patch the fourth week) and measured plasma testosterone, estradiol, LH, FSH, and SHBG levels.

Results: The mean estradiol and testosterone levels during the study ranged between 68.6 ± 38.3 and 12.6 ± 7.21 pg/ml for estradiol and 179 ± 91 and 955 ± 292 ng/dl (mean \pm SD) for testosterone. Levels of testosterone, LH, and FSH were inversely related to peripheral estradiol levels. During letrozole use, the mean plasma estradiol level needed to restore testosterone, LH, and FSH levels to baseline levels was not significantly different from the baseline mean estradiol level.

Conclusions: Local aromatization of testosterone in the hypothalamo-pituitary compartment is not a prerequisite for expression of the inhibitory action of estrogens on gonadotropin secretion in men. Peripheral estradiol levels directly reflect the inhibitory tone exerted by estrogens on gonadotropin release and are a major determinant of peripheral testosterone, LH, and FSH levels. (*J Clin Endocrinol Metab* 91: 3324–3328, 2006)

CIRCULATING TESTOSTERONE levels differ considerably between men (1), and almost 60% of the interindividual variation in testosterone levels is genetically determined (2). The mechanisms behind this genetic determination remain largely unknown. To identify a testosterone level as abnormal, knowledge of these mechanisms is important. One well-known determinant of testosterone levels in healthy men is the body mass index (BMI) (3, 4). The lower testosterone levels in obese men have been attributed to the lower levels of SHBG associated with higher BMI (5) and to increased conversion of testosterone to estradiol in adipose tissue. Aromatization of testosterone can take place throughout the body, including in the brain (6). It is well known that estradiol inhibits gonadotropin release in the male by an action at the hypothalamus and pituitary (7, 8). Aromatase, the enzyme responsible for the conversion of androgens into estrogens has several tissue-specific promoters (9). As a consequence, the extent of androgen aromatization may vary between tissues in one individual, and peripheral estradiol levels might not necessarily reflect estrogen exposure at the level of the pituitary or hypothalamus. Therefore, it remains to be determined whether higher serum estrogen levels in

obese men can be directly related to their lower serum testosterone concentrations. Moreover, in men, gonadotropin and testosterone secretion are powerfully stimulated by administration of an aromatase inhibitor in the face of only moderate decreases of circulating estrogen concentrations (10, 11). This raises the question of whether the observed gonadotropin stimulation during aromatase inhibition can be explained by the decrease of circulating estrogen levels alone or rather reflects local blockade of aromatase activity in the hypothalamo-pituitary tissues.

To get a better understanding of the possible role of the local biosynthesis of estradiol in the hypothalamus or pituitary gland in the regulation of the male hypothalamo-pituitary-gonadal (HPG) axis, we monitored serum gonadotropin and testosterone responses to aromatase inhibition and replacement with different doses of estradiol in young men.

Subjects and Methods

Subjects

The subjects were 10 apparently healthy male volunteers between the ages of 18 and 40 yr. The mean age of the subjects was 29.9 ± 6.47 (range, 20–39) yr; mean BMI was 24.8 ± 2.82 (range, 21.1–29) kg/m^2 . Exclusion criteria were cigarette smoking, pituitary dysfunction, a history of thrombosis, use of any medication, and excessive alcohol abuse. Medical history was not significant, and the result of the physical examination was within normal limits. Levels of testosterone, estradiol, LH, and FSH were within the normal ranges at screening.

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Abbreviations: BMI, Body mass index; HPG, hypothalamo-pituitary-gonadal.

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Study protocol

This study was approved by the Medical Ethics Committee of the Amsterdam Free University Medical Center, and all volunteers gave written informed consent before the start of the study. The study protocol is summarized in Fig. 1. After baseline blood testing, subjects started using letrozole (Novartis AG, Stein, Switzerland), 2.5 mg once daily in the morning for 4 wk. During wk 1–3, estradiol was replaced using estradiol patches (Dermeestril; Sigma- τ Ethirama, Utrecht, The Netherlands) with decreasing doses. The dose of the patches was 100, 50, and 25 μ g estradiol per day, respectively. Patches were applied according to the recommendations of the manufacturer. Patches were replaced on d 4 and 7 of each treatment week or earlier whenever more than 25% of the patch was detached. Blood samples were collected at baseline and at the end of every study week by venipuncture between 0800 and 1100 h to determine LH, FSH, testosterone, estradiol, and SHBG. Blood samples were allowed to clot, and after centrifugation, serum was stored at -20 C until analysis.

