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CLINICAL STUDY

Effects of oral and transdermal estrogen on IGF1, IGFBP3, IGFBP1, serum lipids, and glucose in patients with hypopituitarism during GH treatment: a randomized study

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Abstract

Objective: To evaluate the effects of oral estradiol and transdermal 17β-estradiol on serum concentrations of IGF1 and its binding proteins in women with hypopituitarism.
Design: Prospective, comparative study.
Methods: Eleven patients with hypopituitarism were randomly allocated to receive 2 mg oral estradiol ($n=6$) or 50 μg/day of transdermal 17β-estradiol ($n=5$) for 3 months.
Results: The oral estrogen group showed a significant reduction in IGF1 levels (mean: 42.7%±41.4, $P=0.046$); no difference was observed in the transdermal estrogen group. There was a significant increase in IGFBP1 levels (mean: 170.2%±230.9, $P=0.028$) in the oral group, but not in the transdermal group. There was no significant difference within either group in terms of median IGFBP3 levels. In relation to lipid profiles, there was a significant increase in mean high-density lipoprotein cholesterol levels in the oral group after 3 months of treatment, (27.8±9.3, $P=0.003$). We found no differences in the anthropometric measurements, blood pressure, heart rate, glucose, insulin, C-peptide, or the homeostasis model assessment index after treatment.
Conclusions: Our preliminary data indicate that different estrogen administration routes can influence IGF1 and IGFBP1 levels. These findings in patients with hypopituitarism have an impact on their response to treatment with GH, since patients receiving oral estrogen require increased GH dosage. These results suggest that oral estrogens may reduce the beneficial effects of GH replacement on fat and protein metabolism, body composition, and quality of life.

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Introduction

There is considerable evidence that the use of estrogens by women has an influence on the GH/IGF1 axis (1). Small intervention studies (2, 3, 4, 5, 6, 7, 8, 9, 10) and large cross-sectional studies (11, 12, 13, 14, 15) have demonstrated that oral estrogens reduce serum concentrations of total IGF1. These findings have been observed both in menopausal women and in those with hypopituitarism.

It is presumed that oral estrogen inhibits the secretion/production of IGF1 by means of a first-pass hepatic effect, causing an increase in GH secretion by inhibiting the negative feedback of IGF1 (1, 6). In a study on women with GH deficiency, IGF1 levels were significantly higher during 100 μg transdermal estradiol treatment compared with 2 mg oral treatment (16). In GH-deficient patients, these changes compromise the GH action and, consequently, suppress lipid oxidation and cause changes in body composition (17).

Studies investigating the transdermal administration of estrogen in normal menopausal women have demonstrated that this route of administration has effects on serum IGF1 concentrations. An increase or absence of effect has been demonstrated probably because estrogen does not undergo hepatic metabolism and accesses systemic circulation directly (2, 18, 19, 20, 21).

Around 75% of IGF1 circulates in plasma by formation of a ternary complex with IGFBP3 and the acid-labile subunit. Studies of oral estrogens have provided inconsistent results in relation to IGFBP3 concentrations, with reports of reduced serum levels (5, 7, 13, 22, 23) or an absence of effects (2, 4, 6, 12, 14, 15).

IGFBP1, an insulin-modulated binding protein of IGF, regulates the rate of free IGF1. Serum IGFBP1 levels are inversely related to the development of diabetes mellitus

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Table 1 Sample characteristics at baseline.

Characteristics	Values
n	11
Age (years)[a]	36.1±10.2
Skin color[b]	
White	10 (90.9)
Black	1 (9.1)
Weight (kg)[a]	59.7±10.4
Height (m)[a]	1.55±0.04
BMI (kg/m^2)[a]	24.7±3.9
WHR[a]	0.95±0.04

BMI, body mass index; WHR, waist–hip ratio.
[a]Mean±S.D.
[b]*n* (%).

type 2, particularly in the presence of reduced IGF1 concentrations (24). Helle *et al.* (6) demonstrated a 46% increase in serum IGFBP1 levels after use of oral estrogens, and their findings were later confirmed by other authors (4, 5).

