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Pituitary diseases: long-term clinical consequences

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Chapter 13

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

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ABSTRACT

Objective

Growth hormone (GH)-deficient women using oral estradiol treatment require higher doses of recombinant human GH (rhGH) to achieve similar insulin-like growth factor (IGF)-I levels 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 SHBG concentrations.

Design

Parallel cross-over 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 4 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 (5 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. 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 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 growth hormone replacement, the route of estrogen administration is a determinant of IGF-I levels in hypogonadal, GH-deficient women.



INTRODUCTION

Clinical studies have demonstrated clear effects of estrogens on GH production and IGF-I levels. Estrogens amplify GH releasing hormone (GHRH)- and insulin-stimulated GH release and spontaneous 24h GH production is threefold higher in healthy premenopausal women than in healthy matched men (1;2). Women on estrogen replacement require higher doses of 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 growth hormone deficiency (GHD) on a stable rhGH replacement dose, IGF-I levels increased during a switch from oral to transdermal 17 β -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 (2mg/day and 4 mg/day) and different doses of transdermal estradiol (50 μ g/day and 100 μ g/day) aimed at identical serum estradiol concentrations on serum concentrations of IGF-I, IGFBP-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 μ g/l during the insulin tolerance test (nadir blood glucose <2.2 mmol/l).
- 2) stable rhGH replacement during at least 3 months.
- 3) oral or transdermal estradiol treatment because of gonadotropin deficiency.
- 4) written informed consent.

Patients were enrolled in the two study arms based on their pretreatment (oral estradiol and transdermal estradiol), with a individualized dose of GH replacement aimed at achieving a



normal IGF-I for age. Eighteen patients started with oral estradiol (of whom 15 completed the study), and 5 patients with transdermal estradiol. Clinical details are shown in Table 1. The mean dose of rhGH replacement during the study was 0.75 ± 0.28 mg/day (range 0.3 to 1.3 mg/day). Mean duration of rhGH replacement prior to the start of the study was 6 years (range 1 to 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 cross-over study (Figure 1). Patients were divided into two groups based on the route of administration of estrogens prior to study start. The dose of rhGH 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 carry-over 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, 100 μ g and 50 μ g transdermal estradiol. In group II, the baseline treatment of 50 μ g transdermal estradiol was sequentially increased to 100 μ g 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 4 cycles longer in group I. Study parameters during the two periods of estradiol administration (cycles 1 and 9) did not differ, excluding a carry-over 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, 13 in group I and II and cycle 17 in group I only.

Study medication

Estradiol (Estrofem 2 mg and 4 mg, (Novo Nordisk Farma BV, Alphen aan den Rijn, The Netherlands), Dermestril 50 μ g and 100 μ g (Sigma Tau Ethifarma BV, Assen, The Netherlands)) was given with additional dydrogesteron (Duphaston 10 mg, 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 (8:00 a.m. and 6:00 p.m.). The fasting serum estradiol concentrations were assumed to reflected the 24h concentrations, because the patients received the Estrofem tablets twice daily, and because of the long apparent half-life of the drug (about 16h) 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



Table 13/1: Characteristics of patients with GH and gonadotrophin deficiency included in the study.

