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CLINICAL STUDY

Effects of growth hormone replacement therapy on levels of cortisol and cortisol-binding globulin in hypopituitary adults

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Abstract

Objective: To determine if human growth hormone (hGH) replacement therapy alters pharmacokinetics of hydrocortisone (CS) substitution in hypopituitary adults.

Design: To this aim, we analysed serum and salivary CS profiles 270 min after oral CS administration at baseline and 6 and 12 months after initiation of hGH replacement therapy.

Methods: Serum IGF-I, cortisol-binding globulin (CBG), thyroxine-binding globulin (TBG) and sex hormone-binding hormone (SHBG) were measured using commercially available radioimmunoassays. In-house immunofluorometric assays were employed for measurements of CS and hGH.

Results: hGH replacement did not change total serum CS bioavailability (area under the serum cortisol profile curve). Interference of orally administered CS with salivary measurement of free CS (fCS) caused significant bias. Therefore, fCS levels were calculated from their total CS and cortisol-binding globulin (CBG) levels. CBG decreased by approximately 30% after both 6 and 12 months of hGH replacement therapy ($n = 20$, $P < 0.01$). A significant negative correlation between ΔCBG ($CBG_{6months} - CBG_{baseline}$) and ΔIGF-I ($IGF - I_{6months} - IGF - I_{baseline}$) was observed ($P = 0.04$). The calculated values of free CS tended to increase with physiological hGH replacement, but this effect was marginal and did not reach statistical significance. In contrast to the CBG concentrations, plasma levels of sex hormone-binding globulin and thyroxine-binding globulin were essentially stable.

Conclusion: Given that no clinically relevant alterations in pharmacokinetics of CS were evoked by initiation of hGH replacement in hypopituitary adults, we conclude that CS substitution does not require dose adjustment after initiation of hGH replacement.

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Introduction

Human growth hormone (hGH) is a pleiotropic hormone, influencing numerous metabolic processes and physiological functions in humans. Replacement of hGH in hGH-deficient patients might, therefore, influence the absorption, metabolism, plasma transport and/or clearance of other substituted hormones in patients with hypopituitarism.

Previous studies addressing this subject have produced conflicting results. Weaver *et al.* (1) showed reduced bioavailability of CS between 6 and 12 months on hGH replacement therapy in 19 hypopituitary adults as measured by urinary excretion of cortisol (CS) metabolites and free CS (fCS), although this effect was not considered to be clinically relevant. No significant change in levels of cortisol-binding globulin (CBG) were found in these patients. Rodriguez-Arnao *et al.* (2) reported altered serum CS profiles in 14 patients with hypopituitarism as a result of hGH replacement. The significant reduction in serum concentrations of total CS but not fCS after orally administered CS substitution was attributed to the observed decline in serum CBG levels. In contrast, a decrease in the endogenous CS production rate was demonstrated by Vierhapper and co-workers (3) in eight healthy men using the stable isotope dilution technique and mass spectrometry. Recently, Moore *et al.* (4) showed a dose-dependent inhibition of 11β-hydroxysteroid dehydrogenase 1 (11βHSD1) activity, which in an IGF-I-dependent manner catalyses the interconversion of hormonally active cortisol and inactive cortisone in favour of cortisol. It has also been described that change in body composition has influence on the cortisol to cortisone conversion (5).

To clarify whether the CS replacement dose requires adjustment in adults with hypopituitarism started on hGH replacement therapy, we studied the effects of hGH

Table 1 Patient characteristics of 22 GH-deficient adults: sex, age, age of onset and primary pituitary pathology. Mean age, 37.5 years (range, 22–63 years).