The primary objective of this study was to investigate the interrelationships between estrogen administration routes and concentrations of IGF1 and its binding proteins in patients with hypopituitarism during GH-replacement treatment. In these GH-deficient patients, GH secretion cannot be modulated by estrogen. Therefore, any changes in GH secretion will be caused by other metabolic effects, regardless of GH secretion.

Patients and methods

The study sample comprised 11 female patients with organic and secondary hypopituitarism, aged 18–50 years (Table 1), and selected from a group of 36 women with hypopituitarism treated at the neuroendocrinology clinic at the Hospital de Clínicas de Porto Alegre (HCPA), Southern Brazil. The study was approved by the research ethics committee at HCPA, and all patients who agreed to participate in the study signed a free and informed consent form. Hypopituitarism was diagnosed by assaying the basal hormones T_4, TSH, estradiol, LH, FSH, cortisol, ACTH, and IGF1. GH deficiency was diagnosed by insulin-induced hypoglycemia ($n=5$) or glucagon stimulation test ($n=6$), with patients considered normal if their GH peak was >3 ng/ml (Table 2). All patients were receiving regular treatment to correct other hormone deficiencies. With relation to GH, at the study outset, patients had been on 0.5 IU/day of Norditropin (Novo Nordisk, Sao Paulo, Brazil) for at least 2 months and presented normal IGF1 levels for their ages. The majority of the patients had not been given gonadal steroids (6/11), and those who had taken them (5/11) had had them withdrawn at least 3 months before the study began.

Patients were excluded if they were diabetic, obese, had liver or kidney disease, or if they were <18 or >50 years old. Patients with previous endocrinopathies did not have any active diseases during the study.

The patients selected were randomized to receive equivalent doses of estradiol by different administration routes: 2 mg estradiol orally (Estrofem, Novo Nordisk) or 50 μg/day of transdermal estradiol for 3 months (System, Janssen-Cilag, Sao Paulo, Brazil); subsequently, 1 mg oral norethisterone (Micronor, Janssen-Cilag) was added for a further 3 months to provide endometrial protection.

Assays and methods employed

IGF1 concentrations were determined by IRMA, DSL-5600 Active (Diagnostic System Laboratories, Inc., Webster, TX, USA), with intra- and inter-assay coefficients of variation (CV) of 3.9 and 4.2%, respectively, by the HCPA RIA Laboratory, Porto Alegre, Brazil.

IGFBP3 levels were determined by IRMA, DSL-6600 Active (Diagnostic System Laboratories, Inc.), with intra- and inter-assay CV of 3.9 and 0.6%, respectively, by Instituto Fleury, São Paulo, Brazil.

IGFBP1 concentrations were determined by IRMA, DSL-7800 Active (Diagnostic System Laboratories, Inc.), with intra- and inter-assay CV of 2.7 and 3.6%, respectively, by the Nichols Institute, USA.

The remaining hormone assays were performed at the HCPA RIA Laboratory; estradiol and C-peptide were measured using the chemiluminescence immunoassay (CLIA) method, and insulin was assessed by the RIA method.

Ultra-sensitive C-reactive protein was measured by nephelometry with CardioPhase hsCRP reagents (Dade Behring, Inc., Deerfield, IL, USA); intra- and inter-assay CV were 3.1 and 2.5% respectively.

Table 2 Clinical characteristics of women ($n=11$) with hypopituitarism.

Clinical characteristics	Values
No. of hormone deficiencies[a]	
Gonadotropic	11 (100%)
Thyrotropic	8 (72.7%)
Somatotropic	11 (100%)
Corticotropic	9 (81.8%)
Causes of hypopituitarism[a]	
Cushing's disease	6 (54.5)
Craniopharyngioma	2 (18.2)
Dysgerminoma	1 (9.1)
Empty sella	1 (9.1)
Sheehan's syndrome	1 (9.1)
Surgery[a]	9 (81.8)
Radiotherapy[a]	2 (18.2)
Test[a]	
Glucagon	6 (54.5)
Insulin hypoglycemia	5 (45.5)
Time using GH (months)[b]	7 (2–19)

[a]*n* (%).
[b]Median (minimum−maximum).

