Evidence of oestradiol-induced changes in gonadotrophin secretion in men with feminizing Leydig cell tumours

Jean Marc Kuhn, Lise Duranteau, Max A Rieu, Najiba Lahlou, Marc Roger and Jean Pierre Luton

Service d’Endocrinologie and European Institute for Peptide Research, Université de Rouen, Bois-Guillaume, France; Service d’Endocrinologie, Hôpital Saint Michel, Paris, France; Hôtel-Dieu, Paris, France; Hôpital Saint Vincent de Paul, Paris, France; Centre de Recherches Endocrinologiques, Hôpital Cochin, Paris, France


To study the sex steroid–gonadotrophin relationship, plasma oestradiol (E2), testosterone and gonadotrophin-releasing hormone (GnRH)-induced (100 μg iv) gonadotrophin response were measured in 42 male partners of infertile couples with normal sperm count (group I) and in 21 men with Leydig cell tumour (LCT, group II) in which a basal evaluation was repeated after tumour removal. Plasma free α-subunit (FAS), immunoreactive α-inhibin and luteinizing hormone (LH) pulse analysis were assessed in 10 LCT before and in six of them after surgery. Testosterone was significantly (p < 0.01) lower whereas E2 was significantly (p < 0.001) higher in group II than in group I. Gonadotrophin data were similar in both groups. The mean FAS was higher in group II than in group I and α-inhibin was higher than the normal range in 6/10 LCT. In group II, E2 levels were significantly (p < 0.01) and negatively correlated with testosterone. FSH, GnRH-induced gonadotrophin rise and LH pulse amplitude but not frequency. Significant (p < 0.001) changes were observed after surgery: E2 and α-inhibin fell; testosterone, LH and FSH rose; whereas FAS did not change significantly. The LH pulse amplitude but not frequency increased significantly (p < 0.05). In conclusion E2 oversecreted by LCT decreased LH and testosterone levels concomitantly. The GnRH-induced gonadotrophin level rose and the LH pulse amplitude decreased when the plasma E2 level rose, whereas the pulse frequency remained unaffected. A concomitant increase in α-inhibin and E2 is likely to be responsible for the drop in plasma FSH levels. These data support an action of excessive amounts of E2 at pituitary level, perhaps by decreasing the sensitivity of gonadotrophs to GnRH.

JM Kuhn. Service d’Endocrinologie, Hôpital de Bois Guillaume, 147 avenue du Maréchal Juin, 76230 Bois Guillaume, France

Gynaecomastia occurs in 10–30% of men with Leydig cell tumour (LCT) of the testis (1, 2). Its occurrence seems to be favoured by the drop in testosterone secretion and/or the oestrogen oversecretion by the tumour. Indeed, a rise in plasma oestradiol (E2) levels, contrasting with reduced testosterone levels, was observed frequently in such tumours (3, 4). It was suggested previously that excessive amounts of E2 might alter testosterone synthesis through a direct effect on the testis (5, 6). On the other hand, pharmacological amounts of oestrogens exert a well-known negative feedback effect on gonadotrophin secretion in men (5, 7–9). Oestrogens appear to be able to reduce FSH secretion from the pituitary (10–12) and to decrease the amplitude of the spontaneous LH pulses without a change in LH pulse frequency (13–15). In most LCT studied, the gonadotrophin responses to gonadotrophin-releasing hormone (GnRH) were in the normal range (4), suggesting that the increased E2 secretion does not reduce the pituitary LH content, but rather affects GnRH secretion. Moreover, oestrogen receptors have been localized on hypothalamic neurons implicated in the negative tone exerted on GnRH-secreting cells (16, 17). Furthermore, an oestrogen-responsive element has been identified recently on human GnRH gene (18), and E2 has been demonstrated to be able to decrease GnRH gene expression in mammals (19). Thus, the decrease in plasma testosterone levels observed in oestrogen-secreting LCT might be linked to a drop in gonadotrophin secretion secondary to an inhibitory action at the hypothalamic or at the pituitary level.

