Changes in the bioactivity to immunoreactivity ratio of circulating luteinizing hormone in impotent men treated with testosterone undecanoate

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Abstract. Testosterone undecanoate was administered orally (80 mg twice daily) for 30 days to 10 impotent men with mild Leydig cell failure, age 28 to 42 years. Placebo was administered for 30 days both before and at the end of testosterone undecanoate therapy. Serum levels of bioactive LH, immunoreactive LH and testosterone were determined in basal conditions (day zero), 30 days after the first placebo administration, at the 15th and 30th day of testosterone undecanoate therapy, and at the end of the second treatment with placebo (90th day). Bioactive LH was measured by a sensitive and specific in vitro bioassay based on testosterone production by mechanically dispersed mouse Leydig cell preparations. Immunoreactive LH and testosterone were determined by a double-antibody RIA technique. The results were compared with those obtained in 30 untreated normal young men. In the basal state, serum concentrations of immunoreactive LH were significantly higher in the patients (P < 0.02) than in control subjects, whereas testosterone levels were significantly lower (P < 0.001) in the impotent men. In contrast, bioactive LH levels and the bioactive LH to immunoreactive LH ratios were similar in the two groups. In the patients, at the 15th day of treatment with testosterone undecanoate, serum levels of testosterone and bioactive LH were significantly higher (P < 0.01) than basal values, whereas immunoreactive LH concentrations showed no significant changes. Consequently, the bioactive LH to immunoreactive LH ratios rose significantly (P < 0.01). At the 30th day of treatment with testosterone undecanoate, the mean value of bioactive LH and the mean bioactive LH to immunoreactive LH ratio were significantly higher (P < 0.01) in the patients than in control men, whereas the mean levels of testosterone and immunoreactive LH were similar in the two groups. Neither the first nor the second treatment with placebo changed the hormone values observed in basal conditions. The results support the experimental evidence that androgens may increase the bioactivity of circulating LH.

Experimental evidence indicates that testicular sex steroid hormones act at the pituitary level to increase luteinizing hormone bioactivity. The fall in LH bioactivity to immunoreactivity (B/I) ratio found in male rats after castration was in fact prevented by testosterone (T) (Solano et al. 1980) or dihydrotestosterone (DHT) (Dufau et al. 1982) administration. Few studies, however, have been carried out to demonstrate similar relationships between androgens and the LH bioactivity in men. Beitins et al. (1981) reported a T-induced rise of the LH B/I ratio in a hypogonadal patient with an immunologically active but biologically inactive LH. More recently, we have shown that the B/I ratio of circulating LH rose in response to T administration in a patient with Klinefelter's syndrome (Montanini et al. 1987).
In order to investigate this aspect further, we analysed the effects of testosterone undecanoate (TU) administration on the LH B/I ratios in 10 young impotent men with mild Leydig cell failure, characterized by low T levels, high-normal concentrations of immunoreactive LH (I-LH) and high I-LH to T ratios.

Patients and Methods

Subjects
Ten young men, aged 20 to 42 years (mean ± sd = 30.3 ± 6.6) volunteered for this study. All the patients complained of erectile impotence, along with a variable loss of desire. The time of impotence ranged from 14 to 42 months. A decrease in the number and duration of erectile episodes was demonstrated by nocturnal penile tumescence monitoring in all patients, whereas vascular diseases were excluded after Doppler studies. In the patients, basal morning T concentrations (3.7 to 14.6 nmol/l) were low, when compared with the normal range (16.7 to 31.9 nmol/l) calculated in our laboratory from 80 healthy young men, aged 18 to 46 years. Eight out of the 10 patients had I-LH serum levels (6.0 to 12.0 IU/l) within the normal range (4.0 to 15.0 IU/l), whereas 2 impotent men had increased I-LH concentrations (16.0 and 21.0 IU/l). Nevertheless, the I-LH to T (I-LH/T) ratios were higher in the impotent men (0.74 to 2.69) than in control subjects (0.23 to 0.47), suggesting that an intrinsic Leydig cell defect (Warner et al. 1985) was responsible for the low T serum levels. Basal levels of immunoreactive follicle-stimulating hormone (FSH) were normal (6.0 to 17.0 IU/l) in 8 out of the 10 patients (normal range: 5.0 to 20.0 IU/l). Two patients, who had undergone surgical orchidopexy for bilateral cryptorchidism, had increased serum FSH concentrations (26.0 and 34.0 IU/l). Basal levels of immunoreactive PRL (2.0 to 6.0 nmol/l) and estradiol (E₂) (75.6 to 180.0 pmol/l) were normal in all patients (normal range: PRL = 2.0–6.0 nmol/l, E₂ = 72.0–252.0 pmol/l). The mean values (± sd) of FSH, PRL, and E₂ were, respectively, 13.9 ± 9.4 IU/l, 3.1 ± 1.1 nmol/l and 113.4 ± 37.2 pmol/l. None of the impotent men had diabetes mellitus, thyroid diseases or adrenal dysfunctions, and none of them took any drugs known to alter the endocrine function for at least 6 months prior to the study.