Biochemical tests were performed according to the HCPA General Laboratory's routine methods. The homeostasis model assessment-insulin resistance (HOMA-IR) index was used to detect IR. It was calculated using the formula HOMA-IR = ((fasting glycemia in mg/dl × 0.05551) × fasting insulin in μU/ml)/22.5.

The following clinical variables were assessed: weight, body mass index (BMI = weight/height^2), arterial blood pressure, and waist–hip ratio (the greatest diameter of each circumference was taken into consideration). To investigate possible estrogen-related effects, endometrial thickness was also assessed by transvaginal ultrasound, using a General Electric (GE) LOGIC 200 PRO, with an endocavity transducer at a frequency of 7.5 MHz.

Statistical analysis

Quantitative variables were described as means and S.D. when symmetrical, or as medians and amplitude of variation (maximum − minimum) when asymmetrical. Categorical variables were described in terms of absolute and relative frequencies.

Student's *t*-test was used to compare the groups (oral vs transdermal) in terms of quantitative variables with normal distribution. In cases of asymmetry, the Mann–Whitney *U* test was applied.

To compare baseline assessments vs results after 3 months on estrogen administered by the different routes, the *t*-test for paired samples or the Wilcoxon test was applied, depending on the distribution of each variable.

Pearson's correlation coefficient was used to analyze associations between variations observed in the third month vs baseline data.

The level of significance adopted was 5%, and $P \leq 0.05$ was considered statistically significant. Data were analyzed using the Statistical Package for the Social Sciences (SPSS Inc., New York, NY, USA) version 12.0.

Results

The sample comprised 11 patients with a mean age of 36.1 (±10.2) years and were predominantly of white skin color (90.9% of cases) (Table 1). With relation to the clinical characteristics of the sample, all women showed somatotropic and gonadotropic deficiencies, and 54.5% of them presented Cushing's disease as a previous endocrinopathy, the treatment for which resulted in their hypopituitarism (Table 2).

At the study outset, patients in the oral and transdermal groups were similar in terms of IGF1, IGFBP1, and IGFBP3 levels and other study variables (Table 3).

Over the 3 months of treatment, mean serum estradiol levels increased from 10.1 (5–54.7) to 133.9 pg/ml (7.4–197.9), $P = 0.046$ (median − minimum − maximum) in the oral group. In the transdermal group, mean estradiol levels increased from 12.7 (7.4–33.4) to 36.1 pg/ml (17.4–84.4), $P = 0.043$ (Table 4).

Table 3 Comparison between the two groups at baseline.

Variables	Oral route ($n=6$)	Transdermal route ($n=5$)	*P*
Weight (kg)[a]	60.8 ± 14.2	58.4 ± 3.6	0.724[b]
Height (m)[a]	1.56 ± 0.03	1.55 ± 0.05	0.908[b]
BMI (kg/m^2)[a]	24.9 ± 5.2	24.2 ± 2.2	0.783[b]
WHR[a]	0.97 ± 0.05	0.94 ± 0.04	0.285[b]
Systolic BP (mmHg)[a]	115.5 ± 13.8	123.7 ± 20.9	0.459[b]
Diastolic BP (mmHg)[a]	76.9 ± 12.8	83.3 ± 13.9	0.452[b]
HR (bpm)[a]	81.3 ± 11.8	75.2 ± 11.4	0.405[b]
Endometrial thickness (cm)[c]	0.2 (0.1–0.8)	0.3 (0.1–0.4)	0.931[d]
Estradiol (pg/ml)[c]	10.1 (5–54.7)	12.7 (7.4–33.4)	0.662[d]
IGF1 (ng/ml)[c]	195.6 (40.7–461.5)	166.5 (148.8–340.4)	0.662[d]
IGFBP3 (ng/ml)[c]	3480 (1370–5376)	2877 (2140–4730)	0.662[d]
IGFBP1 (ng/ml)[c]	14.9 (5.0–56.2)	29.9 (5.0–49.7)	0.537[b]
Glucose (mg/ml)[a]	96.5 ± 9.9	84.8 ± 10.7	0.092[d]
Insulin (μIU/ml)[c]	11.9 (2.1–23.1)	3.10 (2.7–24.3)	0.429[b]
C-peptide (ng/ml)[a]	1.52 ± 0.80	1.58 ± 0.85	0.904[d]
HOMA[c]	5.22 (4.72–6.10)	4.38 (4.22–5.55)	0.126[b]
Total cholesterol (mg/dl)[a]	222.0 ± 27.5	235.8 ± 70.6	0.698[b]
HDL (mg/dl)[a]	54.0 ± 19.3	61.4 ± 13.0	0.486[b]
LDL (mg/dl)[a]	188.4 ± 53.1	157.5 ± 80.5	0.464[b]
Triglycerides (mg/dl)[a]	231.2 ± 116.1	192.0 ± 178.4	0.671[b]
Ultra-sensitive C-reactive protein (mg/l)[c]	6.83 (0.54–8.62)	3.16 (1.31–7.31)	0.247[d]
Time on GH (months)[c]	7.5 (3–19)	7.0 (2–12)	0.537[d]
Dose of GH (IU/day)[c]	0.5 (0.25–0.50)	0.5 (0.25–1.00)	0.792[d]