Hormone analysis

Levels of LH, FSH, and SHBG were estimated by luminescence-based immunoassays using an Immulite 2000 (Diagnostic Products Corp., Los Angeles, CA). Inter- and intraassay coefficients of variation were less than 4.9, 5.9, and 6.3%, respectively. Testosterone and estradiol were measured using Coat-a-Count RIA obtained from the same supplier. Variation coefficients for the testosterone assay were less than 7.5%. For the estradiol assay, variation coefficients were 11.8% for levels between 2.2 and 23 pg/ml (8 and 87 pmol/liter) and less than 9.8% for higher concentrations. The lowest detectable level, defined as blank values plus 3 SD of the blank, was 1.9 pg/ml (7 pmol/liter). Finally, cross-reactions were 10% for estrone, 1.8% for estrone glucuronide, and less than 0.6% for all other naturally occurring steroids tested.

Male reference values for these assays were 14–54 pg/ml (50–200 pmol/liter) for estradiol, 1.5–8 IU/liter for LH, 2–7 IU/liter for FSH, 290–860 ng/dl (10–30 nmol/liter) for testosterone, and 0.25–1.75 μ g/dl (10–70 nmol/liter) for SHBG.

Statistics

Mean levels for the analyzed hormones and SHBG were calculated and grouped by study week. Differences between mean levels were tested for significance using ANOVA. We combined all measurements under treatment with letrozole and used linear regression analyses to evaluate the relationships between estradiol and LH, FSH, or testosterone, using estradiol as the independent variable. To obtain a linear relationship between estradiol and other parameters, all were log transformed. To evaluate whether the relationships between estradiol and LH, FSH, or testosterone were different in the samples obtained before and during letrozole use, the baseline levels of estradiol were entered in the previously described regression equations. The resulting predicted values for LH, FSH, and testosterone were compared with baseline levels using the Student's *t* test for paired variables.

Results

Hormone and SHBG levels during the study are summarized in Table 1. Estradiol levels were lowest during treatment with letrozole alone. The daily use of 2.5 mg letrozole was associated with a decline of the mean serum estradiol

concentration of 56%. There was a dose-dependent decrease of estradiol levels during estrogen application. During treatment with letrozole and 100 μ g estradiol per day, the mean peripheral estradiol level was supraphysiological. During the treatment period, the mean testosterone level ranged between below normal and high-normal for young males. Levels of LH, FSH, and testosterone mirrored the fluctuations in peripheral estradiol levels. The mean SHBG levels did not vary significantly during the course of the study.

The relationships between estradiol and LH, FSH, or testosterone were nonlinear; the HPG axis appeared to be extremely sensitive to fluctuations when estradiol levels were low. After logarithmic transformation of the hormone concentrations, linear relationships were obtained between the levels of estradiol and the other hormones during treatment with letrozole and estradiol (*white symbols* in Figs. 2–4). The baseline hormone levels, represented by the *black symbols* in Figs. 2–4, visually fitted the line representing the hormone levels obtained under treatment.

In Table 2, the actual baseline levels of testosterone, LH, and FSH are compared with their respective predicted values. As described, these predicted values were calculated using a regression equation based on the results obtained during treatment with letrozole and various doses of estradiol. None of the differences reached statistical significance.

Discussion

In this study, we evaluated whether local aromatization of testosterone in the hypothalamus or pituitary is of importance for the effect of estradiol on gonadotropin release. When the aromatase inhibitor letrozole was applied to healthy young men, estradiol levels declined by 56%, which is in accordance with published results (12). Aromatase inhibition was associated with increased serum levels of gonadotropins and testosterone, which was expected based on previous reports (8, 12), illustrating the inhibitory action of estradiol on gonadotropin release in men. Although it is tempting to ascribe the increased gonadotropin levels to the lower circulating estradiol levels under treatment, such a conclusion is premature because aromatase activity may be different between tissues because of its different tissue-specific promoters (9). This could result in higher local estradiol concentrations in the pituitary and/or hypothalamus compared with the levels in peripheral blood as has been shown to be the case in mammary tumor tissue in postmenopausal women, in whom tissue levels did not differ from those in premenopausal women despite much lower peripheral estradiol levels (13, 14). Similarly, stimulation of gonadotropin secretion under aromatase inhibition might

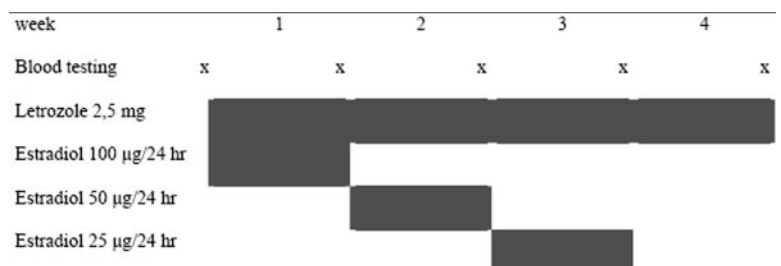


FIG. 1. Study protocol.

