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CLINICAL STUDY

# Discontinuation of estrogen replacement therapy in GH-treated hypopituitary women alters androgen status and IGF-I

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## Abstract

*Objective and design*: Compared with their male counterparts, healthy females secrete more growth hormone (GH) and those with GH-deficiency have lower insulin-like growth factor I (IGF-I) levels and are less responsive to GH substitution. To test whether this gender difference is related to sex hormones we measured androgen status and IGF-I related parameters in 38 hypopituitary women (mean (range) age 41.5 (20–58) years) during continued GH substitution as compared with a control group of 38 healthy women matched for age and menopausal status. Twenty six patients were studied twice: with estrogen replacement and after 28 days of estrogen discontinuation in a randomised design.
*Results*: The patients were androgen deficient compared with controls (median, range), dehydroepiandrosterone sulphate (DHEAS): 185 (99–7800) nmol/l vs 4400 (820–13 000) nmol/l, $P = <0.001$; androstenedione: 0.5 (0.1–7.1) nmol/l vs 4.3 (1.6–8.8) nmol/l, $P = <0.001$; dihydrotestosterone (DHT): 0.13 (0.09–0.54) nmol/l vs 0.55 (0.09–0.89) nmol/l, $P = <0.001$; testosterone: 0.28 (0.09–1.56) nmol/l vs 1.1 (0.71–2.24) nmol/l, ($P = <0.001$); free testosterone: 0.004 (0.001–0.030) nmol/l vs 0.016 (0.001–0.030) nmol/l, $P = <0.001$. The circulating levels of IGF-I, IGF-II, IGF-binding protein 1 (IGFBP-1), and IGFBP-3 did not differ between patients and controls. The subgroup of patients receiving hydrocortisone (HC) replacement ($n = 24$) had significantly lower levels of androgens (suppressed by 80–100%) as well as IGF-I and IGFBP-3 as compared with the patients not receiving HC. IGF-I was correlated to free testosterone in patients ($r = 0.57$, $P = 0.0005$) as well as controls ($r = 0.43$, $P = 0.008$), and free testosterone was a significant positive predictor of IGF-I. Estrogen discontinuation induced an increase in IGF-I ($167 \pm 15$ vs $206 \pm 14$ μg/l, $P = 0.005$ and IGFBP-3 ($3887 \pm 139$ vs $4309 \pm 138$ μg/l, $P = 0.0005$). Estrogen discontinuation was associated with a significant increase in median (range) free testosterone (0.004 (0–0.02) vs 0.0065 (0–0.03) nmol/l, $P = 0.001$) and a significant decrease in median (range) sex-hormone binding globulin (SHBG; 93 (11–278) vs 55.5 (20–142) nmol/l, $P = 0.001$). ΔIGF-I correlated with ΔSHBG ($r = -0.45$ $P = 0.033$) and ΔIGFBP-3 ($r = 0.67$ $P = <0.001$). In a regression model ΔE2, Δtestosterone, ΔSHBG and ΔIGFBP-3 explained 93% of the variation in ΔIGF-I.
*Conclusions*: Androgen levels are low in hypopituitary women and free testosterone correlates with IGF-I. Discontinuation of estrogen replacement in these patients induces elevations in IGF-I as well as free testosterone, and ΔIGF-I correlated positively with Δfree testosterone. These effects may contribute to the gender differences observed in the GH–IGF axis in healthy adults as well as in the responsiveness of hypopituitary patients to GH substitution.

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## Introduction

Recent studies in hypopituitary adults have revealed gender differences in the regulation of serum insulin-like growth factor I (IGF-I) levels. In mid-life adult patients with panhypopituitarism on hormonal substitution excluding growth hormone (GH), serum IGF-I levels are significantly lower in females as compared with males (1–3). Moreover, the increase in serum IGF-I levels following GH substitution is significantly lower in females receiving the same GH dose as their male counterparts (3). The underlying mechanisms are unclear but a direct inhibitory effect of estradiol on hepatic IGF-I production has been suggested inasmuch as oral estradiol administration lowers IGF-I levels in healthy postmenopausal women (4), and the metabolic response to GH is lower in healthy elderly women when co-administered with estrogen (5). A gender difference in serum IGF-I levels is usually not recorded in healthy adults, which may be explained



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by a compensatory elevation in pituitary GH secretion in women (6). The majority of the hypopituitary patients studied so far in previous studies were substituted with sex steroids, precluding an experimental evaluation of the impact of sex steriod treatment on IGF related parameters.