Group	Age	Etiology	Treatment	Pituitary deficiencies besides GH and gonadotropin deficiency	rhGH replacement dose (mg/day)	Completed study
1 OR	21	Craniopharyngeoma	TSS	ACTH, TSH, ADH	1.30	Yes
2 OR	45	M. Sheehan		ACTH, TSH	1.00	No
3 OR	33	M. Cushing	TSS	ACTH, TSH, ADH	1.00	Yes
4 OR	36	Craniopharyngeoma	TCS	ACTH, TSH, ADH	0.53	Yes
5 OR	46	Prolactinoma	TSS	TSH	1.00	Yes
6 OR	64	Arachnoidal cyst of pituitary stalk		ACTH, TSH	0.80	No
7 OR	51	M. Cushing	TSS	ACTH, TSH, ADH	1.07	Yes
8 OR	38	Prolactinoma	TSS & RT	ACTH, TSH	0.50	Yes
9 OR	50	Non-functioning adenoma	TSS	ACTH	0.30	Yes
10 OR	48	Non-functioning adenoma	TSS & RT	ACTH, TSH, ADH	1.00	No
11 OR	37	Prolactinoma	TSS & RT	ACTH, TSH, ADH	0.80	Yes
12 OR	50	M. Sheehan		ACTH, TSH	0.60	Yes
13 OR	32	M. Cushing	TSS & RT	ACTH, TSH, ADH	0.80	Yes
14 OR	52	Non-functioning adenoma	TSS	ACTH, TSH	0.40	Yes
15 OR	40	Prolactinoma	RT	ACTH, TSH	0.67	Yes
16 OR	27	Prolactinoma	TSS & RT	ACTH, TSH, ADH	0.50	Yes
17 OR	49	M. Sheehan		ACTH, TSH	1.20	Yes
18 OR	50	M. Cushing	TSS	ACTH, TSH	0.53	Yes
19 TD	45	Non-functioning adenoma	TSS & RT	ACTH, TSH	0.80	Yes
20 TD	41	Prolactinoma	TSS & RT	ACTH, TSH	0.80	Yes
21 TD	54	M. Cushing	TSS	ACTH, TSH	0.80	Yes
22 TD	49	Epidermoid cyst	TSS	ACTH, TSH	0.33	Yes
23 TD	48	M. Sheehan		ACTH	0.50	Yes

Legend to Table 1: OR: patients starting with oral estrogen treatment; TD: patients starting with transdermal estrogen treatment; TSS Transsphenoidal surgery; RT radiotherapy; TCS transcranial surgery.



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). 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 to mmol/l) was 7.65. Serum IGFBP-3 was measured with an in-house RIA, as described previously (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 radio-immunoassay. 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, 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).

Statistics

Statistical analysis was performed using Systat, version 11 (Systat Software, Richmond, CA). Results are shown as the mean $\pm$ standard error of the mean (SEM), 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 test and the Levene test. 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

Twenty-three patients were included of whom 20 patients completed the four different treatments (Fig. 1). Mean age was 44.2 ± 9.6 years and mean BMI was 28.5 ± 6.7 kg/m². Reasons for withdrawal were aggravated menstrual blood loss (n=1) and fluid retention (n=1), both during 2 mg OR, and personal reasons (n=1) during 100 µg TD estradiol.



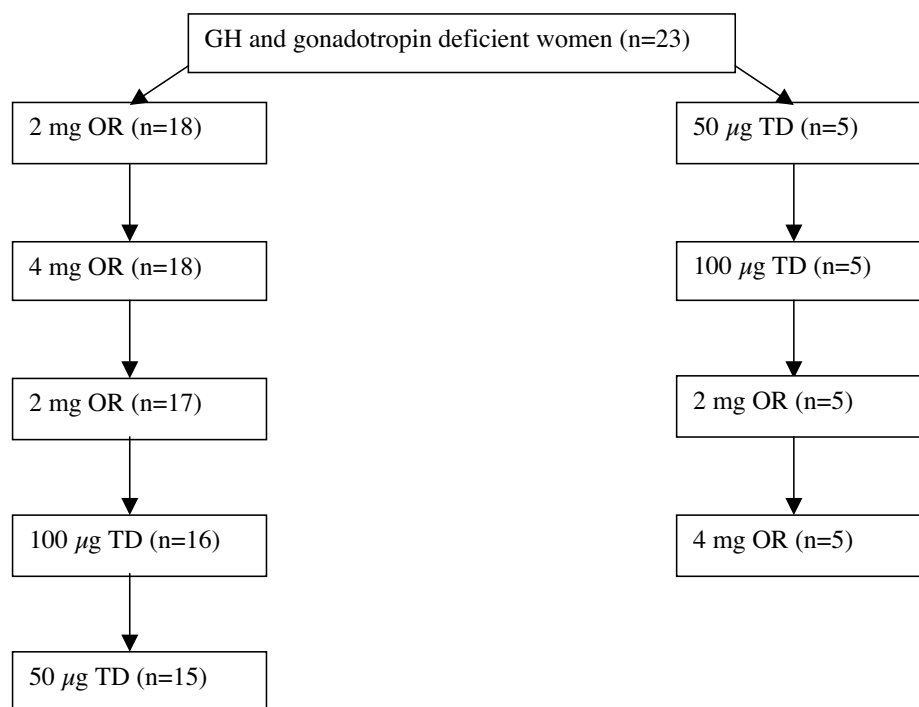


Figure 13/1: Study design of dose and route of estrogen administration in patients with GH and gonadotropin deficiency on stable dose rhGH replacement.