BMI, body mass index; WHR, waist–hip ratio; BP, blood pressure; HR, heart rate; HOMA, homeostasis model assessment.
[a]Mean ± S.D.
[b]Student's *t*-test.
[c]Median (minimum − maximum).
[d]Mann–Whitney *U* test.

Table 4 Comparison between study phases according to route of administration.

	Oral route ($n=6$)			**Transdermal route** ($n=5$)		
Variables	Baseline	After 3 months	*P*	Baseline	After 3 months	*P*
Weight (kg)[a]	60.8±14.2	61.7±15.4	0.280[b]	58.4±3.6	59.1±5.0	0.581[b]
BMI (kg/m^2)[a]	25.0±5.2	25.4±5.7	0.272[b]	24.3±2.3	24.6±2.7	0.593[b]
WHR[a]	0.97±0.05	0.96±0.05	0.576[b]	0.94±0.04	0.94±0.04	0.374[b]
Systolic BP (mmHg)[a]	115.6±13.8	118.2±20.5	0.567[b]	123.7±20.9	121.3±15.7	0.711[b]
Diastolic BP (mmHg)[a]	77.0±12.8	78.9±14.1	0.619[b]	83.3±13.9	86.6±11.8	0.333[b]
HR (bpm)[a]	81.3±11.8	74.0±9.0	0.202[b]	75.2±11.4	75.6±8.0	0.914[b]
Endometrial thickness (cm)[c]	0.2 (0.1–0.8)	0.9 (0.3–1.5)	**0.027**[d]	0.3 (0.1–0.4)	0.6 (0.2–0.8)	**0.042**[d]
Estradiol (pg/ml)[c]	10.1 (5–54.7)	133.9 (7.4–197.9)	**0.046**[d]	12.7 (7.4–33.4)	36.1 (17.4–84.4)	**0.043**[d]
IGF1 (ng/ml)[c]	195.6 (40.7–461.5)	91.3 (34.1–203.9)	**0.046**[d]	166.5 (148.8–340.4)	122.3 (101.9–339.2)	0.500[d]
IGFBP3 (ng/ml)[c]	3480 (1370–5376)	2790 (1530–6330)	0.463[d]	2877 (2140–4730)	3600 (2830–3790)	0.500[d]
IGFBP1 (ng/ml)[c]	14.9 (5.0–56.2)	40.6 (7.9–62.2)	**0.028**[d]	29.9 (5.0–49.7)	12.2 (5–41.1)	0.144[d]
Glucose (mg/ml)[a]	96.5±9.9	89.0±7.5	0.124[b]	84.8±10.7	84.0±6.4	0.830[b]
Insulin (μIU/ml)[c]	11.9 (2.1–23.1)	10.2 (1.8–32.5)	0.893[d]	3.10 (2.7–24.3)	5.79 (2.82–10.9)	0.686[d]
C-peptide (ng/ml)[a]	1.5±0.8	2.0±1.4	0.385[b]	1.6±0.9	1.2±0.5	0.401[b]
HOMA[c]	5.22 (4.72–6.10)	5.04 (4.22–5.38)	0.116[d]	4.38 (4.22–5.55)	4.49 (4.44–5.27)	0.893[d]
Total cholesterol (mg/dl)[a]	222±27.5	239.3±36.4	0.280[b]	235.8±70.6	224.0±71.1	0.083[b]
HDL (mg/dl)[a]	54.0±19.3	68.7±24.0	**0.003**[b]	61.4±13.0	61.4±19.7	1.000[b]
LDL (mg/dl)[a]	188.4±53.1	177.4±56.0	0.531[b]	157.5±80.5	152.5±68.2	0.428[b]
Triglycerides (mg/dl)[a]	231.2±116.1	243.2±124.2	0.679[b]	192.0±178.5	159.4±119.6	0.300[b]
Ultra-sensitive C-reactive protein (mg/l)[c]	6.8 (0.5–8.6)	5.0 (1.5–10.3)	0.917[d]	3.2 (1.3–7.3)	1.1 (0.6–5.5)	**0.043**[d]