The role of testosterone in the regulation of IGF related parameters in adults has not been studied to the same extent. Administration of testosterone increases serum IGF-I levels in normal (7) and hypopituitary (8) men, and in a cross-sectional evaluation of hypopituitary patients with untreated adult GH deficiency (GHDA) serum IGF-I levels were highest in males receiving testosterone substitution (1). Since hypopituitary women exhibit very low androgen levels, it could be speculated that a certain threshold level of testosterone is necessary to permit IGF-I stimulation by GH. The impact of estrogen supplementation on androgen status in hypopituitary women has to our knowledge not previously been assessed.

In the present randomised study we examined the impact of estrogen discontinuation on androgen status and IGF-related parameters in hypopituitary women. The population included patients with and without hydrocortisone substitution, since this feature was assumed to influence androgen levels. All patients had documented GH-deficiency and GH substitution was continued throughout the study to ensure constant GH levels.

## Subjects and methods

### Subjects

Thirty-eight women with a clinical history of hypopituitarism and a mean (range) age of 41.5 (20–58) years from our out-patient clinic participated. The mean (range) duration of pituitary deficiency was 13 (2–43) years. In 28 patients hypopituitarism developed subsequent to pituitary surgery (nonfunctioning adenoma, $n = 21$; ACTH producing adenoma, $n = 4$; craniopharyngeoma, $n = 3$). One patient was diagnosed with empty sella and nine patients had idiopathic hypopituitarism of childhood-onset. All patients had GH-deficiency, which was diagnosed on the basis of a peak in GH of $< 3$ μg/l following an insulin tolerance test or an arginine stimulation test. The diagnosis was reconfirmed in adulthood in all patients with childhood-onset disease, and all patients continued regular GH substitution during the study. All patients had hypogonadism of whom 33 received oral estrogen. Patients with thyroid-stimulating hormone (TSH)-deficiency ($n = 29$), adrenocorticotropic hormone (ACTH)-deficiency ($n = 24$), and antidiuretic hormone (ADH)-deficiency ($n = 13$) continued their regular replacement therapy with levothyroxine, hydrocortisone and desmopressin, respectively. Of the 24 patients with ACTH deficicency, all were substituted with GH, 22 with levothyroxine and oral estrogen (two were postmenopausal and had stopped estrogen treatment). Nine of the 24 were treated with desmopressin. The control group comprised 38 healthy women matched for age and menopausal status. Data on age distribution and body composition in patients and healthy subjects are provided in Table 1.

### Study protocol

In a randomised fashion the patients receiving estrogen treatment were examined twice: i) between tablets two and ten in the estrogen replacement therapy cycle (ET); and ii) after 28 days of estrogen discontinuation (ED). Twelve patients were only studied once, which included those not receiving estrogen treatment ($n = 5$) and seven patients who wished to continue estrogen treatment. The healthy control group was studied once, which was in the early follicular phase of their menstrual cycle, where applicable. The measurements included a fasting morning blood sample and assessment of body composition. The protocol was approved by the Århus County Ethical Scientific committee (no. 1999/4644).

### Methods

Body composition was assessed by means of antropometry (body mass index (BMI), waist/hip ratio) in addition to bioimpedance (RJL BIA-101, RJL Systems Inc., Clinton Township, MI, USA) using Lukarski's equation to calculate fat free mass (FFM) and total body fat and total body water (TBW) (9).