High versus low dose estrogen administration

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

During the high (4 mg) compared to the low (2 mg) oral estrogen dose, serum IGF-I was significantly lower (97 ± 8.4 vs. 140 ± 13.8 µg/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 µg estradiol the lowest mean estradiol and SHBG concentrations and higher mean IGF-I and IGFBP-3 concentrations were measured compared with those obtained during the 100 µg TD dose, the differences were not statistically significant.

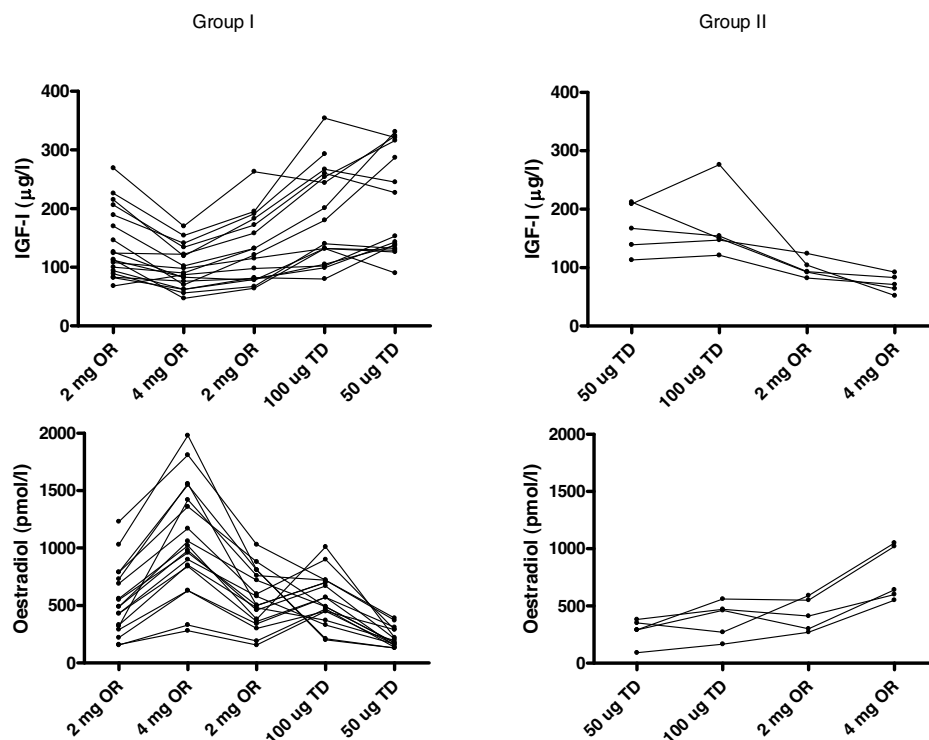


Figure 13/2: Individual plots of IGF-I (upper panels) and estradiol (lower panels) in hypogonadal GH-deficient females during a fixed dose of GH replacement and different doses of oral and transdermal estradiol replacement. Statistical details are listed in Table 2.

Oral (2 mg) versus transdermal (100 μ g) estrogen administration

Estradiol concentrations measured during 2 mg OR and 100 μ g TD estradiol were not statistically different. Despite comparable estradiol concentration, serum IGF-I was significantly lower in the 2 mg OR compared to the 100 μ 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 μ g estradiol dermal patch. Eight patients had 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. During 100 μ g TD and during 50 μ g TD, IGF-I concentrations were above 2 SD scores, indicating clear over-treatment in 3 and 5 cases, respectively. In

Table 13/2: Serum hormone concentrations in female growth hormone-deficient patients during GH and estrogen replacement.

