Administration route-dependent effects of estrogens on IGF-I levels during fixed GH replacement in women with hypopituitarism

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Abstract

Objective: GH-deficient women using oral estradiol treatment require higher doses of recombinant human GH (rhGH) to achieve similar IGF-I levels when compared with men and women on transdermal estradiol replacement. The aim of this study was to evaluate the effects of oral versus transdermal estrogen administration at similar plasma estradiol levels on IGF-I, IGF-binding protein-3, and sex hormone-binding globulin (SHBG) concentrations.

Design: Parallel crossover study in which two groups of hypogonadal and GH-deficient women with fixed and stable rhGH replacement passed through four different estradiol treatment schemes (2 and 4 mg oral, and 50 and 100 μg transdermal estradiol) with a duration of four cycles each to ensure a new steady state. Group I (18 patients using oral estradiol prior to the study) was treated with oral followed by transdermal estradiol and group II (five patients with transdermal estradiol prior to inclusion) with transdermal followed by oral estradiol.

Results: Estradiol concentrations were lowest during 50 μg transdermal and highest during 4 mg oral estradiol treatment. Estradiol concentrations did not differ during 100 μg transdermal and 2 mg oral treatment. Nevertheless, IGF-I levels were significantly higher during 100 μg transdermal when compared with 2 mg oral treatment (P = 0.005 in group I and 0.02 in group II), while SHBG levels were significantly lower (P = 0.002 in group I and P = 0.004 in group II). SHBG and IGF-I concentrations were negatively correlated (R = −0.41, P = 0.0001).

Conclusion: During fixed GH replacement, the route of estrogen administration is a determinant of IGF-I levels in hypogonadal GH-deficient women.

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Introduction

Clinical studies have demonstrated clear effects of estrogens on growth hormone (GH) production and insulin-like growth factor (IGF)-I levels. Estrogens amplify GH-releasing hormone (GHRH) and insulin-stimulated GH release and spontaneous 24 h GH production is threefold higher in healthy premenopausal women than in healthy matched men (1, 2). Women on estrogen replacement require higher doses of recombinant human GH (rhGH) replacement than eugonadal women and men to achieve similar IGF-I concentrations (3–5).

Conversely, discontinuation of oral estrogen substitution increases IGF-I levels during continued substitution with rhGH in female patients with hypopituitarism (6). The route of estrogen administration also affects IGF-I levels. A switch from oral to transdermal estrogen therapy increases IGF-I levels and amplifies the IGF-I response during incremental doses of rhGH (7, 8). In adult women with GH deficiency (GHD) on a stable rhGH replacement dose, IGF-I levels increased during a switch from oral to transdermal 17β-estradiol therapy, however, together with a decrease in serum levels of estradiol (7).

Although it has been suggested that these differential effects of transdermal and oral estradiol on the GH/IGF-I axis are due to a first-pass effect of oral estradiol, prior studies in GH-deficient women on stable rhGH replacement were never aimed at identical serum estradiol concentrations. In order to differentiate between the effects of serum estradiol concentrations per se and the route of estrogen administration on IGF-I levels in hypogonadal GH-deficient women, we designed a study to investigate the effects of different doses of oral estradiol (2 and 4 mg/day) and different doses of transdermal estradiol (50 and 100 μg/day) aimed at identical serum estradiol concentrations on serum concentrations of IGF-I, IGF binding protein-3, and
sex hormone-binding globulin (SHBG) during rhGH replacement with a fixed dose.

**Patients and methods**

**Patients**

Twenty-three women with GHD and gonadotropin deficiency were included. Patients were recruited at the Outpatient Clinic of the Leiden University Medical Center and the University Medical Center in Utrecht, the Netherlands.

Inclusion criteria were:

1) GHD defined by a peak GH concentration $< 3 \mu g/l$ during the insulin tolerance test (nadir blood glucose $< 2.2 \text{mmol/l}$),
2) stable rhGH replacement during at least 3 months,
3) oral or transdermal estradiol treatment because of gonadotropin deficiency, and
4) written informed consent.

Patients were enrolled in the two study groups based on their pretreatment (oral and transdermal estradiol), with an individualized dose of GH replacement aimed at achieving a normal serum IGF-I for age. Eighteen patients started with oral estradiol (of whom 15 completed the study) and five patients with transdermal estradiol.

**Clinical details are shown in Table 1.** The mean dose of rhGH replacement during the study was $0.75 \pm 0.28 \text{mg/day}$ (range 0.3–1.3 mg/day). Mean duration of rhGH replacement prior to the start of the study was 6 years (range 1–13 years). Conventional substitution therapy was monitored and held stable during the study. The study protocol was approved by the local Ethics Committees.

