

## COMMENTS

# A Switch from Oral (2 mg/Day) to Transdermal (50 $\mu$ g/Day) 17 $\beta$ -Estradiol Therapy Increases Serum Insulin-Like Growth Factor-I Levels in Recombinant Human Growth Hormone (GH)-Substituted Women with GH Deficiency

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### ABSTRACT

The response to GH therapy in adults with GH deficiency (GHD) is considerably variable. Generally, the response with regard to serum insulin-like growth factor (IGF)-I concentrations is significantly lower in females compared with males with GHD, which could at least partly be explained by the use of oral estrogen replacement therapy. In the present study, we investigated whether a switch from oral to transdermal estrogen therapy alters serum IGF-I concentrations in women with GHD on stable GH therapy. Six females with GHD and LH deficiency were investigated. During cycles 1 and 2, an oral dose of estradiol was given (2 mg/day), whereas during cycles 3, 4, and 5 estradiol was administered via the transdermal route at a dose of 50  $\mu$ g/day. Serum estrone levels significantly decreased ( $2470 \pm 475$  to  $110 \pm 26$  pmol/L,  $P = 0.005$ ), serum sex hormone-binding globulin

levels significantly decreased ( $102 \pm 13$  to  $63 \pm 7$  nmol/L,  $P = 0.004$ ), and serum estradiol levels also decreased albeit nonsignificantly with transdermal therapy ( $273 \pm 81$  to  $114 \pm 18$ ,  $P = 0.083$ ). Serum IGF-I levels significantly increased after the switch from oral to transdermal estrogen therapy ( $18.7 \pm 1.6$  and  $23.4 \pm 2.5$  nmol/L, respectively,  $P = 0.008$ ). Two of the six patients experienced fluid retention-related side effects, which disappeared after a reduction in dose at the end of the study. The results of the present study suggest that the potency of GH is altered in patients on transdermal compared to oral estradiol therapy. Further investigation should be undertaken to answer the question whether the increase in serum IGF-I levels is due to lower serum levels of estradiol or to differences in the mode of administration of estradiol. (*J Clin Endocrinol Metab* 85: 464–467, 2000)

SINCE THE introduction of recombinant human GH (rhGH), several clinical trials were designed to examine the effect of GH replacement therapy in adults with GH deficiency (GHD). It became apparent that GH treatment has beneficial effects on body composition, quality of life, and bone mass in adults with GHD. For this reason, GH replacement therapy is now an established, registered, and reimbursable therapy in adults with GHD.

The response to GH therapy is considerably variable. Our own data indicate that the response to GH treatment with regard to both serum insulin-like growth factor (IGF)-I concentrations and biochemical parameters of bone turnover was significantly lower in females compared with males with GHD (1, 2). We have also reported that women without oral estrogen replacement therapy (but with transdermal therapy or with no therapy despite LH/FSH deficiency) required lower doses of rhGH compared with women on oral estrogen replacement therapy (3). This finding is also in agreement

with a lower response with the IGF-I generation test in healthy postmenopausal women on oral estrogen therapy compared with women without estrogen replacement as reported by Lieberman *et al.* (4).

Both findings confirm physiological data in healthy controls. First, men and women have similar IGF-I concentrations, but women typically have higher circulating GH concentrations (5). Second, the effect of exogenous estrogen on the GH/IGF-I axis seems to be route dependent (oral *vs.* transdermal) in healthy controls. Oral estrogen suppresses circulating IGF-I levels and increases mean 24-h serum GH concentration in postmenopausal women, whereas women on low-dose transdermal estrogen demonstrate no change in spontaneous GH secretion and an unchanged or even increased serum IGF-I level (6–10).

Based on these observations, we hypothesize that a switch from oral to transdermal estrogen therapy will increase serum IGF-I concentrations in women with GHD on GH therapy.

### Materials and Methods

#### Subjects

Six females with GHD and LH deficiency were investigated (age range, 20–53 yr; body mass index range, 23.9–32.7 kg/m<sup>2</sup>). All patients

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used an oral evening dose of estradiol (2 mg) with additional hydrogesteron for 14 of 28 days. One patient had additional estradiol valerate.

GH substitution therapy was given for at least 1 yr and was tailored according to serum IGF-I levels. Substitution therapy was kept constant for at least 3 months preceding the study (range, 3 to >12 months; median, 8 months).

All patients had additional TSH deficiency, and four of six patients had ACTH deficiency, both of which were substituted as indicated. The etiology of hypopituitarism was a chordoma in one patient and a pituitary adenoma in five patients: morbus Cushing (n = 2), prolactinoma (n = 2), and nonfunctioning-adenoma (n = 1).

