ORAL AND TRANSDERMAL OESTROGEN TREATMENTS HAVE DIFFERING EFFECTS ON GROWTH HORMONE (GH) SENSITIVITY IN HYPOPITUITARY WOMEN RECEIVING GH REPLACEMENT

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ABSTRACT

Background: The route of oestrogen replacement has an influence on GH sensitivity in hypopituitary women but the practical relevance of this effect remains unclear.

Objectives: To compare the effects of oral and transdermal oestrogen replacement on GH requirement in adult females with hypopituitarism receiving GH replacement.

Methods: This cross-sectional, observational study included 69 GH-deficient women each receiving a stable dose of GH replacement therapy. Three groups of patients were investigated: a control population without oestrogen replacement (n = 38), and hypopituitary women receiving oral (n = 18) or transdermal (n = 13) oestrogen substitution. A single pre-prandial blood sample was taken between the hours of 08:30-09:30 from each patient for the measurement of serum IGF-I, serum total cholesterol, serum total triglycerides, serum HDL-cholesterol and serum LDL-cholesterol. The relevant demographic, anthropometric and clinical (e.g. exogenous GH requirement) data were also recorded.

Results: The mean daily GH requirement was 90% higher in the women receiving oral oestrogen compared with the control population (p<0.001), and 53% higher in those receiving transdermal oestrogen compared with the control women (p<0.01). The mean daily weight-corrected GH dose required to achieve target IGF-I levels was higher for oral vs. transdermal subjects (12.0±3.7 µg/kg·day vs. 8.3±3.0 µg/kg·day, respectively, p<0.01). Despite this higher GH dose the mean IGF-I levels were lower in oral vs. transdermal patients (24.2±12.6 nmol/L vs. 35.0±16.1 nmol/L, respectively, p<0.05).

Conclusion: This study shows that oestrogen replacement per se reduces sensitivity to exogenous GH in hypopituitary women. The route of oestrogen replacement is an important influence on GH requirement and those on oral

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oestrogen are clearly more GH resistant than women using transdermal preparations. The route of oestrogen administration is of practical and economic importance in the management of the hypopituitary woman receiving GH replacement.

Keywords: GH replacement; GH sensitivity; hypopituitarism; oral oestrogen; transdermal oestrogen.

BACKGROUND

Hypopituitary adults increasingly receive GH, in addition to sex steroid, glucocorticoid and thyroid hormone replacement. In women oestrogen replacement is most commonly administered via the oral route [1]. Such preparations pass through the gastrointestinal tract and enter the portal circulation to be metabolised in a first pass-effect [1,2]. Transdermal oestrogen regimens, which take the form of patches and gels, avoid hepatic degradation and are considered to achieve a similar oestrogenic response when administered in smaller doses [2].

It is known that oestrogen has an influence on GH sensitivity. Healthy middle-aged women have a 3-fold greater production rate of GH than normal adult males [3], and the average serum GH concentration is higher in females despite similar IGF-I in both genders [3]. Several studies involving adult patients with GH-deficiency (GHD) have also shown a gender-specific response to GH therapy. In a small short-term study Janssen et al. [4] showed that female patients required double the dose of GH replacement to normalise serum IGF-I levels compared with their male counterparts.

Few investigations have examined the effects of oral and transdermal oestrogen on the GH/IGF-I axis in GH-deficient, hypogonadal females. Preliminary studies have suggested that converting from oral to transdermal substitution results in an increased sensitivity to exogenous GH [5,6]. No substantial study has determined whether the route of oestrogen administration is of practical clinical and economic importance in the clinical management of the hypopituitary female receiving GH replacement.

The aim of this study was to determine whether differences in exogenous GH requirement and GH sensitivity exist between GH deficient women using oral and transdermal oestrogen therapies.