BMI, body mass index; WHR, waist–hip ratio; BP, blood pressure; HR, heart rate; HOMA, homeostasis model assessment. Values in bold indicate $P \leq 0.05$.
[a]Mean±S.D.
[b]Student's *t*-test for paired samples.
[c]Median (minimum−maximum).
[d]Wilcoxon test.

Endometrial thickness, analyzed by transvaginal pelvic ultrasound, increased from 2 (1–8) to 9 mm (3–15), $P=0.027$ in the oral group. In the transdermal group, mean endometrial thickness increased from 3 (1–4) to 6 mm (2–8), $P=0.042$.

During treatment with oral estrogens, median IGF1 levels dropped significantly (mean 42.7%±41.4) in relation to baseline values, from 195.6 (40.7–461.5) to 91.3 ng/ml (34.1–203.9), $P=0.046$. In the transdermal group, median IGF1 level at the start of treatment was 166.5 ng/ml (148.8–340.4), and after 3 months of treatment, it was 122.3 ng/ml (101.9–339.2), $P=0.500$. There was also no significant difference within each group with relation to median IGFBP3 levels.

In contrast, we observed significant increases in IGFBP1 levels in the oral group, a mean increase of 170.2% (±230.9), varying from 14.9 (5.0–56.2) at baseline to 40.6 ng/ml (7.9–62.2) at the end of the treatment, $P=0.028$. In the transdermal group, there was a non-significant reduction in IGFBP1 levels, with baseline results at 29.9 ng/ml (5.0–49.7), compared with 12.2 ng/ml (5.0–41.0) after 3 months of treatment, with $P=0.144$.

In relation to lipid profiles, there was a significant increase in mean high-density lipoprotein (HDL) cholesterol levels (27.8%±9.3) in the oral group, from 54.0±19.3 mg/dl at baseline to 68.7±24.0 mg/dl after 3 months of treatment, $P=0.003$.

There were no differences in anthropometric measurements before or after treatment with either administration route, nor were there differences in blood pressures, heart rate, glucose, insulin, C-peptide, or the HOMA index (Table 5).

Ultra-sensitive C-reactive protein results reduced significantly (52.5%±21.0) in the transdermal group, from 3.2 (1.3–7.3) to 1.1 mg/dl (0.6–5.5), $P=0.043$. In the oral group, there was no significant difference in ultra-sensitive C-reactive protein before and after treatment with estradiol.

Changes in IGFBP1 levels had a negative correlation with changes in insulin in the oral group ($r=-0.815$, $P<0.05$). We also observed a strong and significant correlation between changes in triglycerides and IGFBP1 levels ($r=-0.829$, $P=0.042$) in the oral group.

Discussion

In this study, we observed that IGF1 levels were significantly reduced after 3 months using 2 mg oral estradiol, a finding that was not observed after treatment with 50 μg transdermal estradiol. No significant differences were detected in IGFBP3 levels in either of the two treatment groups.

Previous studies with menopausal women have demonstrated different effects on IGF1 levels in response to the two routes of estrogen administration (3, 6, 7, 20, 25, 26). However, the treatment period (2–12 months), the dose of transdermal estradiol (20–100 μg/day), and the types and doses of oral estrogen (ethinylestradiol, 17β-estradiol, estradiol valerate, and conjugated equine

Table 5 Comparison between the two groups in terms of changes after 3 months of treatment.