**Study design**

The study was designed as a parallel crossover study. Patients were divided into two groups based on the route of administration of estrogens prior to beginning the study (Fig. 1). The dose of rhGH that had been individually titrated aimed at achieving a normal serum IGF-I for age at the estrogen treatment used prior to inclusion, and during the study this rhGH replacement dose was kept stable.

To avoid carryover effects and to ensure a new steady state, the duration of each estradiol treatment was four cycles of 28 days. In group I, the baseline oral estradiol treatment of 2 mg was first increased to 4 mg, and thereafter patients passed through the following treatments: 2 mg oral, and 100 and 50 mg transdermal estradiol. In group II, the baseline treatment of 50 mg transdermal estradiol was sequentially increased to 100 mg transdermal, and 2 and 4 mg oral estradiol. Because of the necessity to achieve a new steady state at the highest oral estradiol dose, the protocol was four cycles longer in group I. Study parameters during the

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Characteristics of patients with growth hormone (GH) and gonadotropin deficiency included in the study.</th>
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<tbody>
<tr>
<td>Group</td>
<td>Age</td>
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<td>1 OR</td>
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<td>3 OR</td>
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<td>64</td>
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<td>7 OR</td>
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<td>20 TD</td>
<td>41</td>
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<td>21 TD</td>
<td>54</td>
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<td>22 TD</td>
<td>49</td>
</tr>
<tr>
<td>23 TD</td>
<td>48</td>
</tr>
</tbody>
</table>

OR, patients starting with oral estrogen treatment; TD, patients starting with transdermal estrogen treatment; TSS, transsphenoidal surgery; RT, radiotherapy; TCS, transcranial surgery.
two periods of estradiol administration (cycles 1 and 9) did not differ, excluding a carryover effect.

Study parameters were measured on day 12 of cycle 1, before dydrogesteron was added, and during each fourth cycle of 28 days of stable estrogen therapy during the subsequent cycles 5, 9, and 13 in group I and II and cycle 17 in group I only.

**Study medication**

Estradiol (2 and 4 mg Estrofem; Novo Nordisk Farma BV, Alphen aan den Rijn, The Netherlands and 50 and 100 μg Dermestril; Sigma Tau Ethifarma BV) was given with additional dydrogesteron (10 mg Duphaston, Solvay Pharmaceuticals, Weesp, The Netherlands) from days 15 to 28. Transdermal estrogen patches were used every Monday and Thursday at fixed time points. Tablets were taken every day at fixed time points (0800 and 0600 h). The fasting serum estradiol concentrations were assumed to reflect the 24 h concentrations, because the patients received the Estrofem tablets twice daily, and because of the long apparent half-life of the drug (about 16 h) due to the extended resorption phase (9).

**Study parameters and assays**

Study parameters were serum levels of IGF-I, IGF-BP3, estradiol, and SHBG. All serum samples were obtained in the fasting state. The serum samples were immediately centrifuged and stored at −20 °C until analysis. All samples of all subjects were analyzed simultaneously at the end of the study.

Serum IGF-I was measured using an immunometric technique on an Advantage Chemiluminescence System (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). The lower limit of detection was 6.0 ng/ml and the intra-assay variation (n = 250) was 8.0 and 6.0% at mean plasma levels of 30 and 450 ng/ml respectively. The inter-assay variation was 8.7, 5.8, and 6.5% at mean IGF-I plasma levels of 33, 174, and 445 ng/l respectively (n = 115). The conversion factor (ng/ml–mmol/l) was 7.65. Serum IGFBP-3 was measured with an in-house RIA, as previously described (10). The lower limit of detection was 0.002 mg/l (absolute concentration) and the inter-assay variation was 7.5, 5.7, and 7.4% at mean plasma IGFBP-3 levels of 0.97, 2.0, and 3.0 mg/l respectively (n = 44). Estradiol was measured after diethyl ether extraction and Sephadex chromatography using an in-house competitive RIA. The lower limit of detection for estradiol was 20 pmol/l (2 ml sample). The inter-assay variation was 12 and 3% at 80 and 660 pmol/l respectively (n = 45, resp. 25). We measured SHBG with an immunometric technique on an Immulite analyzer (Diagnostic Products Corporation, Los Angeles, CA, USA). The lower limit of detection was 5 nmol/l and inter-assay variation was 5.5, 4.1, and 5.3% at 14, 34, and 91 nmol/l respectively (n = 23).