### Assays

Serum IGF-I, -II, and GHBP were measured by in-house noncompetitive time resolved immunofluorometric assays (TR-FIA). Serum insulin-like growth factor binding protein-3 (IGFBP-3) was measured in an immunoradiometric assay (Diagnostic System Laboratories Inc, Webster, TX, USA). Serum IGFBP-1 was measured by ELISA (Medix Biochemica, Kainainen, Finland).

**Table 1** Age distribution and body composition in hypopituitary and healthy (control) patients.

| | Hypopituitary | Control | *P* |
|---|---|---|---|
| *n* | 38 | 38 | |
| Age (years) | 41.5 (20–58) | 40.5 (19–57) | 0.957 |
| Weight (kg) | 69.9 (49.5–113) | 62.2 (46.0–92.5) | <0.001 |
| BMI (kg/m$^2$) | 25.9 (19.8–42.9) | 22.1 (17.6–30.6) | <0.001 |
| Waist/hip ratio | 0.84 (0.67–0.99) | 0.76 (0.67–0.95) | <0.001 |
| Total body fat (kg) | 31.5 (7.15–62.1) | 21.0 (5.27–52.6) | <0.001 |
| Fat free mass (kg) | 41.6 (33.7–55.7) | 41.4 (32.0–69.2) | 0.669 |
| Total body water (l) | 30.6 (24.8–41.1) | 30.4 (23.5–51.1) | 0.640 |

BMI, body mass index. Values are means (range).

Dehydroepiandrosterone sulphate (DHEAS), α-4-androstendione (A), testosterone (T), dihydrotestosterone (DHT), sex-hormone binding globulin (SHBG) and 17β-estradiol (E2) were measured by an in-house radioimmunoassay (RIA) after extraction end subsequent celite chromatography (10). Free testosterone (fT) is estimated using a method described by Bartsch, based on measurement of SHBG, total T and DHT and use of the law of mass action, using the binding constant of T and DHT to SHBG, and including a calculation of binding of T to albumin (assuming a constant association constant for albumin). Binding to cortisol-binding globulin is thought to be negligible (11). This method for estimating fT is essentially similar to the method suggested by Vermeulen *et al.* to be the most reliable estimate of fT and correlates closely with direct measurement of fT by equilibrium dialysis (12). Inter- and intra-assay coefficients of variations, respectively were as follows: DHEAS: 11.5%, 8.5%; A: 11.4%, 9.4%; T: 13.8%, 8.2%; fT: 6.4%, 4.7%; DHT: 11.0%, 9.1%; SHBG: 7.5%, 5.2%; E2: 10.5%, 7.4%. The sex hormone assays are accredited after the ISO-17 025 standard. Samples with undetectably low levels were set to the detection limit.

### *Statistics*

Statistic calculations were done with SPSS for Windows version 10.0 (SPSS, Chicago IL, USA). Data was checked for normality with Kolmogorov–Smirnov's test and by plotting. Androgens and estrogen were non-parametrically distributed. Comparisons between groups were done with students unpaired *t*-test, or the Mann–Whitney U test, as appropriate. Data are expressed as means± S.E.M, or median (range), as appropriate. Spearman correlation analysis was used for examining relations between IGF-I, sex hormones and other variables. Multiple linear regression analysis was performed with IGF-I as the dependent variable. Statistical significance was assumed for *P* less than 5% ($P < 0.05$).

## Results

### *Population characteristics (Table 1)*

The patients were more obese in terms of total body weight, BMI, total fat mass and central adiposity as compared with the control group. The subgroup of hydrocortisone (HC) treated patients ($n = 24$) did not differ regarding age and body composition compared with the subgroup of patients with sufficient adrenal function (SAF, $n = 14$) (data not shown).