SUBJECTS AND METHODS

Subject

This cross-sectional, observational study included 69 adult hypopituitary females (52±14 yrs, mean ± standard deviation [SD]) with severe GH-deficiency (defined as a peak GH <9 mU/L (3 mcg/L) during an insulin tolerance test with nadir plasma glucose ≤2.2 mmol/L). The women were re-
cruiited from the endocrine clinics at Guy’s & St. Thomas’ NHS Foundation Trust using an electronic database. The participants were divided into three groups, which included 31 subjects with coexisting hypogonadism and receiving oestrogen treatment, and 38 exogenous oestrogen-naïve controls. Of those women using oestrogen preparations, 18 were taking oral oestrogen and 13 were on transdermal replacement. The process of GH titration was performed according to standard practice [7], with all patients receiving stable substitution therapy (i.e. taking a constant dose of rhGH) for at least 6 weeks before the study-date. Research ethics approval was granted by the St. Thomas’ Hospital Ethics Committee; subjects provided written informed consent.

Criteria for eligibility included an age-range set at 18-85 years and the use of an oestrogen preparation for a minimum of 3 months prior to study. The patients were also receiving stable hormone replacement for any other pituitary deficiencies. Exclusion criteria included pregnancy and factors that may have affected the efficacy of oral oestrogen substitution, such as vomiting and diarrhoea. Patients who were not receiving a stable dose of GH for at least 6 weeks prior to the study-date were excluded.

Data collection

Patients were identified using an in-house electronic patient record (Diabeta3®). GH dose and oestrogen status were recorded. A fasting venous blood sample was taken between 08.30 and 09.30 from each subject. All patients were laid recumbent on a hospital bed at 45º and after a minimum of 30 minutes, a pre-prandial blood sample was collected for the following measurements: total serum cholesterol, total serum triglycerides, serum high density lipoprotein (HDL)-cholesterol, serum low density lipoprotein (LDL)-cholesterol and serum IGF-I.

At the time of sample collection, weight and height were measured using a balance and stadiometer, respectively; the body mass index (BMI) was then calculated by applying the formula weight/height\(^2\) (kg/m\(^2\)). Body composition was also assessed using bioelectrical impedance (Model TBF-300, Body Composition Analyser, Tanita, UK).

Blood collection

A 21 gauge cannula was passed into the antecubital fossa and kept patent by flushing with a single bolus of 5ml 0.9% saline. After 30 minutes, with the intention of removing the saline from within the line, 2.5ml of blood were initially discarded. Depending on the analyte in question, blood samples were then drawn into the relevant vacutainer® tube.
Storage and Analysis

The vacutainers were immediately deposited in a refrigerator at 4°C and the blood was allowed to clot for 30 minutes. The samples were subsequently spun at 3000rpm and 4°C for 10 minutes in a refrigerated centrifuge (Model BR401, Billinghurst, UK), thus separating the plasma from the cells. 1ml-aliquots of the recovered supernatant containing the isolated plasma were pipetted into the appropriate 2ml storage tubes (Sarstedt, UK).

Samples were stored in a freezer at -20°C for no longer than 3 months prior to analysis. Before each assay, the samples were thawed for one hour. The plasma was subsequently transferred into labelled cuvettes and centrifuged at 2500rpm and 4°C for 10 minutes.

Assays

Serum levels of total cholesterol and total triglycerides were evaluated using an automated enzymatic colorimetric assay (Roche Diagnostics, Indianapolis, USA). An automated homogenous enzymatic colorimetric test (Roche Diagnostics, Indianapolis, USA) was used to determine the concentration of serum HDL-cholesterol. LDL-cholesterol was calculated from quantitative measurements of plasma triglycerides, total cholesterol and HDL-cholesterol via the Friedewald equation [8]. Serum IGF-I level was measured using the automated Immulite IGF-I analyser (Diagnostic Products Corporation (DPC), Los Angeles, CA, USA), which is a solid-phase, enzyme-labelled chemiluminescent immunometric assay.

Statistical Analysis

Statistical analyses were performed using a statistical software package (SPSS 13.0, Inc., Chicago, IL, USA). An alpha-level of 0.05 (two-tailed) was set a priori as the criterion for statistical significance. The Kolmogorov-Smirnov test was first applied to define normality of distribution within each arm. The significance of differences between the three parametric groups was determined using the independent samples t-test assuming equal variances. Results are expressed as mean ± SD.

RESULTS

Demographic and Clinical Information

The subjects’ demographic and clinical details are summarised in Table 1. Those receiving oestrogen substitution were younger than their non-oestrogen-substituted counterparts: control vs. oral (p<0.001); control vs. transdermal (p=0.004, Table 1). However, the ages of those patients taking
oral and transdermal therapy were similar (p=0.7). The durations of GHD within each group were also similar (Table 1).