Sex	Age	Onset	Hypopituitarism	Diagnoses
Male	52	Adult	Complete	Non-secreting adenoma
Male	58	Adult	Complete	Non-secreting adenoma
Male	40	Adult	Complete	Chondroma
Female	40	Adult	Complete	Craniopharyngeoma
Female	61	Adult	Complete	Prolactinoma
Female	38	Adult	Complete	Prolactinoma
Female	41	Adult	Complete	Craniopharyngeoma
Male	28	Child	Complete	Idiopatic
Male	26	Child	Complete	Craniopharyngeoma
Male	22	Child	Complete	Maldescensus of pituitary
Male	28	Child	Complete	Empty sella
Male	28	Child	Complete	Empty sella
Male	31	Child	Complete	Dysgerminoma
Male	33	Child	Complete	Craniopharyngeoma
Male	63	Adult	GH, LH/FSH, ACTH	Prolactinoma
Male	62	Adult	GH, LH/FSH, ACTH	Idiopathic
Male	40	Adult	GH, LH/FSH, TSH	M. Cushing
Male	22	Child	Isolated	Maldescensus of pituitary
Male	26	Child	Isolated	Trauma
Male	22	Child	Isolated	Birth trauma
Female	32	Child	Isolated	Silver–Russel syndrome
Female	32	Child	Isolated	Silver–Russel syndrome

replacement therapy on profiles of total and free serum CS as well as on levels of the major serum hormone-transport proteins.

Patients and methods

Patients

Twenty-two adult patients with hypopituitarism were enrolled in the study, six patients with isolated GH deficiency and 16 subjects with multiple pituitary hormone deficiencies including the adrenal axis. GH deficiency was proven by insulin tolerance testing (ITT) and arginine stimulation test after at least 6 months of stable substitution with CS, gonadal steroids, thyroxine and/or vasopressin, when subjects were deficient. Studies were performed both before and after 6 and 12 months of hGH replacement therapy. Isolated GH deficiency was proven by a normal adrenocorticotrophin hormone (ACTH) and CS reserve and reduced GH peak (<3.0 ng/ml) in insulin tolerance testing (ITT, 0.1 U/kg body weight) and a normal increase of thyroid-stimulating hormone (TSH) and luteinising hormone/follicle-stimulating hormone (LH/FSH) in thyrotropin-releasing hormone (TRH) and gonadotropin-releasing hormone (GnRH) testing. Patient characteristics are listed in Table 1. Dosage of glucocorticoid replacement therapy was 25 mg hydrocortisone p.o., distributed as 10, 5, 5 and 5 mg over the course of day. rhGH replacement was adjusted to individual IGF-I values with the objective of attaining the 50th percentile of the age-adjusted normal range (0.003 to 0.011 mg/kg/day). CBG, TBG and SHBG could be analysed only in 20 patients (5/15) due to insufficient aliquots of serum samples. Patients with abnormal liver or renal function tests were excluded. Written informed consent was obtained from all patients. The study was approved by the ethical committee of the Faculty of Medicine at the Ludwig-Maximilians University, Munich.

Methods

Serum IGF-I, CBG, TBG and SHBG were measured using commercially available radioimmunoassays. (IGF-I: Mediagnost, Tuebingen, Germany; CBG: Medgenix Diagnostics, Fleurus, Belgium; TBG: Cis Bio International, Gif-Sur-Yvette, France; SHBG: DELFIA, Wallac Oy, Turku, Finland). In-house time-resolved fluorescence immunoassays were employed for the measurement of serum and salivary CS (intra- and interassay co-variances were 5.3 and 7.2% respectively, for serum, 5.1 and 8.2% respectively, for saliva) (6).