### *Sex hormones (Fig. 1)*

In the patient group as a whole the circulating androgen levels were reduced compared with the control group (range): DHEAS: 185 (99–7800) nmol/l vs 4400 (820–13 000) nmol/l (Fig. 1A), $P = <0.001$; A: 0.5 (0.09–7.11) nmol/l vs 4.3 (1.59–8.78) nmol/l (Fig. 1B), $P = <0.001$; DHT: 0.13 (0.09–0.54) nmol/l vs 0.545 (0.09–0.89) nmol/l (Fig. 1C), $P = <0.001$; T: 0.275 (0.09–1.56) nmol/l vs 1.1 (0.71–2.24) nmol/l (Fig. 1D), $P = <0.001$; fT: 0.004 (0.001–0.03) nmol/l vs 0.0155 (0.001–0.03) nmol/l (Fig. 1E), $P = <0.001$. By contrast, the levels of E2 as well as SHBG did not differ between patients and controls (range): E2: 0.25 (0.03–5.57) pmol/l vs 0.165 (0.04–1.37) pmol/l, $P = 0.214$; SHBG 93 (11–278) nmol/l vs 80 (26–365) nmol/l, $P = 0.981$. A substantial proportion of patients receiving hydrocortisone replacement displayed undetectable levels of circulating androgens, and their median levels were significantly lower as compared with the patients not receiving hydrocortisone (Fig. 1). Moreover, androgen levels among the patients not receiving hydrocortisone showed a considerable overlap with the control group, and the difference in median levels between these two groups only reached statistical significance for A, T and DHT (Fig. 1).

### *IGF and binding proteins (Table 2)*

When considering all patients together, their levels of circulating IGF-I, IGF-II, IGFBP-1 and -3 were not significantly different from the control group. Subdividing patients according to hydrocortisone therapy revealed lower IGF-I and IGFBP-3 levels in HC patients as compared with SAF patients. The serum levels of GHBP were higher in the patients as compared with the control group. This difference was also present when GHBP was divided by body weight (data not shown).

### *Correlations and regressions*

Age was correlated to IGF-I in controls ($r = -0.57$, $P = 0.0005$), but not in patients ($r = -0.19$, $P = 0.279$). GHBP correlated to indices of fat mass, i.e. in patients TBF ($r = 0.60$, $P = 0.0005$) and in controls BMI ($r = 0.56$, $P = 0.0005$). DHEAS ($r = -0.50$ $P = 0.002$), A ($-0.47$, $P = 0.004$), and DHT ($-0.52$, $P = 0.001$) declined with age in patients, and in controls (DHEAS: $-0.58$, $P = 0.001$; A: $r = -0.64$, $P = 0.0005$; DHT: $r = -0.62$, $P = 0.0005$). In hypopituitary patients free testosterone and SHBG showed reciprocal relationships to several IGF related parameters. IGF-I: fT, $r = 0.57$, $P = 0.001$ (Fig. 2); SHBG: $r = -0.62$, $P = 0.0005$; IGFBP-1: fT, $r = -0.33$, $P = 0.055$, SHBG: $r = 0.44$, $P = 0.008$; IGFBP-3: fT, $r = 0.55$, $P = 0.001$; SHBG: $r = -0.66$, $P = 0.0005$. In the control group this relationship only achieved statistical significance for IGF-I (fT: $r = 0.43$, $P = 0.008$ (Fig. 2); SHBG: $r = -0.35$, $P = 0.030$) and IGFBP-1 (fT: $r = -0.60$, $P = 0.0005$; SHBG: $r = 0.40$, $P = 0.014$).