Body Composition & Lipid Measurements

Most body composition variables did not differ between the three groups (Table 2). Weight (p=0.044), lean body mass (LBM, p=0.019) and waist measurement (p=0.025) were lower in the oral oestrogen group compared with controls. Total cholesterol, HDL-cholesterol and LDL-cholesterol were similar for all three groups (Table 3). In contrast, the triglyceride concentration for those applying transdermal preparations was less than the level recorded in the control population (p=0.045).

Serum IGF-I levels

Serum IGF-I levels were similar in the oral oestrogen vs. control groups (24.2±12.6 vs. 26.4±11.5 nmol/L, respectively, p=0.53, Figure 1), but IGF-I levels were higher in those receiving transdermal substitution (35.0±16.1 nmol/L) compared to the other two groups (vs. control: p=0.041; vs. oral: p=0.046). All three mean IGF-I values fall within the boundaries of the same reference range, adjusted for mean age: 11.0-43.9 nmol/L for females aged between 41-61 years.

Daily GH dose

Oral oestrogen replacement was associated with a significantly increased daily GH requirement compared to the control population (0.76±0.19 vs. 0.40±0.17 mg/day, respectively, p<0.001). This represented a mean percentage increase of 90%. Transdermal therapy was also associated with an increased daily GH dose compared to the control group, although to a lesser extent (0.61±0.24 vs. 0.40±0.17 mg/day, respectively, p=0.001), representing an increase of 53%. Oral oestrogen replacement significantly increased the daily weight-corrected GH dose compared to the control group by 114% (12.0±3.7 vs. 5.6±3.3 µg/kg·day, respectively, p<0.001, Figure 2). Transdermal therapy also increased the weight-corrected dose compared to the control group, although to a lesser extent (48%; 8.3±3.0 vs. 5.6±3.3 µg/kg·day, respectively, p=0.014). The difference in dose between the two oestrogen-substituted groups was significant (oral vs. transdermal: 12.0±3.7 vs. 8.3±3.0 µg/kg·day, respectively, p=0.006).

DISCUSSION

This cross-sectional, observational study examined the clinical effect of oestrogen replacement on GH sensitivity in women with hypopituitarism. Both oral and transdermal oestrogen treatments resulted in reduced sensitivity
to exogenous GH, with larger doses of GH required to achieve titrated target IGF-I concentrations. Important differences were seen between the two oestrogen modalities, with the increase in daily weight-corrected GH requirement more marked in oral vs. transdermal treated patients (114% vs. 48%, respectively).

A number of previous studies have demonstrated that hypopituitary women using transdermal oestrogen require less GH than their counterparts treated with oral oestrogen [5,6,9,10]. These studies however included far fewer patients and are not necessarily reflective of conventional clinical practice. In contrast, this investigation offers a direct comparison between the two oestrogen preparations in the “real-life” clinical setting. A study of six GH-deficient females with hypogonadism demonstrated that switching the modality of oestrogen treatment from the oral to transdermal route increased the serum IGF-I level despite an unchanged GH dose [5]. A further small open-label study demonstrated the effect of oral oestrogen in reducing IGF-I levels compared with transdermal oestrogen across a range of GH replacement doses [9].

Oestrogen is actively metabolised by the hepatic cytochrome system, resulting in a first pass effect [11]. When given orally, relatively large oral doses of oestrogen are administered in order to achieve desired therapeutic systemic levels. This results in supraphysiological oestrogen levels in the portal circulation that affects aspects of hepatic function [11] and may suppress hepatic IGF-I synthesis. [9,10,12]. Kelly and others [12] observed an equal dissociation of the GH/IGF-I axis following oral administration of three different oestrogen formulations. The group compared ethinyl oestradiol (20µg/day), conjugated equine oestrogen (1.25mg/day) and oestradiol valerate (2mg/day) in a randomized, crossover study involving six healthy postmenopausal women. They observed a decrease in IGF-I and an increase in GH and growth hormone-binding protein (GH-BP) for all treatment groups [12]. These findings indicate that oral oestrogen reduces GH sensitivity and decreases IGF-I concentration. In the current study the decreased GH dose necessary to result in a higher IGF-I concentration in those receiving transdermal compared with oral oestrogen indicates that the reduction in GH sensitivity is less significant when transdermal oestrogen is used in hypopituitarism. Transdermal preparations deliver oestrogen directly into the systemic circulation, bypassing the liver and avoiding the first-pass effect associated with oral substitution [11].