The present study was done in men with oestrogen-secreting LCT in order to evaluate the effect of increased secretion of E2 from the tumour upon gonadotroph cell secretions, i.e. LH, FSH and free α-subunit (FAS), as assessed by the response to acute administration of GnRH used to assess the sensitivity of gonadotrophs to GnRH and by an LH pulsatility study.
Subjects and methods

Subjects studied

Sixty-three adult men were studied, divided into groups I and II.

Group I included 42 male partners (aged 25–39 years) of infertile couples. They were taking no medication and did not have any history of disease. Clinical examination did not show any abnormality. All had a normal sperm count and normal cell motility and morphology, and infertility was reported to a female origin. Testosterone, E2, LH, FSH, immunoreactive α-inhibin (α-INH) and FAS were measured in three different samples under basal conditions from 08.00 to 09.00 h. Ten of them gave their informed consent for GnRH testing (100 µg iv Stimulon, Roussel, Paris, France) and for a pulsatility study that was carried out as follows: after a basal sampling (08.00 h), blood was drawn at 15-min intervals for 8 h. The plasma LH level was measured in all samples and the plasma FSH level at 1-h intervals.

Group II included 21 men (aged 21–54 years) complaining of bilateral gynaecomastia. The patients did not receive any medication and did not have any history of disease or treatment. The diagnosis of LCT was based on clinical and echographic examination of the testes and on hormone data, i.e. increased E2 responses to hCG and β-hCG < 5 IU/l (5). The diagnosis was confirmed in all cases by pathological examination of the removed testis. In all cases, testosterone, E2, LH and FSH (18/21 patients) levels were measured in three different samples under basal conditions from 08.00 to 09.00 h then, following GnRH administration (100 µg iv), blood was collected at 15, 30 and 60 min after injection. Data obtained in some of these patients were published previously (5). Basal hormone levels (testosterone, E2, LH and FSH) were assayed again at least 1 month after surgical removal of the testis tumour.

Ten patients in whom plasma FAS and α-INH also were measured under basal conditions gave their informed consent for a study of spontaneous LH pulsatility, which was performed as in control subjects. Six of them had the same hormone measurements at least 1 month after surgery.

Hormone assays

Plasma testosterone levels were measured by radioimmunoassay after chromatography on a Celite column (Touzart Matignon, France) as described previously (4); the normal range in adult men was 14.7–36.7 nmol/l. Oestradiol was measured by radioimmunoassay as described previously (20); normal values in adult men were below 157 pmol/l. Both LH and FSH were measured by immunometric assay (Amerlite, Kodak, France). The cross-reactivities of FAS and the free β-subunit of LH, FSH and TSH were less than 2%. For LH and FSH the intra-assay variations were 3.8 and 5.9%, respectively, and the interassay variations were 7.9 and 7.5%, respectively. All samples of pulsatility study from the same subjects were analysed in duplicate in the same assay run and in the same laboratory, as for each hormone assay.

Immunoreactive α-INH was measured by radioimmunoassay as published previously (21), using antiserum no. 1989 raised against purified 31-kD bovine INH (22). Purified bovine 31-kD INH iodinated by the lactoperoxidase method (23) was used as a tracer. A pool of human follicular fluid was used as a standard. Its bioactivity was 2.8 × 105 mU/l as assessed by in vitro bioassay with dispersed rat pituitary cells. In this radioimmunoassay 1 mU of the human follicular fluid was equipotent to 8 pg of recombinant human INH. The standard pool was diluted in plasma from castrated subjects in order to achieve the same protein content as in the sample assay tubes. Bovine activin A and the α- and βA-subunits of INH produced by reduction and alkylation showed less than 0.5% cross-reactivity with dimeric INH using this antiserum (22), but the recombinant α-subunit of human INH exhibited a complete cross-reactivity (24). Purified gonadotrophin. FSH, LH and hCG did not cross-react with this antiserum. Plasma dilution curves paralleled the standard curve. The intra- and interassay coefficients of variation at the level 4.2 × 103 mU/l were 2.1 and 6.5%, respectively. The sensitivity was 2.8 × 104 mU/l. The normal range in adult men is 3.3 × 105–7.1 × 105 mU/l.