**Statistical analysis**

Statistical analysis was performed using Systat, version 11 (Systat Software, Richmond, CA, USA). Results are shown as the mean ± S.E.M., unless specified otherwise. A P value < 0.05 was assumed to represent a significant difference.
Patients were divided into two groups based on prior oral or transdermal estrogen treatment (see study design) and groups were analyzed separately. Raw data were logarithmic-transformed and equality of the variances at each time period was verified with the Bartlett and the Levene tests. The serial data of both groups were analyzed by ANOVA with repeated measures with a general linear model. The statistical significance between the contrasts was corrected with the Bonferroni procedure for multiple comparisons in the post hoc tests in group I.

Results

Patients

Of the 23 patients included, 20 completed the four different treatments (Fig. 1). Mean age was $44.2 \pm 9.6$ years and mean body mass index (BMI) was $28.5 \pm 6.7$ kg/m$^2$. Reasons for withdrawal were aggravated menstrual blood loss ($n = 1$) and fluid retention ($n = 1$), both during 2 mg oral (OR), and personal reasons ($n = 1$) during 100 $\mu$g transdermal (TD) estradiol.

High-versus low-dose estrogen administration

The individual hormone concentrations in the two groups are plotted in Fig. 2 and the relevant statistical details are listed in Table 2. The lowest estradiol concentrations were measured during 50 $\mu$g TD estradiol administration and the highest during 4 mg OR. Estradiol concentrations were approximately twofold higher during the higher OR and TD doses when compared with lower OR and TD doses. The mean serum estradiol concentrations were not statistically different between the 2 mg OR and the 100 $\mu$g TD treatment period ($P = 1.0$).

During the high oral estrogen dose (4 mg) when compared with the low (2 mg), serum IGF-I was significantly lower ($97 \pm 8.4$ vs $140 \pm 13.8$ $\mu$g/l, $P = 0.003$ in group I and $P = 0.04$ in group II), while SHBG concentration was higher ($P < 0.002$) and serum IGFBP-3 was not significantly different.

Although during the transdermal dose of 50 $\mu$g estradiol the lowest mean estradiol and SHBG concentrations and higher mean IGF-I and IGF-BP3 concentrations were measured when compared with those obtained during the 100 $\mu$g TD dose, the differences were not statistically significant (please refer to Table 2).

Oral (2 mg) versus transdermal (100 $\mu$g) estrogen administration

Estradiol concentrations measured during 2 mg OR and 100 $\mu$g TD estradiol were not statistically different. Despite comparable estradiol concentration, serum IGF-I was significantly lower in the 2 mg OR when compared with the 100 $\mu$g TD period ($P = 0.005$ in group I and $P = 0.02$ in group II), while SHBG concentrations were significantly higher ($P = 0.002$ in group I and $P = 0.004$ in group II). Serum SHBG and IGF-I concentrations were
negatively correlated in a linear model ($R = -0.41$, $P = 0.0001$).

**Side effects**

Patients of group I especially had physical complaints during the use of the 50 mg estradiol dermal patch. Eight patients experienced muscle pains, two had arthralgias, and four carpal tunnel syndrome. The complaints quickly disappeared when the patients were switched again to oral estradiol after completion of the trial. In group II IGF-I concentrations were above two standard deviation scores during 100 and 50 mg TD, indicating clear over-treatment in three of five cases respectively.

**Discussion**

This study was designed to differentiate between the effects of serum estradiol concentrations *per se* and the route of estrogen administration on IGF-I levels in women with GH and gonadotropin deficiency. Each subject was kept on a fixed dose of rhGH during the whole study. Oral administration of estradiol resulted in lower IGF-I levels when compared with transdermal

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**Table 2** Serum hormone concentrations in female growth hormone (GH)-deficient patients during GH and estrogen replacement.