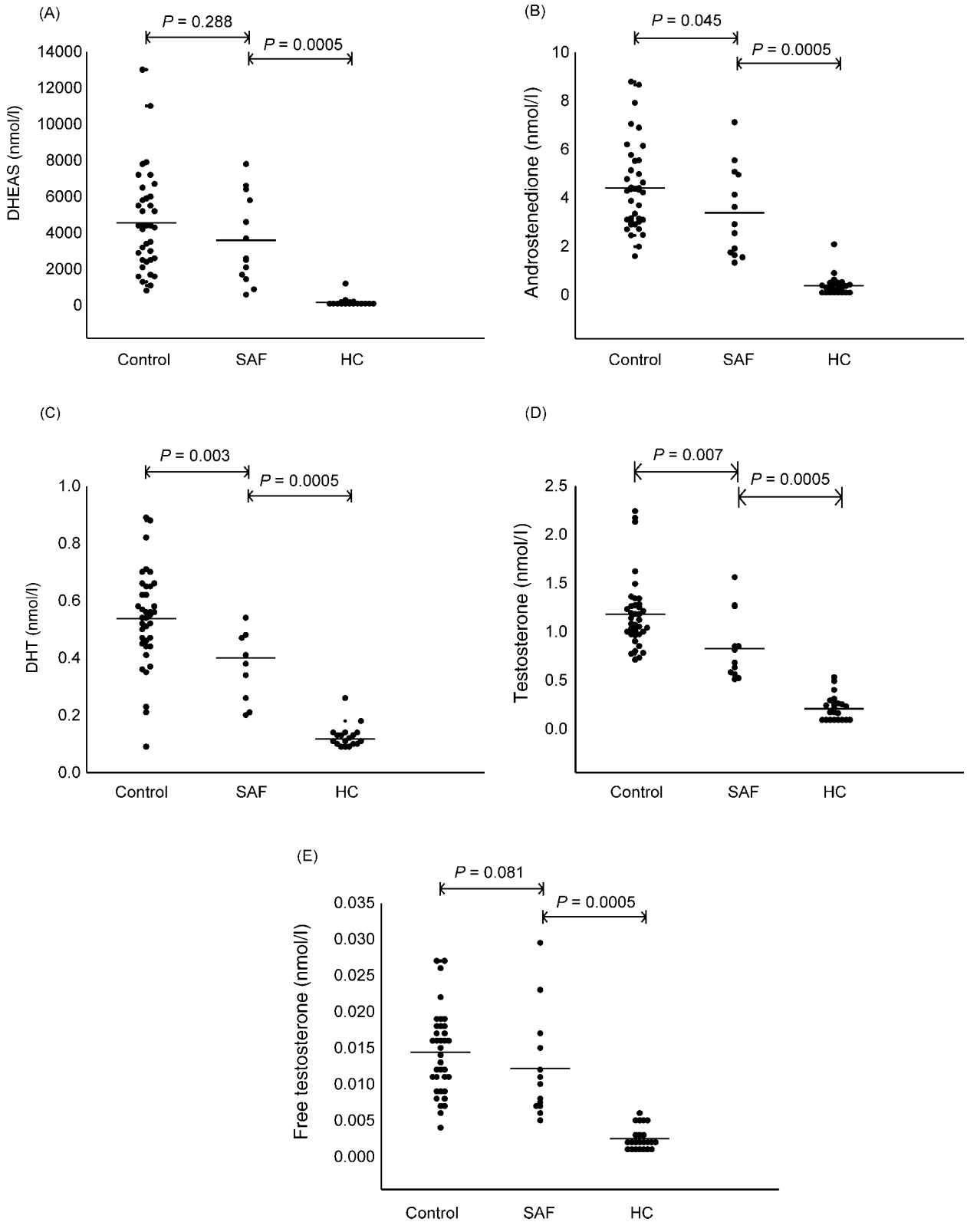


**Figure 1** Single measurements (●) and mean levels (–) of circulating DHEAS (A), androstendione (B), DHT (C), testosterone (D) and free testosterone (E) levels in healthy female controls (Control), in women with hypopituitarism but sufficient adrenal function (SAF) and in hypopituitary women recieving hydrocortisone treatment (HT). The level of significance between the studied groups is indicated in the figures.

**Table 2** Circulating levels of insulin-like growth factor (IGF)-I and IGF binding proteins (IGFBP) in controls and treated subgroups of hypopituitary patients.

| | Hypopituitarism | Control | *P* | SAF | HC | *P* |
|---|---|---|---|---|---|---|
| *n* | 38 | 38 | | 14 | 24 | |
| IGF-I (μg/l) | 164±13 | 178±8 | 0.509 | 201±14 | 145±16 | 0.033 |
| IGF-II (μg/l) | 1045±43 | 1052±23 | 0.892 | 1153±48 | 989±57 | 0.069 |
| IGFBP-1 (μg/l) | 3.25 (0.50–24.46) | 4.94 (0.71–17.26) | 0.334 | 1.81 (0.50–24.46) | 3.39 (0.60–17.78) | 0.375 |
| IGFBP-3 (μg/l) | 3751±148 | 3997±73 | 0.251 | 4149±135 | 3542±203 | 0.021 |
| GHBP (nmol/l) | 2.69±0.17 | 1.38±0.10 | <0.001 | 2.37±0.25 | 2.86±0.23 | 0.272 |

SAF, sufficient adrenal function; HC, hydrocortisone treated patients.
Values are means±S.E.M. (range).