Plasma immunoreactive FAS levels were measured by a previously described radioimmunoassay (25). The intra- and interassay coefficients of variation were 5.2 and 11.5%, respectively, at the level 0.3 IU/l and 4.0 and 7.2% at the level 3.5 IU/l. The detection limit was 0.04 IU/l. The cross-reactivity of human pituitary LH, FSH and TSH purified on phenyl-sepharose columns (Pharmacia-France, Saint Quentin en Yvelines, France) was less than 2%. The normal range in adult men is 0.2–0.7 µg/l.

Luteinizing hormone pulse detection

Pulses were analysed using the Cluster pulse detection algorithm (26). This program identifies peaks by searching for significant increases and decreases within a hormone concentration series. A cluster configuration of 2 × 2 (two test samples for the nadir and two for the peak) and a t value of 2.0 × 2.0 were chosen to minimize false positive and false negative errors for 14-min data series (31). At each time point the actual experimental error was estimated (from the duplicate pulse analysis) as a constant of 10%.

The pulse frequency was defined as the number of pulses per total observation time (8h) and was extrapolated to 24 h. The pulse amplitude is the difference in hormone levels between the peak maximum and the preceding nadir.
Statistics
Areas under the curve (AUC) were calculated with the trapezoidal rule. Statistical differences were evaluated by applying Student's t-test for paired and unpaired data, as appropriate. Correlations between plasma hormone levels were examined by linear regression study after verification that the normal distribution of sample values was not precluded. Results are given as means ± SEM.

Results
Comparison between group I and group II patients before surgery
Basal hormone levels. The mean plasma testosterone level was significantly (p < 0.01) lower in group II (11.7 ± 1.2 nmol/l) than in group I (26.5 ± 2.4 nmol/l). By contrast, the mean plasma E2 level was significantly (p < 0.001) higher in group II (241.9 ± 18.2 pmol/l) than in group I (95.1 ± 11.4 pmol/l).

The means of plasma LH and FSH levels were no different between group I or group II (LH = 3.1 ± 0.2 and 3.3 ± 0.5 IU/l, respectively; FSH = 2.6 ± 0.2 and 2.2 ± 0.3 IU/l, respectively).

![Fig. 1](image1.png)
**Fig. 1.** Correlation between plasma 17-β oestradiol and either plasma testosterone (upper panel) or FSH levels (lower panel) in men with Leydig cell tumours of the testis studied before surgery.

![Fig. 2](image2.png)
**Fig. 2.** Correlations between 17-β oestradiol and the area under the curve response (AUC) of either LH or FSH to GnRH in men with Leydig cell tumours of the testis studied before surgery.

The mean plasma FAS level was significantly (p < 0.005) higher in group II (0.74 ± 0.14 µg/l) than in group I (0.48 ± 0.04 µg/l).

The mean plasma α-INH was significantly (p < 0.001) higher in group II (978.3 ± 261.6 U/l) than in group I (460.3 ± 26.8 U/l). Plasma immunoreactive α-INH levels were higher than the normal range in six of 10 men with LCT.

Hormone responses to acute administration of GnRH. The mean peak response of LH to GnRH was similar (p > 0.05) in group II (21.3 ± 2.4 IU/l) and in group I (18.8 ± 2.0 IU/l). However, the mean peak response of FSH to GnRH was significantly (p < 0.05) lower in group II (3.5 ± 0.5 IU/l) than in group I (5.0 ± 0.7 IU/l).

Luteinizing hormone pulsatility studies. The mean of the LH pulse frequency was no different between group I and group II (3.1 ± 0.5 and 3.0 ± 0.3 pulse/8 h, respectively). The mean LH pulse amplitude was similar in group II (2.4 ± 1.4 IU/l) and in group I (2.3 ± 0.3 IU/l).