TABLE 1. Mean hormone and SHBG levels in studied subjects (mean \pm SD)

| | Baseline | Letrozole + estradiol 100 | Letrozole + estradiol 50 | Letrozole + estradiol 25 | Letrozole |
|--|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|
| Estradiol [pg/ml (pmol/liter)] ^a | 28.7 \pm 7.81 (106 \pm 29) | 68.6 \pm 38.3 (252 \pm 141) | 43.7 \pm 17.9 (161 \pm 65.7) | 26.0 \pm 12.3 (95.4 \pm 45.0) | 12.6 \pm 7.21 (46.2 \pm 26.5) |
| LH (IU/liter) ^a | 4.32 \pm 2.11 | 1.89 \pm 0.93 | 2.91 \pm 1.46 | 4.71 \pm 1.98 | 14.5 \pm 6.01 |
| FSH (IU/liter) ^a | 4.30 \pm 2.18 | 1.40 \pm 0.93 | 2.10 \pm 0.77 | 4.08 \pm 1.83 | 12.0 \pm 6.05 |
| Testosterone [ng/dl (nmol/liter)] ^a | 503 \pm 97 (17.4 \pm 3.37) | 179 \pm 91 (6.21 \pm 3.16) | 339 \pm 86 (11.8 \pm 2.98) | 658 \pm 196 (22.8 \pm 6.79) | 955 \pm 292 (33.1 \pm 10.1) |
| SHBG [μ g/dl (nmol/liter)] | 0.72 \pm 0.23 (28.8 \pm 9.23) | 0.78 \pm 0.26 (31.2 \pm 10.6) | 0.81 \pm 0.26 (32.5 \pm 10.6) | 0.76 \pm 0.25 (30.3 \pm 10.1) | 0.68 \pm 0.21 (27.3 \pm 8.37) |

^a Values in SI units are given in parentheses.

^a $P < 0.001$ for differences among groups.

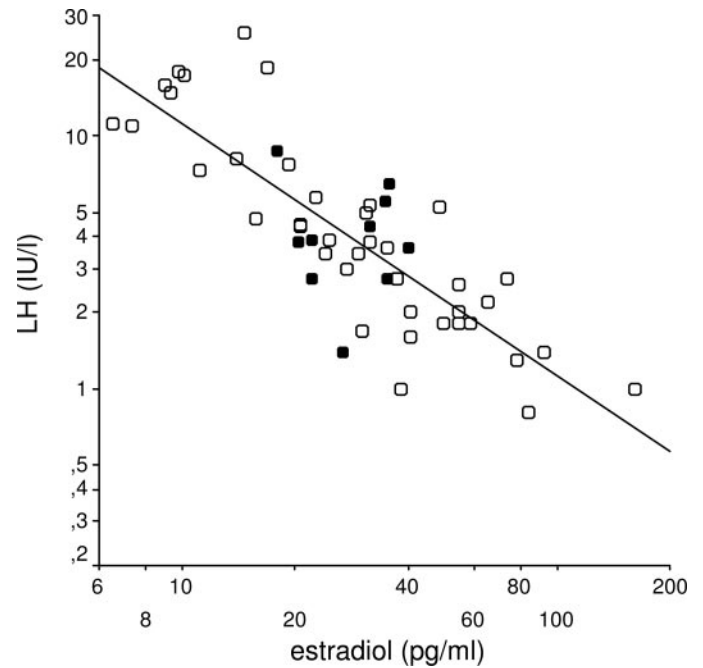


FIG. 2. The relationship between plasma estradiol and LH levels in 10 male subjects. *White symbols*, Hormone levels obtained under treatment with letrozole and various doses of estradiol; *black symbols*, baseline hormone levels. Pearson's coefficient of correlation for the relationship between estradiol and LH during letrozole use (*white symbols*) is -0.87 ($P < 0.001$).