In a multiple linear regression model with IGF-I as the dependent variable, age, status (i.e. patient or a control), BMI, IGFBP-3 and fT were independent explanatory variables ($R = 0.735$, $P < 0.0005$) predicting 54.1% of the variation in IGF-I with no further contribution of SHBG or E2.

## *Effects of discontinuation of estrogen replacement*

***Body composition*** Estrogen discontinuation did not significantly influence body composition (estrogen treatment (ET) vs. estrogen discontinuation (ED) (data not shown).

***Sex hormones*** Estrogen discontinuation was associated with a significant increase in the level of free testosterone and a significant decrease in SHBG (fT: 0.004 (0–0.02) nmol/l (ET) vs 0.0065 (0–0.03) nmol/l (ED), $P = 0.001$; SHBG: 93 (11–278) nmol/l (ET) vs 55.5 nmol/l (20–142) (ED), $P = 0.001$] (Fig. 3). By contrast the levels of DHEAS, A, T, and DHT were almost identical in the two situations (data not shown). The median levels of DHEAS were also unaffected by estrogen discontinuation in the subgroup of patients not receiving hydrocortisone ($n = 8$) (DHEAS: 3150 (600–7800) nmol/l (ET) vs 3200 (670–7200) nmol/l (ED), $P = 0.973$).

***IGF and binding proteins*** Estrogen discontinuation was followed by an increase in IGF-I, IGF-II and IGFBP-3 and a decrease in IGFBP-1 (Table 3). Moreover, the ratio of IGF-I:IGFBP-3 was also increased following estrogen discontinuation. With the exception of IGFBP-1, these changes appeared to be independent

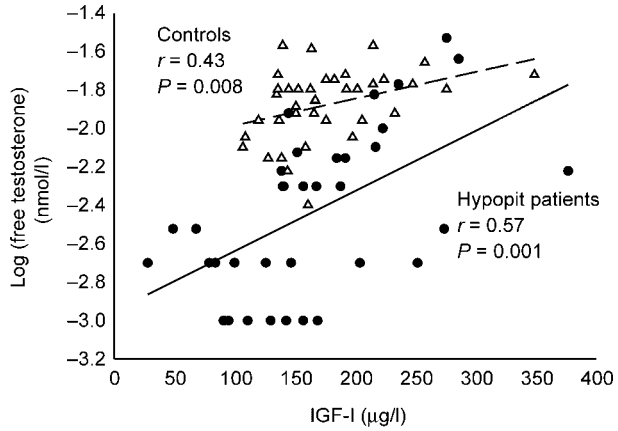


**Figure 2** Correlation between free testosterone (logarithmic transformed data) and IGF-I in female hypopituitary (Hypopit) patients (●, solid line) and in age matched controls (△, dashed line). Regression coefficients and level of significance are given in the figure.