Variables	Changes after 3 months of treatment			
	Oral route Mean±S.D.	Transdermal route Mean±S.D.	95% CI	*P*[a]
BMI (kg/m^2)[b]	0.37±0.74	0.30±1.17	0.07 (−1.24 to 1.38)	0.909
WHR[b]	−0.003±0.01	0.006±0.01	−0.009 (−0.03 to 0.009)	0.285
Systolic BP (mmHg)[b]	2.7±10.7	−2.3±13.1	5.0 (−11.2 to 21.2)	0.502
Diastolic BP (mmHg)[b]	1.9±8.7	3.3±6.8	−1.4 (−12.3 to 9.4)	0.770
HR (bpm)[b]	−7.3±12.2	0.4±7.8	−7.7 (−22.1 to 6.7)	0.255
Endometrial thickness (cm)[c]	0.62±0.43	0.26±0.18	0.36 (−0.11 to 0.83)	0.120
Estradiol (pg/ml)[c]	96.9±68.1	24.9±17.0	72 (0.72 to 143.2)	**0.048**
IGF1 (ng/ml)[c]	−132.8±122.1	−14.4±67.3	−118.4 (−257.4 to 20.6)	0.086
IGFBP3 (ng/ml)[c]	−293.5±904.4	152.6±752.9	−446.1 (−1597.4 to 705.2)	0.404
IGFBP1 (ng/ml)[c]	17.2±15.0	−8.7±14.9	25.9 (5.4 to 46.4)	**0.019**
Glucose (mg/ml)[b]	−7.5±9.9	−0.8±7.8	−6.7 (−19.1 to 5.7)	0.252
Insulin (μIU/ml)[c]	0.92±7.7	−0.79±7.3	1.71 (−8.6 to 12.0)	0.717
C-peptide (ng/ml)[b]	0.46±1.19	−0.34±0.82	0.8 (−0.62 to 2.2)	0.233
HOMA[c]	−0.42±0.55	−0.04±0.43	−0.38 (−1.06 to 0.31)	0.249
Total cholesterol (mg/dl)[b]	17.3±35.1	−11.8±11.5	29.1 (−8.2 to 66.4)	0.111
HDL (mg/dl)[b]	14.7±6.7	0.0±8.9	14.7 (4.0 to 25.3)	**0.012**
LDL (mg/dl)[b]	−11.0±40.0	−5.0±12.7	−6.0 (−48.4 to 36.4)	0.757
Triglycerides (mg/dl)[b]	12.0±67.0	−32.6±61.2	44.6 (−43.8 to 133)	0.283
Ultra-sensitive C-reactive protein (mg/l)[c]	−0.38±2.7	−1.67±0.96	1.29 (−1.6 to 4.2)	0.344

CI, confidence interval; BMI, body mass index; WHR, waist–hip ratio; BP, blood pressure; HR, heart rate; HOMA, homeostasis model assessment. Values in bold indicate $P \leq 0.05$.
[a]Value calculated with Student's *t*-test for independent samples.
[b]Mean±S.D.
[c]Median (minimum−maximum).

estrogens) differed between studies. Furthermore, most of these studies were not placebo controlled. Instead, they were longitudinal or cross-sectional studies involving small groups of patients.

Those earlier studies demonstrated 15–40% reductions in IGF1 levels after the use of oral estrogens (3, 6, 7, 20, 25, 26). In our experiment, we observed a 42% reduction in IGF1 levels after 2 mg oral estradiol was given. Helle *et al.* (6) employed the same type and dose of oral estrogen and observed a 16% reduction in the IGF1 levels of menopausal women. Therefore, the accentuated decrease in IGF1 observed in our experiment appears to be the result of having studied patients with GH deficiency, i.e. patients in whom secretion of GH could not increase in compensation.

The various effects of different routes of estrogen administration could be the result of different effects on the hepatic synthesis of IGF1. Transdermal estrogens are not subjected to the effects of the first pass through the liver and, therefore, do not cause the hepatic changes that result in reduced IGF1 (6, 7, 20, 26). Another study has also demonstrated that intranasal administration of 17β-estradiol does not modify serum IGF1 levels (27).