The precise mechanism by which oestrogen acts to provoke a decrease in IGF-I levels remains unclear. Evidence suggests that this may involve an immediate interaction with hepatocytes, which down-regulates the transcriptional action of GH via the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway [13]. Oestrogen stimulates the hepatic expression of a family of molecules, the suppressors of cytokine signalling (SOCS), namely SOCS-2 and SOCS-3, that inhibit GH activation of JAK/STAT signalling [13]. An oestrogen-induced increase in SOCS-2
mRNA has also been demonstrated in human embryonic kidney cells, and that the inhibitory effects of oestrogen on GH signalling were lost in cells lacking SOCS-2 [14]. Rather than directly inhibiting hepatic IGF-I synthesis, oral oestrogen replacement may decrease GH availability by augmenting production of GH-BP, thus lowering in vivo hormonal bioactivity [12,15]. Weissberger et al. [15] were unable to demonstrate any changes in GH-BP using transdermal 17β-oestradiol. These findings suggest that (a) GH-BP is an oestrogen-sensitive protein of hepatic origin and (b) the liver serves as the predominant source of circulating GH-BP in humans [12]. Weissberger et al. [15] also postulated that the first-pass metabolism of oral oestrogens may alter the synthesis of other liver derived proteins, as confirmed by Kam and co-workers [16]. They revealed that exogenous oestrogens exert a route-dependent effect on all three components of the IGF-I ternary complex [16]. Circulating IGF-I is mainly bound to IGF-binding protein-3 (IGFBP-3) and the acid labile subunit (ALS), both of which are produced by the liver under the influence of GH [11]. Unlike transdermal substitution, oral oestrogen significantly reduced serum levels of IGF-I, IGFBP-3 and ALS in healthy postmenopausal women; oral therapy exerted similar effects in hypogonadal GH-deficient females, indicating that the effects are unrelated to GH [16].

Previous crossover studies [17,18] in postmenopausal women have shown that transdermal oestrogen patches delivering 100µg/day of 17β-oestradiol and oral-conjugated oestrogen 1.25mg daily induce a significant and parallel reduction in the concentrations of LH and FSH. These findings indicate that at the doses used in clinical practice these preparations have similar systemic bioequivalence of oestrogen action [17,18].

Both oestrogen modalities exerted marginal effects upon the serum lipid profile and most body composition parameters. The reduced LBM observed in those patients receiving oral oestrogen replacement reflects previous findings [17,19]. As circulating IGF-I mediates the anabolic actions of GH [11], this is possibly the result of long-term suppression of IGF-I levels [17,19]. In addition, oral oestrogen therapy diminishes the incorporation of leucine into protein (an index of protein synthesis) via a mode of action supposedly unrelated to changes in IGF-I [9]. The decreased waist circumference of oral vs. control subjects was unexpected, as previous evidence [6] indicates that GH-deficient women taking oral oestrogens show less reduction in waist and hip measurements and a greater waist/hip ratio after one year of GH treatment compared with eugonadal, oestrogen-naïve patients. In contrast, waist and hip circumferences were similar for patients using patch therapy and those with normal gonadal function [6], similar to the findings in the current study.

In conclusion, the route of oestrogen replacement has substantial effects on components of the GH/IGF-I axis, and is as an important determinant of exogenous GH sensitivity. The choice of oestrogen substitution for a hypopituitary female is influenced by a number of factors, such as age, personal preference, smoking history, BMI and oestrogen-dependent cancer risk. Many young women are treated with combined oral contraceptive prepara-
tions. In standard practice women approaching the age of the natural menopause are more likely to take conjugated equine oestrogen or synthetic oestrogens. Topical transdermal products are generally used in women around the time of menopause. This study provides clear evidence that patients are more sensitive to exogenous GH, and thus require a lower dose to achieve effective replacement, when taking transdermal rather than oral oestrogen therapy. This should form a real consideration when choosing an appropriate oestrogen modality for the hypopituitary female receiving GH replacement. Although cost is not a primary influence in choosing oestrogen treatment, the current study suggests that oral oestrogen use results in an increased annual cost of GH therapy per individual of £1,265 (British National Formulary, September 2008) compared with transdermal preparations. It has been postulated that switching the route of oestrogen substitution from oral to transdermal for all GH-deficient, hypogonadal women living in the Unites States could yield an annual saving of approximately US$110-250 million [11]. The route of oestrogen treatment is of practical and economic importance in the treatment of hypopituitary women receiving GH replacement.