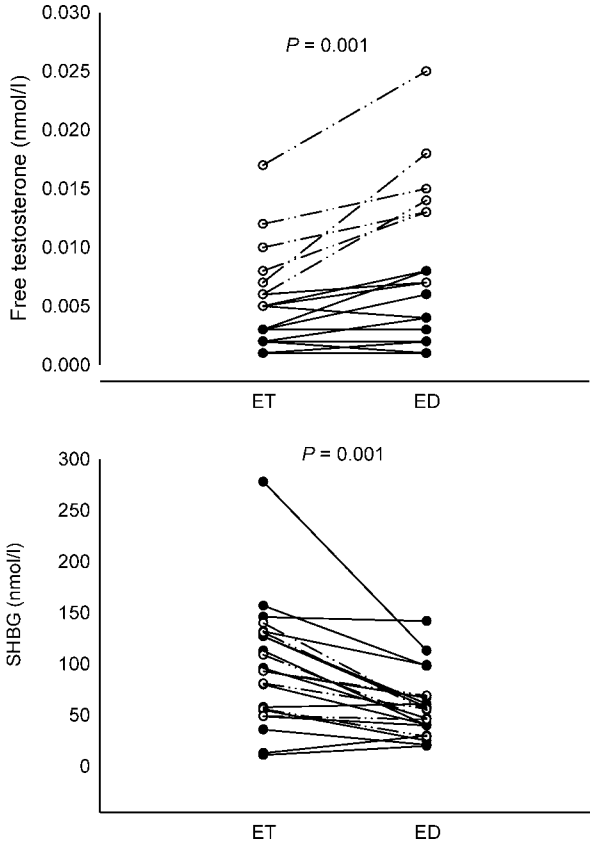


**Figure 3** Free testosterone and SHBG levels in female hypopituitary patients during oral estrogen replacement therapy (ET) and after 28 days of estrogen discontinuation (ED). Patients are divided according to hydrocortisone substituted (●, dashed line) and sufficient adrenal function (○, solid line). Level of significance is given in the figures.

of whether the patients received hydrocortisone replacement or not (ΔIGFBP-1: $-6.7\pm2.1$ nmol/l (SAF) vs $-2.7\pm0.8$ nmol/l (HC), $P=0.046$). Serum GHBP levels did not differ between the two situations (data not shown).

Estrogen discontinuation-induced changes (Δ-values) in serum E2 correlated with ΔSHBG ($r=0.56$, $P=0.007$) and ΔfT ($r=0.45$, $P=0.30$) and ΔT ($r=0.59$, $P=0.004$). ΔIGF-I correlated with ΔSHBG ($r=-0.45$, $P=0.033$) and ΔIGFBP-3 ($r=0.67$, $P=<0.001$).

In a backward regression model ΔSHBG and ΔIGFBP-3 were the main contributors to the variation in ΔIGF-I (explaining 86% of the variation). Adding ΔE2 and ΔT to the model, 93% of the variation in ΔIGF-I could be explained ($R=0.964$, $P<0.001$).

## Discussion

The aim of the present study was to examine the impact of estrogen replacement therapy on androgen status and IGF-related parameters in hypopituitary women. Previous studies on the interaction between estrogen and IGF-I have not included measurements of androgen levels, and have either comprised estrogen treatment in healthy postmenopausal women (15–19), or studies in hypopituitary patients continuing sex steroid replacement with or without concomitant GH substitution. Our study was therefore designed to evaluate the effects of estrogen replacement on androgen levels and IGF related parameters in GH-treated hypopituitary females. Our data confirmed the increased prevalence of reduced androgen levels in hypopituitary women, which was particularly pronounced in patients receiving hydrocortisone replacement. Discontinuation of estrogen replacement was associated with significant elevations in the circulating levels of free testosterone as well as IGF-I, IGF-II, and IGFBP-3. Regression analysis indicated that the increase in IGF-I was partly dependent on the changes in testosterone and SHBG.

The cross sectional data of the present study support and extend previous reports of androgen deficiency in hypopituitary women (2, 17–18). In our subgroup of patients receiving hydrocortisone substitution the androgen levels were reduced by 80–100% compared with early follicular values observed in healthy subjects. The corresponding reduction in patients not receiving hydrocortisone replacement was 30–40%. These data are in accordance with those obtained after experimental suppression of adrenal function by dexamethasone in healthy pre-menopausal women (19), and after suppression of ovarian function in endometriosis with GnRH agonist treatment (20). Estrogen discontinuation was associated with a significant increase in free testosterone together with a decline in SHBG, whereas the other androgen related parameters were unchanged. We therefore propose that the main impact of exogenous estrogen on androgen status in our patients was elevation of SHBG production (14), rather than a suppression of residual ovarian function. Our study does not confirm a stimulatory effect of estrogen on adrenal androgen synthesis, as previously reported (21). From a clinical point of view our data emphasise that pronounced androgen deficiency is predominantly recorded in female patients replaced with hydrocortisone.