	2 mg OR	4 mg OR	50 µg TD	100 µg TD
OR to TD group I				
IGF-I (µg/l)	140 ± 13.8 ^{a,c}	97 ± 8.4 ^b	206 ± 23	186 ± 21
Estradiol (pmol/l)	540 ± 70 ^{d,f}	1070 ± 111	221 ± 23	552 ± 57 ^e
IGFBP-3 (mg/l)	2.1 ± 0.1	1.9 ± 0.1	2.4 ± 0.2 ^g	2.3 ± 0.2
SHBG (nmol/l)	135 ± 13 ^{h,k}	174 ± 14 ^k	65 ± 6	95 ± 12
TD to OR group II				
IGF-I (µg/l)	99 ± 7.1 ^{m,l}	72 ± 7	168 ± 19	170 ± 27
Estradiol (pmol/l)	424 ± 64 ^{n,o}	772 ± 108	280 ± 51	385 ± 72
IGFBP-3 (mg/l)	1.8 ± 0.1	1.6 ± 0.1	2.0 ± 0.1	2.1 ± 0.1
SHBG (nmol/l)	120 ± 26 ^{q,r}	153 ± 28	70 ± 13	88 ± 21

Legend to Table 2: Data are shown as mean ± SEM. Data were analyzed by ANOVA for repeated measures. Significance was tested with the Bonferroni correction for multiple comparisons. ^a: p=0.005 vs. 100 µg TD; ^b: p<0.0001 vs. 50 µg and 100 µg TD; ^c: p=0.003 vs. 4 mg OR; ^d: p<0.0001 vs. 4 mg OR; ^e: p<0.0001 vs. 50 µg TD; ^f: p=0.01 vs. 50 µg TD; ^g: p<0.001 vs. 4 mg OR; ^h: p<0.002 vs. 4 mg OR; ^k: p=0.002 vs. 50 and 100 µg TD; ^l: p=0.04 vs. 4 mg OR; ^m: p=0.02 vs. 100 µg TD; ⁿ: p=0.007 vs. 4 mg OR; ^o: p=0.07 vs. 50 µg TD; ^q: p=0.004 vs. 100 µg TD; ^r: p=0.02 vs. 4 mg OR.

the other group the most important complaints were increase in weight and emotional lability during the high dose estradiol period.

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 compared with transdermal 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 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 recombinant human growth hormone replacement.

Serum IGF-I concentrations decreased during 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 cross-over 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 cross-over 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 cross-over study compared IGF-I concentrations with 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 IGF-I, which acts upon the somatotrope and hypothalamic centers 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 applied in the present study.

Estradiol is rapidly absorbed from the gastro-intestinal 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 sulphate 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 only be reached during oral estradiol treatment 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 highest during oral estrogen treatment compared with transdermal treatment. Nevertheless, the inhibitory effect on serum IGF-I concentration can also be accomplished by using large (nonphysiological) doses of transdermal estradiol, thus underscoring that the hepatic estradiol effects on IGF-I, SHBG, CBG, clotting factors, and CRP are liver-specific rather than route-specific (21). In the model we used, the differential effects of oral and transdermal estrogen administration on IGF-I levels were explained by different degrees of estrogen exposure of the liver. We found the well known dose dependent estrogen effects on serum IGF-I, but also a ~25% mean reduction of IGF-I by the first pass effect of oral administration.

Several animal studies have shown the relationship between estrogen treatment and hepatic IGF-I RNA expression. In ovariectomized rats, replacement with estradiol dose-dependently suppressed hepatic IGF-I liver mRNA expression and plasma IGF-I concentrations (22;23). Recently, the molecular mechanism underlying the hepatic effect of estrogen on IGF-I synthesis was discovered. Growth hormone signaling via the JAK-STAT pathway is inhibited by the suppression of JAK2 phosphorylation through stimulation of SOCS-2 (24). These basal mechanisms



Table 13/3: Summary of intervention studies with estrogens in GH and gonadotropin deficient women.