*Design of the study*

The duration of the study was five menstrual cycles of 28 days each. Estradiol was given with additional hydrogesteron (Duphaston 10 mg) from day 15 to day 28. During cycles 1 and 2, a daily oral dose of estradiol (2 mg Zumenon) was given, whereas during cycles 3, 4, and 5 estradiol was given via the transdermal route (Menorest 50, two times per week). A higher dose of transdermal estradiol was given in the one patient with additional estradiol valerate (progynova) until the switch (Menorest 75, two times per week). The dose of GH was kept constant within one individual during the whole duration of the study

Patients were seen in the outpatient clinic in the morning fasting, on days 12 (10–14) and 26 (24–28) of cycles 1 and 5 and on day 12 (10–14) of cycles 2 and 4. Body composition and blood pressure were measured, and blood was withdrawn for the measurement of serum IGF-I, IGF binding protein-3 (IGFBP-3), estradiol, and estrone levels.

*Body composition*

Weight was measured with a minimum of clothes to the nearest 0.1 kg. Height was measured barefoot to the nearest 0.001 m. The body mass index was calculated as weight (kilograms)/height (meters)<sup>2</sup>.

Body impedance was measured with a Human-IM Scan multifrequency impedance analyzer (Dietosystem, Milan, Italy), using a tetrapolar electrode placement with a surface area of 5 cm<sup>2</sup> at the left side of the body. Total body water (TBW) and extracellular water were calculated using the equation developed by Deurenberg *et al.* (11) based on a healthy control group, which is the most appropriate in adults with GHD, as reported previously (12). Free-fat mass was estimated by assuming a hydration of 73% (13). Mean body weight, TBW, extracellular water, and fat percentage during the oral estrogen phase were compared with mean levels during the transdermal phase.

*Assays*

The total serum IGF-I concentration was determined by RIA (INCSTAR Corp., Stillwater, MN) after extraction and purification on ODS-silica columns (Dia Sorin, Düsseldorf, Germany). The efficiency of separating the individual components of the IGF-I complex was demonstrated by the absence of IGFBP-3 in the eluate. The interassay coefficient of variation was less than 11%. The detection limit was 1.5 nmol/L. The measurement of serum IGFBP-3 was performed by RIA (Nichols Institute Diagnostics, San Juan Capistrana, CA), with an interassay coefficient of variation below 6.8% at different levels. The limit of detection was 0.08 mg/L.

Total estradiol concentrations were determined by RIA using a coated tube-technique (Orion Diagnostica, Espoo, Finland) with a sensitivity of 5 pmol/L. The interassay coefficient of variation (CV) is less than 8% at different levels. Cross-reaction of estrone is below 1% (to convert to pg/mL divide by 3.671). The addition of supraphysiological amounts of SHBG to serum did not influence the results.

Estrone was determined by RIA with double-antibody precipitation of separation of the bound and free fractions (DSL, Webster, TX). The interassay CV is less than 10% at different levels. The sensitivity is 5 pmol/L. Cross-reaction of estradiol is 1.25% (to convert to pg/mL divide by 3.7).

Serum SHBG concentrations were measured with a highly specific solid-phase two-site chemiluminescent immunometric assay (Immulite; DPC, Los Angeles, CA). The interassay CV is less than 9% at different levels. The sensitivity is 0.2 nmol/L.

*Statistics*

Results are given as mean ± SEM. Data were analyzed by ANOVA with repeated measures. Contrasts between relevant time points were subsequently calculated. Because these analyses demonstrated the absence of an increase in hormone concentration during either oral or transdermal estrogen therapy, most results are expressed as the mean of the average individual data during the experimental periods. The level of significance was 0.05. The calculations were performed with Systat, release 9 (SPSS, Inc., Chicago, IL).

**Results**

*Serum IGF-I and IGFBP-3 levels*

Changes in serum IGF-I of individual patients during the study are given in Fig. 1. The ANOVA revealed highly significant increases in circulating concentrations after the switch from oral to transdermal estrogen administration ( $P = 0.002$ ). IGF-I concentrations did not change significantly ( $P = 0.2$ ) during oral estrogen therapy, and this was also true for the transdermal estrogen application period ( $P = 0.089$ ). The mean average individual serum IGF-I levels significantly increased after the switch from oral to transdermal estrogen therapy ( $18.7 \pm 1.6$  to  $23.4 \pm 2.5$  nmol/l,  $P = 0.008$ ).

Serum IGFBP-3 levels did not significantly change during the study (ANOVA,  $P = 0.170$ ). The mean average individual concentrations were  $2.6 \pm 0.15$  and  $3.0 \pm 0.3$  mg/L during oral and transdermal therapy, respectively ( $P = 0.151$ ).