The patients continued GH substitution in a daily dosage tailored to achieve serum IGF-I levels within the normal range. Nevertheless, the patients receiving concomitant hydrocortisone replacement achieved lower mean levels of IGF-I and IGFBP-3 as compared with the subgroup of patients who were considered ACTH-sufficient. The impact of glucocorticoids on serum IGF-I levels is complex, but there is no convincing evidence to suggest a suppression. We did observe that IGF-I correlated positively with fT and negatively with SHBG, both of which are in accordance with previous data in hypopituitary patients not receiving GH substitution (2). Furthermore, fT contributed as an explanatory variable in a multiple regression model to predict IGF-I.

Discontinuation of estrogen replacement was associated with significant increases in the serum concentrations of IGF-I, IGF-II, and IGFBP-3, together with a decrease in IGFBP-1. The changes in IGF-I are comparable to those reported following oral estrogen administration in healthy postmenopausal women (4, 13–16); more importantly, our data suggests that the previously reported age-dimorphism in the IGF-I response to GH to a large extent is attributable to estrogen. On the other hand, we observed a 23% increase in IGF-I following estrogen discontinuation, whereas the gender difference in the responsiveness to GH has been reported to exceed 50% (3). In contrast to our data, the studies comparing male and female patients on GH therapy used significantly higher GH dosages and achieved supra-physiological IGF-I levels (3, 22), which makes direct comparisons difficult. In a multiple regression model the changes in SHBG and testosterone were significant predictors of the estrogen-induced change in IGF-I. We therefore propose that both estrogen and testosterone are significant determinants of serum

**Table 3** Levels of circulating IGFs and IGFBPs in hypopituitary patients after estrogen treatment and discontinuation of treatment.

| | ET | ED | *P* |
|---|---|---|---|
| IGF-I (μg/l) | 167±15 | 206±14 | 0.005 |
| IGF-II (μg/l) | 1082±34 | 1145±42 | 0.049 |
| IGFBP-1 (μg/l) | 3.22 (0.50–24.46) | 1.99 (0.35–12.74) | 0.001 |
| IGFBP-3 (μg/l) | 3887±139 | 4309±138 | 0.001 |
| GHBP (nmol/l) | 2.6±0.2 | 2.5±0.2 | 0.103 |
| Molar IGF-I/IGFBP-3 | 0.042±0.003 | 0.047±0.003 | 0.022 |

$n=26$. ET, estrogen treatment; ED, estrogen discontinuation.
Values are means±S.E.M. (range).

IGF-I levels, which may explain the gender related differences in IGF-I levels revealed in hypopituitary patients both before and after GH substitution. There is experimental evidence to suggest that estrogen directly inhibits the hepatic production of IGF-I and IGFBP-3 (23–24), and hence also the corresponding circulating levels. By contrast, the mechanisms by which testosterone stimulates IGF-I are not understood. In adults with intact pituitary function the effects of sex steroids on hepatic IGF-I production are masked by a compensatory increase in GH secretion in females.

GHBP levels were higher in the patients group, which was partly explained by differences in body composition. Since treatment with oral estrogens are known to increase GHBP levels (13) this may in addition explain the higher levels of GHBP in the patient group. However, GHBP levels were found to be unaltered after discontinuation of estrogen treatment.

In summary, the present data clearly demonstrates that exogenous estrogen suppresses the circulating levels of IGF-I, IGF-II, and IGFBP-3 through GH-independent mechanisms. We also report evidence to suggest that testosterone is a significant and positive determinant of IGF-I. In combination these effects may to a large extent account for the gender differences observed in both the GH–IGF axis in healthy adults and in the responsiveness of hypopituitary patients to GH substitution.

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