Study protocol
Each patient received placebo for 30 days, then TU (Andriol™, Organon, Rome, Italy), 80 mg twice each day, po, for 30 days, and placebo again for 30 days. Venous blood samples for endocrine evaluation (3 samples at 15-min intervals), drawn between 08.00 and 09.00 h after an overnight fast, were obtained prior to the first placebo administration (day zero), 30 days after placebo (30th day), during TU therapy (45th day), and at the end of TU (60th day) and placebo (90th day) treatment. Blood samples were allowed to clot at room temperature and centrifuged. Sera were kept frozen at −20°C until assayed for bioactive LH (B-LH), I-LH, T, FSH, PRL and E₂ as described below.

B-LH, I-LH and T values, measured in basal conditions and after placebo and TU administration, were compared with the basal concentrations obtained in 30 normal volunteering young men (mean age ± sd = 23.8 ± 4.5 years). In each control subject, 3 venous blood samples were drawn at 15-min intervals between 08.00 and 09.00 h after an overnight fast.

The investigation was conducted in accordance with the principles of the Declaration of Helsinki II.

Hormone assays
A sensitive and specific in vitro bioassay method, based on T production by preparations of mechanically dispersed mouse Leydig cells (Celani et al. 1984), was employed to assess B-LH serum levels. The 2nd IRP bio 78/549 (NIBSC, London) was used as the standard preparation. The volumes of serum employed in the bioassay ranged from 0.01 to 0.02 ml/tube, in a final assay volume of 0.3 ml/tube. As suggested by Rajalakshmi et al. (1979), using these low volumes of serum, the contribution of the preformed T to the total T production by mouse interstitial cells is negligible. Serum I-LH concentrations were determined by a double-antibody radioimmunoassay technique (Celani et al. 1984). The standard employed was a pituitary preparation calibrated by RIA against the 2nd IRP HMG (Biodata, Rome, Italy). Since 78/549 and the Biodata standard gave parallel dose-response curves in the bioassay, a conversion factor of 1.07 (± 0.05), obtained by repeated determinations of the biopotencies between the two reference preparations, was employed to convert the bioassay results, expressed in terms of 78/549, to equivalents of the 2nd IRP HMG (Celani et al. 1984). Serum T was assayed after ether extraction by a RIA method using an antibody against T-3-carboxyoxime BSA (Celani et al. 1987). The sensitivity, intra- and inter-assay coefficients of variation were, respectively: 29 × 10⁻⁶ IU, 4.1% and 10.9% for LH in vitro bioassay; 500 × 10⁻⁶ IU, 8.1% and 10.4% for LH RIA; 3 × 10⁻⁶ nmol, 6.2% and 11.5% for T RIA. Serum concentrations of FSH, PRL and E₂ were assayed using commercial kits. The maximum intra- and inter-assay variations were 11 and 13%, respectively.