require inhibition of local aromatase activity in the hypothalamo-pituitary tissues besides the achieved moderate lowering of peripheral estradiol serum concentrations. If local aromatization would play a role in feedback on LH and FSH levels, gonadotropin release under aromatase inhibition would be attenuated by estradiol administration only if the resulting peripheral estradiol levels reached the levels normally present in the brain. By inhibiting aromatase activity while at the same time replacing peripheral estradiol levels, the extent of brain aromatization can be evaluated by comparing the estradiol level needed to restore pretreatment LH and testosterone levels with the baseline serum estradiol concentration in the absence of aromatase blockade. This study shows that during aromatase inhibition in men, replacement of the peripheral estradiol concentration to baseline levels is sufficient to normalize gonadotropin and testosterone levels. These results are in accordance with observations in aromatase-deficient men treated with estradiol (15–17). In these men, gonadotropin and testosterone levels responded to estradiol replacement, achieving a circulating estradiol level in the physiological range. However, all showed normal or only moderately elevated levels of LH and testosterone at baseline in the absence of circulating estradiol indicative of an impaired function of their HPG axes. Moreover, FSH levels did not decrease to normal levels after estradiol replacement in two of the patients (15, 16), probably as a result of low inhibin B levels associated with severe impairment of spermatogenesis.

Our study shows that the male HPG axis is very sensitive to circulating estradiol levels. The relationship between estradiol and testosterone or gonadotropin levels was nonlin-

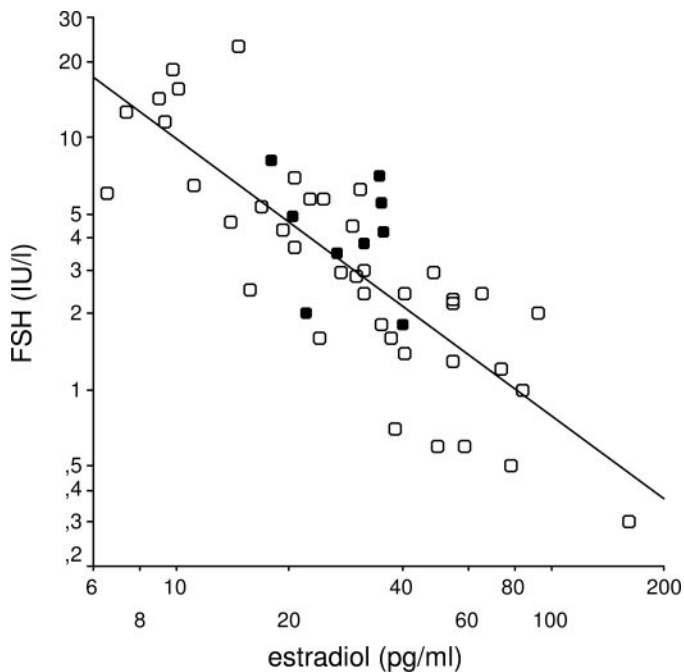


FIG. 3. The relationship between plasma estradiol and FSH levels in 10 male subjects. *White symbols*, Hormone levels obtained under treatment with letrozole and various doses of estradiol; *black symbols*, baseline hormone levels. Pearson's coefficient of correlation for the relationship between estradiol and FSH during letrozole use (*white symbols*) is -0.84 ($P < 0.001$).