Oral estrogens provoke increases in HDL cholesterol without altering the remainder of the lipid profile. Although treatment with GH may result in worsened carbohydrate metabolism and cause IR (28), our data did not reveal changes in the levels of glycemia, insulin, C-peptide or the HOMA index in response to oral estrogens.

In our sample, oral estrogens provoked a significant increase in IGFBP1 levels, which did not take place with transdermal estrogens. Another study with menopausal women showed significant increases in IGFBP1 after 3 months' use of 2 mg oral estradiol valerate (25). A 46% increase in serum IGFBP1 levels was observed by Helle *et al.* (6) after oral estrogen was given to eight women who had had undetectable serum progesterone levels; the suggestion was made that this increase was due to the oral therapy.

Some authors have shown a positive correlation between low IGFBP1 levels and intermediate markers for cardiovascular disease in non-diabetic patients (29, 30). Another study has also demonstrated a positive correlation between low serum IGFBP1 levels and macrovascular disease and hypertension in diabetic patients, suggesting that elevated concentrations of IGFBP1 could protect against cardiovascular disease, reducing the mitogenic potential of IGFs in the vasculature (31). Low levels of IGF1 and IGFBP1, together with elevated ultra-sensitive C-reactive protein levels, have also been implicated in the pathogenesis of metabolic syndrome and cardiovascular disease (32). In our experiment, we did not observe changes in ultra-sensitive C-reactive protein, in spite of the reduction in IGF1 and the increase in IGFBP1 with the use of oral estrogens.

Because all patients included in our sample had hypopituitarism and were receiving a fixed dose of

GH (0.5 IU/day), the variations observed in IGF1 and IGFBP1 levels were not the result of possible central effects of estrogens modifying GH secretion. In fact, these effects appear to depend primarily on the hepatic actions of oral estrogens, also responsible for the increase in HDL cholesterol (25). Moreover, since the variation in IGFBP1 was inversely correlated with variations in insulin levels in the group given oral estrogens, we might expect a reduction in insulin secretion, with a resulting increase in glycemia and/or changes in its peripheral action. However, to the extent that glycemic levels remained unaltered, with lower insulin levels, we could infer that there was an improvement in peripheral insulin action, although not expressed in the HOMA index. Nevertheless, irrespective of the mechanism involved, increases in IGFBP1 and reductions in serum IGF1 levels resulting from the oral administration of estrogen establish a situation of relative resistance to the action of GH. These changes could be reflected clinically, with the emergence of certain abnormalities associated with GH deficiency, such as increase in trunk fat, increase in body weight, changes in the lipid profile, increase in C-reactive protein levels, and abnormalities in glucose metabolism, which were not observed in our study. These clinical abnormalities may not have occurred because of the 3-month follow-up period, which was possibly insufficient for some of these changes to be observed.

We comment about the possibility of a type 2 error in our study. Also, although the doses of estrogen administered to both groups (33) were equivalent (a daily dose of 2 mg oral estradiol and a daily dose of 50 μg transdermal estradiol), the mean serum estradiol concentration in the oral group reached higher levels, though within normal reference ranges. However, the difference between estradiol levels might have had an influence on the outcome measures because the changes in estradiol levels after 3 months of treatment were 96.9 ($\pm$68.1) and 24.9 pg/ml ($\pm$17.0), $P=0.048$, in the oral and transdermal groups respectively. In addition, the changes in IGF1 levels after 3 months of treatment were not different between the two groups, -132.8 ($\pm$122.1) in the oral group and -14.4 ng/ml ($\pm$67.3) in the transdermal group, $P=0.086$ (Table 5).

In conclusion, our preliminary data showed that the administration of oral estrogen to patients with hypopituitarism significantly reduces the GH action, modifying total serum IGF1 levels and possibly its free fraction. These findings suggest that estrogen replacement should be transdermally administered to these patients, with a consequent reduction in the doses of GH required to obtain adequate IGF1 levels. This could potentially reduce the cost of treatment and amplify the metabolic and physiological actions of GH, with favorable repercussions for the correction of the clinical status and the improvement of the quality of life of these patients.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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