Statistics
The results are expressed as mean values ± sd. For all hormones assayed, each value was the arithmetic mean of 3 samples taken at 15-min intervals. The B-LH/I-LH (B/I) ratio was calculated for each individual serum sample. Unpaired Student's t-test, Dunnet's test and linear regression analysis were employed for statistical evaluation. P-values less than 0.05 were considered significant.
Table 1.
Effects of placebo and testosterone undecanoate (TU) administration on bioactive (B)-LH, immunoactive (I)-LH, B/I and testosterone (T) values in 10 impotent men. Comparison with the mean basal values in 30 untreated control subjects.

<table>
<thead>
<tr>
<th>Basal values</th>
<th>Placebo</th>
<th>Impotent men</th>
<th>Placebo</th>
<th>Control subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-LH (IU/l)</td>
<td>19.3 ± 9.4</td>
<td>23.1 ± 11.9</td>
<td>40.3 ± 17.2** d</td>
<td>36.2 ± 16.1*</td>
</tr>
<tr>
<td>I-LH (IU/l)</td>
<td>11.1 ± 4.3b</td>
<td>12.0 ± 3.7c</td>
<td>9.9 ± 4.1</td>
<td>8.7 ± 2.8</td>
</tr>
<tr>
<td>B/I ratio</td>
<td>1.9 ± 1.1</td>
<td>2.0 ± 1.1</td>
<td>4.6 ± 2.7** c</td>
<td>4.2 ± 1.7**</td>
</tr>
<tr>
<td>T (nmol/l)</td>
<td>9.3 ± 3.3d</td>
<td>9.7 ± 3.4d</td>
<td>17.0 ± 5.4** c</td>
<td>22.7 ± 7.6**</td>
</tr>
</tbody>
</table>

Values are means ± sd. * P<0.05 and ** P<0.01 vs basal values (Dunnet’s test); a: P<0.05, b: P<0.02, c: P<0.01 and d: P<0.001 vs control subjects (unpaired Student’s t-test).

Results
As evident from Table 1, mean T concentrations in basal conditions were significantly lower in the patients (P<0.001) than in control subjects, whereas mean I-LH serum levels were significantly higher (P<0.02) in the former group. The I-LH/T ratios were also significantly higher in the impotent men (1.34 ± 0.69) than in the controls (0.35 ± 0.15, P<0.001). No significant differences for B-LH and LH B/I values were observed between the two groups.

In the patients, the first placebo administration did not significantly alter the hormonal parameters. In contrast, after 15 days of TU therapy, T concentrations rose significantly from basal values (P<0.01). This rise was coupled with a significant increase in B-LH levels (P<0.01), whereas the TU-induced decrease of I-LH concentrations was mild and not significant. Consequently, a significant increase in LH B/I ratios (P<0.01) was observed. At the end of TU treatment, T, B-LH and LH B/I values were still higher than those in the basal state (P<0.01, P<0.05 and P<0.01, respectively). At this time, mean B-LH and LH B/I values were significantly higher in the patients (P<0.001) than in controls.

Table 2.
Basal B-LH, I-LH, B/I and T values observed in the individual impotent man before and at the end of treatment with testosterone undecanoate (TU) (80 mg twice daily).

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (years)</th>
<th>Before TU treatment</th>
<th>At the end of TU treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B-LH (IU/l)</td>
<td>I-LH (IU/l)</td>
<td>B/I ratio</td>
</tr>
<tr>
<td>1</td>
<td>26</td>
<td>30.1</td>
<td>12.0</td>
</tr>
<tr>
<td>2</td>
<td>29</td>
<td>11.4</td>
<td>9.0</td>
</tr>
<tr>
<td>3</td>
<td>32</td>
<td>25.7</td>
<td>6.0</td>
</tr>
<tr>
<td>4</td>
<td>28</td>
<td>7.2</td>
<td>8.5</td>
</tr>
<tr>
<td>5</td>
<td>31</td>
<td>35.3</td>
<td>16.0</td>
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<tr>
<td>6</td>
<td>42</td>
<td>15.7</td>
<td>10.0</td>
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<tr>
<td>7</td>
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<td>21.0</td>
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</tr>
<tr>
<td>8</td>
<td>40</td>
<td>10.9</td>
<td>9.0</td>
</tr>
<tr>
<td>9</td>
<td>25</td>
<td>11.3</td>
<td>11.0</td>
</tr>
<tr>
<td>10</td>
<td>30</td>
<td>24.0</td>
<td>9.0</td>
</tr>
</tbody>
</table>

| X ± sd      | 30.3 ± 9.3  | 11.1 ± 1.9 | 9.3 ± 3.5 | 22.7 ± 7.6 |

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trol men, whereas mean T and I-LH levels were similar in the two groups. After the second placebo administration, all the hormone values became similar to those observed in basal conditions (Table 1). Basal B-LH, I-LH, B/I and T values observed in each individual patient before and at the end of treatment with TU are presented in Table 2.