fCS was calculated from the measured total CS and CBG concentrations using the following standard equation (7): $U^2K(1+N)+U(1+N+K+(T-C))-C=0$. In this equation U represents the molar concentration of free cortisol, C the molar concentration of total cortisol, and T the concentration of CBG. K corresponds to the affinity of CBG for cortisol at 37 °C ($3\times10^{-7}\,M^{-1}$) and N to the proportion of albumin-bound to unbound cortisol (1.74). The equation can be solved for U in the following way:

$$U=\sqrt{Z^2+\frac{C}{(1+N)K}}-Z,$$

where

$$Z=\frac{1}{2K}+\frac{T-C}{2(1+N)},$$

or quantitatively

$$U=\sqrt{Z^2+0.0122C}-Z,$$

where

$$Z=0.0167+0.182(T-C).$$

Therefore

$$U=\sqrt{(0.0167+0.182(T-C))^2+0.0122C}-(0.0167+0.182(T-C)).$$

Serum profiles were collected after a fasting and drug restriction period of 12 h (except desmopressine) between 0800 h and 1230 h. After intravenous catheterisation of an antecubital vein followed by a recovery period of 20 min to avoid stress-induced bias, baseline serum and plasma samples were collected. Immediately thereafter patients on CS substitution

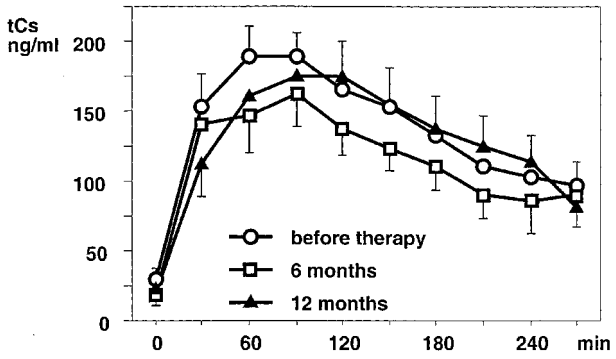

Figure 1 Levels of total serum cortisol (tCs) after oral intake of 10 mg hydrocortisone, measured at 30 min intervals for a total of 270 min in 16 GH-deficient adults with secondary adrenal insufficiency prior to hGH replacement therapy. The three curves represent the values before hGH replacement therapy and after 6 and 12 months hGH.

therapy received a 10 mg tablet of CS and extensively rinsed their mouth with water for at least 5 min. CBG concentrations in serum were measured at the beginning of each collecting period.

Patients were seated in a comfortable, reclined position and samples were collected in 30 min intervals over 270 min. Saliva samples for fCS measurements were collected using saliva sampling devices consisting of a cotton swab and a polystyrene tube (Sarstedt, Nuembrecht, Germany) as previously described (8).

Statistics

All measurements are given as means ± S.E.M. Statistical tests were one-way ANOVA, Wilcoxon, Spearman rank and Kruskal–Wallis test as indicated (StatView, SAS Institute Inc., Version 5.0).

Results

In patients with secondary adrenal insufficiency prior to hGH replacement therapy ($n = 16$), total serum CS (tCS) levels after oral intake of 10 mg CS consistently peaked after 90 min (8 am basal values of 29.50 ± 7.56 ng/ml, 9.30 am values 189.69 ± 17.34 ng/ml). Six and 12 months after initiation of hGH replacement therapy, no significant differences in the kinetics of increase or in the maximum peak values of the tCS serum profiles after oral CS substitution were found (Spearman rank test, $P = 0.73$) (Fig. 1). This suggests that hGH replacement does not exert a negative influence on enteral resorption or metabolism of CS.

This was also true when the total amounts of CS reaching the systemic circulation were estimated by calculation of mean areas under the serum cortisol profile curves (Spearman rank test, $P = 0.32$). Patients with endogenous CS production ($n = 6$) also showed no differences in their tCS profiles during hGH substitution (data not shown).

To test for alterations of CS bioavailability after hGH replacement, salivary levels of CS, reflecting the bioactive fraction of the circulating hormone after free diffusion through epithelial cells of the salivary gland (9), were analysed in parallel. Preliminary studies revealed that salivary CS measurements are prone to significant bias by contamination with traces of the CS tablets depending on how long and intensively the patients rinsed their mouth after oral CS administration (data not shown). Thus, salivary fCS levels were evaluated in only five patients who did not require cortisol replacement (Fig. 2). Similar patterns of calculated free serum cortisol and cortisol in saliva were observed. The elevated levels at study onset are in keeping with the stress response to blood sampling. The delay in the CS peak in saliva to 30 min reflects the delay in transport of CS from serum to saliva.