Author	Year of publication	Number of subjects	Design	Outcome
Kam (12)	2000	9	Single intervention study, 4 weeks treatment with oral estradiol valerate, 2 mg/day	Compared to baseline: IGF-I ↓, IGFBP-3 ↓, ALS ↓
Wolthers (8)	2001	8	Crossover study, randomized sequence, 8 weeks oral estradiol valerate 2 mg/day and 8 weeks of transdermal 17β-estradiol 100 µg/day, during 2 nd month of each estrogen phase rhGH administration in a stepwise incremental regimen.	Compared to baseline: during OR IGF-I ↓, during TD IGF-I ↔. RhGH administration: during OR IGF-I and increment in IGF-I lower compared to TD.
Christiansen (6)	2005	26	Withdrawal study, patients with oral estrogens were studied between tablet 2 and 10 in the estrogen replacement cycle and after 28 days of estrogen discontinuation	Discontinuation: SHBG ↑, IGF-I ↑, IGFBP-3 ↑. Regression: change in SHBG and change in IGF-I (86% of were main contributors in change in IGF-I (86% of variation)
Janssen (7)	2000	6	Crossover study, 2 cycles oral estradiol (2mg/day), then switch 3 cycles of transdermal estradiol (50 µg/day)	Switch from OR to TD estrogen: IGF-I ↑, IGFBP-3 ↔, estradiol ↔ (P=0.067), estrone ↓, SHBG ↓
Gibney (11)	2005	12	Crossover randomized study, 4 wk treatment with 17β-estradiol (2mg, followed by 4 mg) or raloxifene (selective estrogen receptor modulator with tissue-specific estrogen agonistic and antagonistic effects, 60 mg, followed by 120 mg)	Estradiol: IGF-I ↓, IGFBP-3 ↓ Raloxifene: IGF-I ↓, IGFBP-3 ↑

Legend to table 3: Summary of intervention studies in GH and gonadotropin deficient women. ↓ significant decrease, ↔ no change, ↑ significant increase.



explain the gender difference that is observed in GH sensitivity for example in the treatment of GH-deficient patients (25).

IGF binding protein (BP) 3 concentrations were lowest during 4 mg oral estrogen therapy, but the changes in this study were limited and also other studies report conflicting data on the estrogen effect on IGFBP-3. Either no effect (in normal pre- and postmenopausal women) (13;15) or a decrease was observed (in postmenopausal women and hypogonadal GH-deficient patients after oral estrogen administration) (11;12), while no changes were seen after transdermal estrogen administration. Although the limited changes in IGFBP-3 may be caused by the decrease of IGF-I, direct inhibition of IGFBP-3 synthesis and release from Kupfer cells is also possible (24). It is important to note that IGFBP-3 is of limited use in clinical practice to detect changes in disease activity of GH disorders.

The changes in SHBG levels after estrogen treatment were found to be dose dependent, with the highest SHBG concentrations during oral estrogen treatment. Serum SHBG concentrations correlated negatively with IGF-I concentrations, as reported by others in healthy subjects and in GHD (6;26). Moreover, SHBG levels were lower during transdermal administration of 100 µg estradiol compared with oral administration of 2 mg estradiol, despite similar circulating estradiol concentrations. Therefore, the route of estrogen administration is also a determinant of SHBG levels.

In this study, we could not include naïve untreated gonadotropin and GH-deficient premenopausal women. Consequently, a randomization procedure was not possible and the preference of patients and physicians for oral estradiol substitution is reflected in the unbalanced number of patients between the two groups. Because of the limited number of patients (in group II) and the four different estrogen treatments that had to be compared, the groups were separately analyzed, although no group effects or carry-over effects were present. Although probably due to the low number of patients in group II some of the changes did not reach significance, it is interesting to note that the changes in this group were comparable to the changes observed in group I. The additive value of group II is that the results of this study are both valid for interventions leading to IGF-I concentrations in the supraphysiological range (group I) as in the subphysiological range (group II). When the two groups were analyzed together, the conclusions remained unaltered (data not shown), particularly for the comparisons of IGF-I, IGFBP-3, and SHBG during 2 mg oral estrogen treatment and 100 µg transdermal estrogen treatment.

From a cost-effective point of view of GH substitution transdermal estrogen replacement is preferred. Patients who are switched from oral to transdermal estrogen replacement require ~ 0.3 mg GH less per day which on a nation wide scale is a considerable cost reduction (27). Leung et al. have calculated a cost reduction for the USA population of \$110 billion or approximately \$4400 per patient (27).

In summary, the route of estrogen administration is a determinant of serum IGF-I concentrations in adult women with GH and gonadotropin deficiency during fixed rhGH replacement.



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