Correlations between hormone parameters in group I. The E2 levels were correlated negatively with testosterone levels (r = -0.48, p < 0.001; Fig. 1). Plasma E2 levels
also were correlated negatively with basal FSH levels ($r = -0.63, p < 0.001$; Fig. 1), as well as with the AUC response of LH ($r = -0.47, p < 0.01$) and of FSH ($r = -0.46, p < 0.01$; Fig. 2) to acute administration of GnRH. In addition, E$_2$ levels were correlated negatively with LH pulse amplitude ($r = -0.63, p < 0.05$; Fig. 3). In contrast, a positive correlation ($r = 0.59, p < 0.005$; Fig. 4) was found between plasma testosterone levels and the AUC response of LH to GnRH. A significant ($p < 0.05$) correlation also was found between the AUC of the LH response to acute stimulation with GnRH and the AUC of the LH pulsatility study.

Fig. 3. Correlations between 17-ß oestradiol and either LH pulse frequency (upper panel) or LH pulse amplitude (lower panel) in men with Leydig cell tumours of the testis studied before surgery.

Fig. 4. Correlation between plasma testosterone levels and the area under the curve response (AUC) of LH to GnRH in men with Leydig cell tumours of the testis studied before surgery.

Fig. 5. Plasma 17-ß oestradiol, FSH, LH and testosterone levels in 17 patients with Leydig cell tumour of the testis, studied before and after removal of the tumour. ** $p < 0.01$. 

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(p < 0.05, respectively (Fig. 5).

In the six patients who underwent a postsurgical pulsatility study, the LH pulse amplitude increased from 2.14 ± 0.10 before surgery to 3.63 ± 0.07 IU/l (p < 0.05) after surgery, whereas the LH pulse frequency did not change (3.83 ± 0.31 vs 3.67 ± 0.33 pulses/8 h) after tumour removal (Fig. 6).

In the same six patients the mean FAS level increased from 0.72 ± 0.12 to 1.28 ± 0.36 µg/l but did not change significantly, and the plasma α-INH level decreased from 1087 ± 166 to 633 ± 68 U/l (p < 0.01) after surgery (Fig. 6).

Discussion

We report here a highly significant negative correlation between plasma E2 and testosterone levels in patients with LCT revealed by gynaecomastia, suggesting that increased E2 production originating from the tumour might be responsible for the fall in testosterone secretion by the normal endocrine testis. The E2 oversecretion may decrease testosterone secretion either through a direct action on testosterone synthesis or through inhibition of gonadotrophin secretion, as shown by the effects of endogenous or exogenous oestrogens in men (5–9).

In subjects with LCT, hCG administration induced a dramatic E2 rise whereas hCG-induced testosterone secretion was similar to that seen in normal men (3, 4, 27); this does not favour a direct effect of E2 on testosterone synthesis at a testicular level. On the other hand, despite normal basal LH levels, the negative correlations found between plasma E2 levels and LH secretory parameters (AUC after GnRH and spontaneous pulse amplitude), as well as between E2 levels and FSH secretory parameters, strongly argue for an action of the tumour-produced E2 on the gonadotrophs. Hormone data obtained after removal of the tumour are consistent with such a mechanism.

Exogenously given oestrogen is able to reduce the importance of the LH surge in response to endogenous GnRH (14, 28, 29). Such an action of E2 may be due to a reduction of the sensitivity of the gonadotroph cells to GnRH. Oestrogen receptors have been localized in the pituitary gonadotrophs in mammals (17, 18, 30), and it was shown previously that E2 decreases gonadotroph sensitivity and responsiveness to GnRH through a receptor-mediated mechanism in both rat and man (12, 31, 32). As a consequence of LH suppression, testosterone secretion is reduced in a dose-dependent manner, as evidenced by the positive correlation found between testosterone and the AUC of LH after GnRH stimulation. For similar reasons, the drop in basal plasma FSH levels and the decrease in FSH rise following acute administration of GnRH, observed in our LCT subjects, may be the result of an inhibitory