*Serum estradiol, estrone, and SHBG*

Plasma estradiol concentrations tended to be higher during oral therapy than during transdermal administration (ANOVA,  $P = 0.067$ ). The mean estradiol level during oral therapy was 273 pmol/L with a large SE (81 pmol/L). Two of the six patients had estradiol levels above the normal range of the follicular phase of the menstrual cycle of healthy individuals (normal range, 70–250 pmol/L). During transdermal therapy, mean estradiol levels tended to be lower than

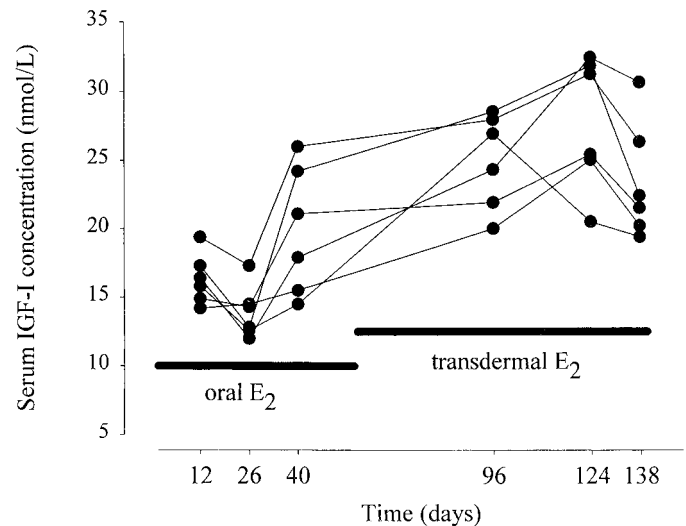


FIG. 1. Changes in serum IGF-I concentration during oral and transdermal estradiol therapy in GHD patients. Data of the six individual patients are shown. The increase in levels during transdermal administration is significant (ANOVA,  $P = 0.002$ ). Blood samples at time points 26 and 138 were taken during the administration of progestagen.

during oral therapy ( $114 \pm 18$  pmol/L,  $P = 0.083$ ). One patient had a mean level of estradiol below the normal range of the follicular phase.

Serum estrone levels significantly decreased as expected (ANOVA,  $P < 0.0005$ ). The individual mean concentrations during oral and transdermal therapy were  $2470 \pm 475$  and  $110 \pm 26$  pmol/L respectively;  $P = 0.005$ .

Serum SHBG levels significantly decreased (ANOVA,  $P < 0.0005$ ). The individual mean concentrations fell from  $102 \pm 13$  to  $63 \pm 7$  nmol/L ( $P = 0.004$ ).

The percentage change in estradiol, estrone, or SHBG was not significantly correlated with the percentage change in serum IGF-I levels.

#### *Body composition and blood pressure/heart frequency*

No significant differences were found in mean body weight during the oral estrogen phase compared to that during the transdermal phase ( $74.6 \pm 4.5$  and  $75.0 \pm 5.3$  kg, respectively;  $P = 0.683$ ). In addition, no significant changes were found in TBW ( $35.8 \pm 1.1$  and  $36.9 \pm 1.8$ , respectively;  $P = 0.241$ ) or fat percentage ( $33.3\% \pm 4.1$  and  $31.0 \pm 5.6\%$ , respectively;  $P = 0.260$ ).

No significant changes were found in systolic or diastolic blood pressure or in mean heart frequency.

#### *Side effects*

During transdermal estrogen therapy, two of the six patients reported side effects related to fluid retention. One patient reported severe pain in the hands and arms, which started  $\pm 2$  weeks after switching to the transdermal route. These complaints related to a carpal tunnel syndrome and had been present in a mild form for some months but had not been until then reported to the physician. At the end of the study, the dose of rhGH was halved (to 1.6 IU), and complaints decreased within 2 weeks. The other patient reported muscle stiffness  $\pm 6$  weeks after the switch to transdermal estrogen. The dose of rhGH was reduced from 2.8 IU to 2.4 IU at the end of the study, and complaints disappeared subsequently.

### **Discussion**

GH therapy in adults with GHD should be individualized because there is a large interindividual variability in response to treatment. We previously reported that males are generally more responsive to GH treatment than females (1), and in the present study we found that the response to treatment with regard to serum IGF-I levels can be improved by administering estrogen therapy transdermally (with lower serum estradiol levels), rather than orally, in hypopituitary females.

Several studies have demonstrated that estrogen has important effects on the GH/IGF-I axis. Oral estrogen therapy increases GH secretion and decreases IGF-I levels in postmenopausal women (8, 14–16). This finding suggests that estrogen therapy inhibits GH stimulation of IGF-I secretion with consequent diminished feedback on the hypothalamic-pituitary axis, resulting in increased GH levels. Kelly *et al.* (14) investigated the effect of three different estrogen for-

mulations and reported that in postmenopausal women all three had suppressive effects on serum IGF-I with increased GH levels. However, the suppressive effect was highest with ethinyl-estradiol (20 ug), followed by conjugated equine estrogen (1.25 mg), and the least suppressive effect was with estradiol valerate (2 mg), although dose-related factors could not be ruled out because there were also differences in the suppressive effect on LH and FSH.