Serum FSH, PRL and E₂ levels did not shown any significant change throughout the study period.

When B-LH and LH B/I values were plotted against T concentrations, significant positive relationships were found (r = 0.664, \( P < 0.001 \), and \( r = 0.534, \ P < 0.001 \), respectively).

**Discussion**

It has been emphasized that sex steroid hormones are able to modulate the bioactivity of gonadotropins relative to their immunoreactivity. Estrogen administration to patients with Turner's syndrome (Lucky et al. 1979; Celani et al. 1985) or to postmenopausal women (Chang et al. 1984) was followed by a significant fall in the B/I ratios of circulating LH. This was ascribed to a direct negative effect of estrogens on LH bioactivity at the pituitary level and/or to the estrogen-dependent reduction of the LH secretion rate (Dufau et al. 1982). An opposite action of androgens, i.e. a positive effect on LH bioactivity, has been suggested by indirect evidence. The progressive rise of the LH B/I ratios found in normal boys during sexual maturation (Lucky et al. 1980; Celani et al. 1983) showed a positive relationship with the parallel increase in T serum levels (Marrama et al. 1983). In contrast, a negative relationship between the failing T levels and the B/I ratios of circulating LH molecules was demonstrated in ageing men (Marrama et al. 1984). As pointed out by Warner et al. (1985), however, two possible explanations for these results may exist. The rising T levels in boys and the low T concentrations in elderly men could have increased or reduced, respectively, the LH B/I ratios. Alternatively, the increase or decrease of LH bioactivity could be responsible for the opposite changes in T concentrations.

The TU-induced enhancement of LH biopotency described in hypogonadal men by Beitins et al. (1981) and by Montanini et al. (1987), as well as the results of the present study, are consistent with the former hypothesis. The 10 impotent men with mild Leydig cell failure had low T serum levels and high I-LH serum concentrations, when compared with normal men. The LH B/I ratios, however, were similar in the two groups. According to Dufau et al. (1982), the latter finding could be related to the increased rate of LH secretion from the pituitary, responsible for the release of LH molecules with high bioactivity. After TU administration, a significant rise in T and B-LH serum concentrations was observed, whereas I-LH serum levels showed no significant changes. Therefore, the B/I ratio of circulating LH rose significantly in response to TU therapy. Because I-LH serum levels were relatively unaffected by TU administration, in agreement with experimental studies (Dufau et al. 1982), it seems that androgen therapy per se, rather than changes in the LH secretion rate, was responsible for the increased LH B/I ratios.

The TU-induced rise of LH bioactivity could be due to the release from the pituitary into the circulation of more glycosylated LH molecules (Dufau et al. 1982). The more glycosylated gonadotropins possess in fact an increased biological activity, both in vivo (decreased metabolic clearance rate) (Vaitukaitis et al. 1976) and in vitro (enhanced cyclic AMP production) (Manjunath & Sairam 1982). Unfortunately, no studies on the chromatographic behaviour and isoelectric focusing profiles of the hormone after TU therapy have been carried out in men to confirm this suggestion.

The choice of different standard preparations for the bioassay and the immunoassay of LH in our study seems not to be involved in the changes of LH B/I ratios. It has been reported, in fact, that the age- and sex-related differences in LH B/I ratios, induced by variations in the steroid 'milieu', persist regardless the standard preparations used (Lichtenberg et al. 1982; Celani et al. 1983).

In conclusion, it appears that androgens are able to exert a direct positive modulatory effect on the bioactivity of human LH. Further studies, however, are needed to explain the biochemical basis of this action.

**References**


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