Group II after surgery

The E2 level dropped to 107.2 ± 8.1 pmol/l (p < 0.001) and the testosterone level increased significantly (p < 0.001) to 20.1 ± 2.0 nmol/l (Fig. 5). Plasma E2 levels were normalized in all patients, and plasma testosterone levels were normalized in all but three patients in whom testosterone remained below the

normal range. Basal plasma LH and FSH levels increased and reached 7.1 ± 0.8 and 8.6 ± 1.9 IU/l (p < 0.01), respectively (Fig. 5).

Fig. 6. LH pulse amplitude and frequency and plasma immunoreactive inhibin and free α-subunit levels in six patients with Leydig cell tumour of the testis, studied before and after removal of the tumour. *p < 0.05. **p < 0.01.
action of E$_2$ at the pituitary level. Oestrogen-induced suppression of pituitary FSH synthesis and secretion has been suggested previously by in vitro and in vivo studies (10–12, 33).

It should be noted that in most of our cases plasma LH and FSH levels were either in the normal range or moderately decreased. Both androgens and oestrogens can act at the pituitary level. As suggested previously in hCG-secreting tumours, it is possible that, at a plasma E$_2$ level similar to that observed in LCT, only a combined oversecretion of both testosterone and E$_2$ can lead to complete suppression of gonadotrophin secretion (34).

The frequency of spontaneous LH pulses reflects endogenous GnRH secretion (28, 35, 36). In men with LCT, the LH pulse frequency was no different from that in healthy men and was not modified after hemicastration. These results agree with several studies showing no E$_2$-induced change in LH pulse frequency in men (7, 13, 37). Thus, changes in LH and FSH secretion observed in men with LCT do not appear to be linked to an action of E$_2$ on the rhythm of pulsatile GnRH secretion.

Elevated plasma immunoreactive α-INH levels were found in six of 10 patients studied. The antiserum used cross-reacts with the free α-subunit of INH and the elevated plasma α-INH levels may be due partly to an increase in free α-subunit secretion as well as an increase in the dimeric form. However, the presence of circulating free α-subunit of INH has not been demonstrated clearly in the human male (24). The increase in plasma α-INH levels found in LCT suggests either that α-INH may be secreted by the tumour cells, as shown for normal Leydig cells (38), or that tumoural Leydig cells may stimulate α-INH secretion by normal Sertoli cells (39). Inhibin may play a role in decreasing plasma FSH levels in men with LCT, as shown in the macaque and in the rat (40, 41). No significant correlation, however, was found between plasma FSH and INH levels in our patients.

Elevated plasma FAS levels found in men with LCT by comparison to healthy men may be secondary to the decrease in plasma testosterone levels. Testosterone has been shown to reduce FAS through a direct effect on the pituitary, whereas E$_2$ did not change the secretion of FAS or its mRNA levels in rat pituitary cells in culture (42). Such a regulatory mechanism might explain the fact that in our patients high F$_2$ levels were associated with the decrease in GnRH-induced LH secretion and that FAS levels were increased simultaneously as a consequence of low testosterone levels. After surgery, the gonadotroph cell secretions were increased as a consequence of hemicastration and it is well known that FAS secretion is enhanced together with gonadotrophin secretion in such a situation.

In summary, our data show that in subjects with feminizing Leydig cell tumour of the testis, E$_2$ oversecreted by the tumour induces a significant decrease in LH secretion, probably at pituitary levels, with a concomitant reduction in plasma testosterone levels. Leydig cell tumour therefore seems to be a unique model of indirect modulation of testosterone secretion by endogenous E$_2$. On the other hand, FAS secretion seems to be more sensitive to the reduction of testosterone levels than to the increase in E$_2$ levels. In addition, we report here for the first time an increase in α-INH secretion in LCT, which would be responsible, in association with increased plasma E$_2$ levels, for the reduction of both basal FSH levels and FSH responsiveness to GnRH.

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