Interestingly, transdermal estrogen has been reported to increase serum IGF-I levels, without a change in GH secretion (7). The differences between the oral and transdermal routes of estrogen may reflect differing effects of high *vs.* low estrogen concentrations in the target tissue either on IGF-I secretion or on hepatic-derived binding proteins for GH or IGF-I. It is also possible that differences in dose or formulation of estrogen can explain differences in IGF-I suppression, although similar gonadotropin suppression was also reported (7). A more recent study in healthy postmenopausal women reported that transdermal estrogen had a suppressive effect on serum IGF-I levels similar to that of oral preparations when given in doses that result in high serum estradiol levels during oral and very high levels during transdermal therapy (17). Mean 24-h GH concentration was similarly increased after oral and transdermal estradiol therapy compared to the untreated state.

The direct effect of estradiol on serum IGF-I levels could, thus, be biased by changes in GH secretion. The present study was undertaken in females with hypopituitarism, including LH/FSH deficiency and GHD. All patients were substituted with rhGH for at least 2 years at a dose that was aimed at normalizing serum IGF-I levels. In addition, all females had been on oral  $17\beta$ -estradiol therapy for some time. During the study, patients were switched to a transdermal formulation of  $17\beta$ -estradiol, without any change in the dose of rhGH. Therefore, this study was an appropriate model to investigate the effect of the application route of  $17\beta$ -estradiol, without any bias deriving from the GH status. Serum IGF-I levels increased when patients were switched from the oral to the transdermal form of  $17\beta$ -estradiol. However, levels of estradiol tended to be lower during transdermal compared to oral substitution of  $17\beta$ -estradiol. Further investigation is, thus, required to test whether the application route and/or the dose played a role in the increase in serum IGF-I concentrations observed with transdermal estrogen therapy.

Women with (hypogonadotropic) hypogonadism require sex hormone replacement for relief of symptoms (*e.g.* vaginal dryness) and for prevention (or at least limitation) of the long-term consequences of estrogen deficiency (*e.g.* osteoporosis). No data are available on the dose of estradiol for "physiological" replacement. Generally, 2 mg oral estradiol is given, which results in high normal levels of serum estradiol and very high levels of serum estrone. A more physiological replacement is reached with transdermal estradiol substitution, with both (low) normalized levels of serum estradiol and serum estrone. When the goal of substitution is to normalize serum estradiol and serum estrone levels, transdermal substitution is a better choice, and when oral therapy is given, doses lower than 2 mg should be considered.

In postmenopausal women, the transdermal patch (50 µg/day) was able to increase bone mineral density of the lumbar spine after 2 years of treatment, with a modest additional clinical benefit by increasing the dosage to 75 or 100 µg (18). No differences were reported between transdermal and oral estradiol with regard to bone mineral density and/or bone metabolism in postmenopausal women (19). With regard to lipid metabolism, oral estradiol generally results in more beneficial effects than transdermal therapy (20), which might at least partly be due to differences in serum estradiol levels. In the present study, no change was found in body weight, TBW, or fat percentage. In a larger group of postmenopausal women, oral estrogen was found, however, to increase fat mass and decrease lean body mass when compared with transdermal estrogen therapy, although dose-related factors could not be excluded (21).

Two of the six patients in the present study developed side effects related to fluid retention during transdermal estrogen therapy. GH dose was decreased at the end of the study, and side effects readily disappeared in both patients, suggesting that the side effects were related to GH therapy and not to transdermal estrogen therapy. Prescribing transdermal estrogen therapy, or possibly lower doses of estrogen, instead of the regularly used oral estrogen, to GH-treated patients, could thus have positive financial consequences. However, possible smaller beneficial effects of lower levels of estradiol with regard to bone mineral density and cardiovascular effects remain to be examined.

Estrogen replacement therapy is commonly prescribed in combination with a progestin to prevent endometrial hyperplasia or carcinoma. Additional oral intake of dydrogesterone did not significantly influence serum IGF-I or IGFBP-3 levels in this small number of patients, confirming data from others in healthy postmenopausal women (9).

In the present study, hypopituitary females on stable GH therapy were switched from an oral to a transdermal 17β-estradiol formulation. An increase in serum IGF-I concentrations was consequently observed, suggesting increased effectiveness of GH treatment. Further investigations should be undertaken to answer the question whether this finding is as a result of lower serum levels of estradiol or route-dependency. This could imply that doses of rhGH necessary to keep serum IGF-I within the normal reference range may be lowered in women on transdermal estrogen. The possible reduction in the rhGH dose would have a clear impact on the financial aspects of rhGH therapy in women with GHD. Additional studies are required to answer the question whether lower doses of estradiol therapy would be sufficient in hypopituitary patients.

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