REFERENCES

[8] Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of prepara-
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**TABLES & FIGURES**

*Table 1* Subjects’ demographic and clinical summary

<table>
<thead>
<tr>
<th>Route of oestrogen therapy</th>
<th>Demographics</th>
<th>Clinical Information</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age (yrs)</td>
<td>Duration of GHD (yrs)</td>
</tr>
<tr>
<td>Control</td>
<td>58 ± 13</td>
<td>16 ± 9</td>
</tr>
<tr>
<td>Oral</td>
<td>44 ± 11**</td>
<td>19 ± 12</td>
</tr>
<tr>
<td>Transdermal</td>
<td>45 ± 13*</td>
<td>20 ± 9</td>
</tr>
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</table>

*p<0.01 compared to the control group; **p<0.001 compared to the control group; IGHD (isolated GHD); MPHD (multiple pituitary hormone deficiency)

*Table 2* Body composition variables grouped according to route of oestrogen therapy

<table>
<thead>
<tr>
<th>Route of oestrogen therapy</th>
<th>Wt (kg)</th>
<th>BMI (kg/m²)</th>
<th>Fat (%)</th>
<th>FM (kg)</th>
<th>LBM (kg)</th>
<th>Waist circum. (cm)</th>
<th>Hip circum. (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>76.9 ± 18.6</td>
<td>29.1 ± 6.5</td>
<td>40.2 ± 9.8</td>
<td>32.2 ± 13.9</td>
<td>44.8 ± 8.1</td>
<td>101 ± 18</td>
<td>105 ± 12</td>
</tr>
<tr>
<td>Oral</td>
<td>66.7 ± 14.3*</td>
<td>26.1 ± 5.6</td>
<td>38.8 ± 13.6</td>
<td>27.2 ± 15.0</td>
<td>39.4 ± 6.9*</td>
<td>89 ± 16*</td>
<td>98 ± 14</td>
</tr>
<tr>
<td>Transdermal</td>
<td>74.7 ± 15.5</td>
<td>29.4 ± 5.5</td>
<td>40.0 ± 7.1</td>
<td>29.8 ± 10.2</td>
<td>43.1 ± 6.1</td>
<td>96 ± 9</td>
<td>103 ± 8</td>
</tr>
</tbody>
</table>

*p<0.05 compared to the control group
### Table 3  Lipid concentrations

<table>
<thead>
<tr>
<th>Route of oestrogen therapy</th>
<th>Total Chol (mmol/L)</th>
<th>Total Trigs (mmol/L)</th>
<th>HDL-Chol (mmol/L)</th>
<th>LDL-Chol (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.6 ± 1.3</td>
<td>1.97 ± 1.03</td>
<td>1.51 ± 0.52</td>
<td>3.19 ± 1.30</td>
</tr>
<tr>
<td>Oral</td>
<td>5.5 ± 1.3</td>
<td>1.80 ± 1.12</td>
<td>1.60 ± 0.36</td>
<td>3.07 ± 0.88</td>
</tr>
<tr>
<td>Transdermal</td>
<td>4.9 ± 0.6</td>
<td>1.31 ± 0.37*</td>
<td>1.56 ± 0.32</td>
<td>2.77 ± 0.57</td>
</tr>
</tbody>
</table>

*p<0.05 compared to the control group

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**Figure 1** Serum IGF-I concentration in hypopituitary women grouped according to the route of oestrogen therapy

![IGF-I Concentration Chart](chart.png)

*p<0.05 compared to the control group; $p<0.05$, oral vs. transdermal
Figure 2 Weight-corrected daily GH dose in hypopituitary women grouped according to route of oestrogen therapy

* p<0.05 compared to the control group; ** p<0.001 compared to the control group; $ p<0.01, oral vs. transdermal