<table>
<thead>
<tr>
<th></th>
<th>2 mg OR</th>
<th>4 mg OR</th>
<th>50 µg TD</th>
<th>100 µg TD</th>
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</thead>
<tbody>
<tr>
<td>OR to TD group I</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>IGF-I (µg/l)</td>
<td>140 ± 13.8* †</td>
<td>97 ± 8.4†</td>
<td>206 ± 23</td>
<td>186 ± 21</td>
</tr>
<tr>
<td>Estradiol (pmol/l)</td>
<td>540 ± 70*&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1070 ± 111</td>
<td>221 ± 23</td>
<td>552 ± 57&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>IGFBP-3 (mg/l)</td>
<td>2.1 ± 0.1</td>
<td>1.9 ± 0.1</td>
<td>2.4 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>135 ± 13&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>174 ± 14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>65 ± 6</td>
<td>95 ± 12</td>
</tr>
<tr>
<td>TD to OR group II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGF-I (µg/l)</td>
<td>99 ± 7.1&lt;sup&gt;d&lt;/sup&gt;&lt;sub&gt;&lt;sup&gt;a&lt;/sup&gt;&lt;/sub&gt;</td>
<td>72 ± 7</td>
<td>168 ± 19</td>
<td>170 ± 27</td>
</tr>
<tr>
<td>Estradiol (pmol/l)</td>
<td>424 ± 64&lt;sup&gt;d&lt;/sup&gt;&lt;sub&gt;&lt;sup&gt;a&lt;/sup&gt;&lt;/sub&gt;</td>
<td>772 ± 108</td>
<td>280 ± 51</td>
<td>385 ± 72</td>
</tr>
<tr>
<td>IGFBP-3 (mg/l)</td>
<td>1.8 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>120 ± 26&lt;sup&gt;j&lt;/sup&gt;&lt;sub&gt;&lt;sup&gt;i&lt;/sup&gt;&lt;/sub&gt;</td>
<td>153 ± 28</td>
<td>70 ± 13</td>
<td>88 ± 21</td>
</tr>
</tbody>
</table>

Data are shown as mean±S.E.M. Data were analyzed by ANOVA for repeated measures. Significance was tested with the Bonferroni correction for multiple comparisons. *$P=0.005$ vs 100 µg TD; †$P<0.0001$ vs 50 µg and 100 µg TD; *$P=0.003$ vs 4 mg OR; †$P<0.0001$ vs 50 µg TD; †$P=0.01$ vs 50 µg TD; †$P<0.001$ vs 4 mg OR; †$P<0.001$ vs 50 µg TD; †$P=0.007$ vs 4 mg OR; †$P=0.07$ vs 50 µg TD; †$P=0.004$ vs 100 µg TD; †$P=0.02$ vs 100 µg TD; †$P=0.007$ vs 4 mg OR; †$P=0.07$ vs 50 µg TD; †$P=0.004$ vs 100 µg TD; †$P=0.02$ vs 4 mg OR.
administration, in accordance with previous studies that were not aimed at achieving comparable estradiol concentrations (7, 8). IGF-I levels were higher during transdermal administration of 100 μg 17β-estradiol when compared with oral administration of 2 mg 17β-estradiol while circulating estradiol concentrations were similar. Therefore, we conclude that the route of estradiol administration is a determinant of IGF-I levels during fixed rhGH replacement.

Serum IGF-I concentrations decreased when increasing oral dose of estrogen treatment or increased after discontinuation of estrogen replacement as shown by studies in GH-deficient women (Table 3 (6, 11, 12)). In two other crossover studies aimed at unraveling the effects of different routes of estrogen administration on IGF-I concentrations, patients received both transdermal and oral estrogen replacement (Table 3 (7, 8)). In the first crossover study, IGF-I levels increased in women on a fixed rhGH replacement dose during the switch from oral to transdermal estrogen therapy, but after the switch serum levels of estradiol decreased, which could have contributed to the IGF-I increase (7). The second crossover study compared the IGF-I concentrations with the baseline values. In that study GH administration increased IGF-I levels in a stepwise dose-dependent manner during both estrogen administration routes, but IGF-I concentrations were lower during oral estradiol than transdermal administration at all used GH doses (8). Also in healthy postmenopausal women with an intact GH/IGF-I axis, oral estrogen administration reduces IGF-I concentrations, whereas transdermal estrogen administration has a variable effect (13–15).

The hypothalamic–somatotrope–IGF-I axis is primarily driven by GHRH, ghrelin, and somatostatin and restrained by the negative feedback of liver-derived estradiol and inhibited by ghrelin and somatostatin, in a complex interplay (1). In addition, other hormones and metabolic signals modulate this system. Pathophysiological studies in postmenopausal women have revealed a central GH-stimulating role for estradiol (16, 17). Therefore, for precise studies of IGF-I modulation by estrogens it is crucial that GH is fixed, as in the present study.

Estradiol is rapidly absorbed from the gastrointestinal tract, but undergoes extensive first-pass effects resulting in the conversion into various metabolites. In pigs, only 6% of orally administered estradiol is present as such in the portal vein, whereas the remainder is metabolized into estrone and glucuronide and sulfate conjugates of estradiol and estrone (18). Most of the portal estradiol is rapidly cleared into the systemic circulation (19). Therefore, similar plasma estradiol concentrations can be reached only during oral estradiol treatment when compared with transdermal estradiol treatment at the expense of high estrogen exposure to the liver. In accordance with this notion, we found that SHBG concentrations, a reflection of estrogen exposure of the liver (20), were the highest during oral estrogen administration.
Route-dependent effects of estradiol


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