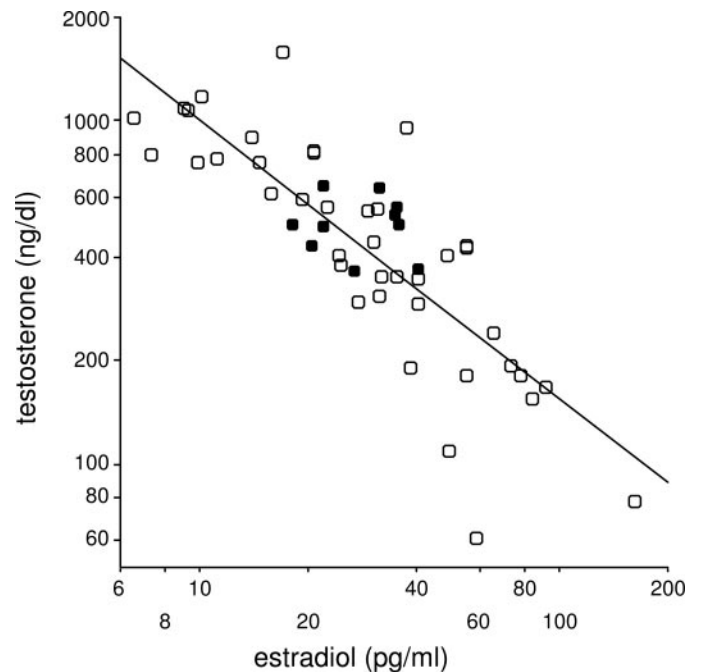


FIG. 4. The relationship between plasma estradiol and testosterone levels in 10 male subjects. *White symbols*, Hormone levels obtained under treatment with letrozole and various doses of estradiol; *black symbols*, baseline hormone levels. Pearson's coefficient of correlation for the relationship between estradiol and testosterone during letrozole use (*white symbols*) is -0.80 ($P < 0.001$).

ear. Higher estradiol levels have increasingly less effect on circulating LH, FSH, and testosterone levels. Varying peripheral estradiol concentrations in the male physiological range resulted in testosterone levels in the low-normal to high-normal range. Although testosterone has an estradiol-independent effect on gonadotropin release (18, 19), high testosterone levels did not prevent gonadotropins from increasing in response to low estradiol levels.

Under normal conditions, only 10–20% of circulating estradiol is directly secreted by the testes; the remaining 80% is the product of peripheral aromatization of testosterone or conversion of estrone (20). The adrenal glands also contribute to circulating estradiol levels, through the production of estrone and androstenedione, which can be converted to estradiol and testosterone, respectively (6). The production of estradiol depends on the activity of the testes and the adrenal glands and the activity of converting enzymes (21). The estradiol concentration will also be determined by the plasma volume and the metabolic clearance rate. The result is a complex interaction between peripheral levels of estradiol and testosterone. The estradiol concentration is largely dependent on testosterone as a precursor but may also inhibit testosterone production through its effect on gonadotropin

release. This explains why in most cross-sectional studies testosterone and estradiol concentrations are positively associated (21). An important difference between the normal physiological situation and the conditions during our study is that here estradiol concentrations were not determined by the serum testosterone concentration. It might be speculated that under normal circumstances, the relation between estradiol and testosterone will weaken the overall effect of estradiol on gonadotropin and testosterone levels. If androgen aromatization increases, for instance as a result of weight gain, higher estradiol levels will result in lower testosterone levels, thereby decreasing the amount of precursor for estradiol synthesis. Obesity is clearly associated with lower levels of both total and free testosterone (4, 22), and the results of the present study tend to support the assumption that this is at least partly mediated by the increased estrogen levels associated with obesity.

This raises an intriguing question: is the male HPG axis primarily driven by circulating testosterone or by estradiol? The results of the present study make a good case for estradiol. However, the increased gonadotropin and testosterone levels in the presence of normal estrogen levels in adult androgen-insensitive subjects indicate that there must be a

TABLE 2. Actual and predicted values for testosterone, LH, and FSH at baseline (mean \pm SD)

| | Actual value | Predicted value | P for difference |
|-----------------------------------|--------------------------------|---------------------------------|------------------|
| LH (IU/liter) | 4.32 \pm 2.11 | 4.27 \pm 1.25 | 0.94 |
| FSH (IU/liter) | 4.30 \pm 2.18 | 3.26 \pm 1.05 | 0.17 |
| Testosterone [ng/dl (nmol/liter)] | 503 \pm 97 (17.4 \pm 3.37) | 438 \pm 105 (15.2 \pm 3.64) | 0.18 |

Values in SI units are given in parentheses.

contribution of an androgen-receptor-mediated effect (23). Also, Hayes *et al.* (24) demonstrated an estradiol-independent effect of testosterone on LH but not on FSH release by the pituitary. Nevertheless, it remains to be determined whether the circulating testosterone, when varied within the male physiological range, has an estradiol-independent, clinically relevant effect on gonadotropin release in men.

In conclusion, circulating estradiol appears to be an important determinant of gonadotropin and plasma testosterone levels in men. The plasma estradiol concentration may help in clinical decision making concerning the cause of low or low-normal testosterone levels in men. However, prerequisite for a correct interpretation is that the estradiol assay used performs adequately in the male physiological range (25) and that the estradiol reference range in men for this assay has been established.

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Address all correspondence and requests for reprints to: W. de Ronde, Department of Endocrinology, VU University Medical Center, de Boelelaan 1117, P.O. Box 7057, 1007 MB Amsterdam, The Netherlands. E-mail: p.deronde@vumc.nl.

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