The bioactive fraction of serum CS (fCS) was assessed by measuring the concentration of CBG, the major plasma CS-binding protein, and calculating the unbound CS concentration (10). Compared with baseline levels, the CBG levels after both 6 and 12 months of hGH therapy in ACTH-deficient as well as in ACTH-sufficient patients decreased by approximately 30% (Kruskal–Wallis test, $P < 0.01$) (Fig. 3). This effect was mediated by hGH replacement, as a significant negative

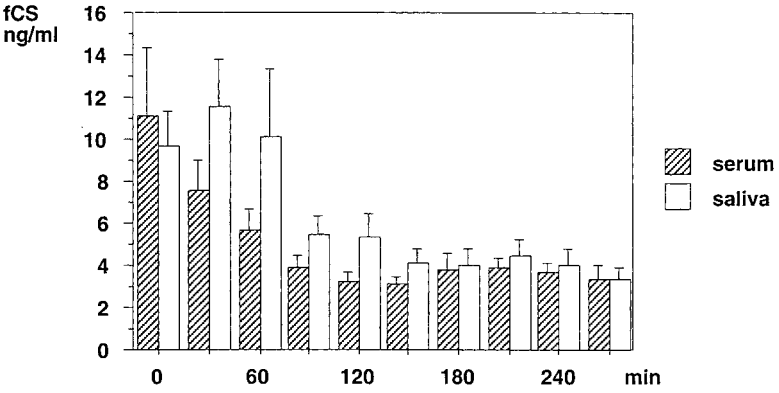

Figure 2 fCS levels, measured in saliva and calculated from serum, in five adults with isolated GH deficiency. The same pattern of free serum CS and CS in saliva can be seen. The delay in the CS peak in saliva to 30 min reflects the delay in transport of CS from serum to saliva at study onset due to first blood sampling.

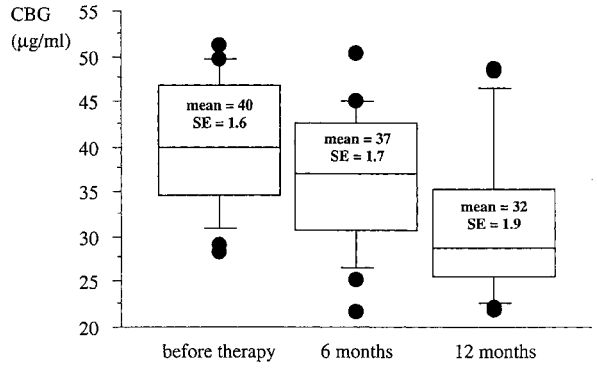

Figure 3 Box plot (5th, 25th, 50th, 75th and 95th percentile with outliers) of CBG levels in 20 GH-deficient adults (15 patients with multiple pituitary hormone deficiency, five subjects with isolated GH deficiency) before hGH replacement therapy and after 6 and 12 months hGH. Kruskal–Wallis test indicates statistical significance of decrease ($P < 0.01$).

correlation between ΔCBG ($CBG_{6months} - CBG_{baseline}$) and individual ΔIGF-I ($IGF - I_{6months} - IGF - I_{baseline}$) was found ($n = 20$, $P = 0.04$, Spearman rank correlation coefficient) (Fig. 4). Bias to CBG concentrations due to metabolic factors was minimised by the 12 h fasting and drug restriction period of the euthyroid patients. The calculated fCS levels on hGH therapy in both patient groups tended to be slightly higher than the corresponding pre-therapy values, however, these differences did not reach statistical significance (Wilcoxon test, $P = 0.09$) (Fig. 5). This was also true when the bioactive cortisol in saliva was determined (Fig. 6).

To determine if hGH replacement affects the plasma levels of other specific hormone binding proteins, the serum concentrations of thyroxine-binding globulin and sex-hormone binding globulin were determined.

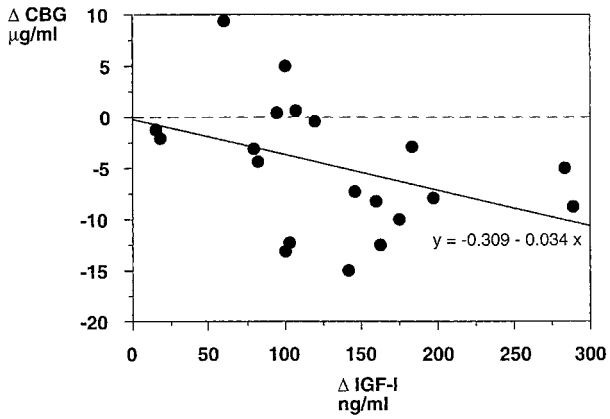

Figure 4 Individual increase of IGF-I compared with individual changes of CBG levels in 20 GH deficient patients during hGH replacement therapy. A significant negative correlation between ΔCBG ($CBG_{6months} - CBG_{baseline}$) and ΔIGF-I ($IGF - I_{6months} - IGF - I_{baseline}$) was found ($n = 20$, $P = 0.04$, $\rho = -0.48$, $y = -0.309 - 0.034x$, Spearman rank correlation coefficient).

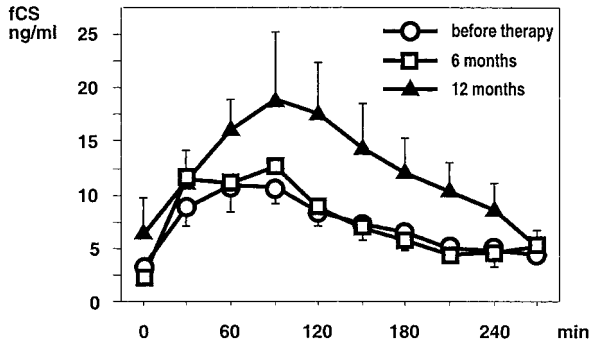

Figure 5 Calculated levels of unbound serum cortisol after oral intake of 10 mg hydrocortisone in 15 GH-deficient adults with secondary adrenal insufficiency, measured at 30 min intervals for a total of 270 min. The three curves represent the values before hGH replacement therapy, after 6 months and after 12 months. The area under the curve (AUC) after 12 months GH replacement tends to be slightly higher than the corresponding pre-therapy AUC, however this difference does not reach statistical significance (Wilcoxon test, $P = 0.09$).

However, hGH replacement did not alter the binding capacity of plasma for thyroxine or testosterone.

Discussion

During the last decade, clinical studies on hGH replacement therapy have revealed an immense variety of growth hormone-dependent physiological mechanisms (11). However, few studies have examined the possible interference of growth hormone replacement therapy with the pharmacodynamics and -kinetics of other hormones (CS, thyroxine and sex hormones) (12–14).

We aimed to investigate the effects of hGH replacement on pharmacokinetics and need for adjustment of CS substitution in hypopituitary adults. We found that growth hormone substitution does not significantly

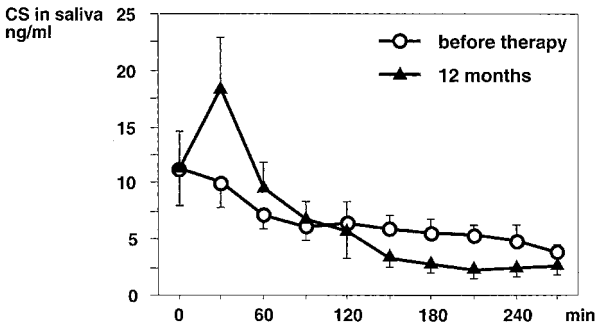

Figure 6 fCS levels in saliva in five adults with isolated GH deficiency before hGH replacement therapy and after 12 months hGH. The AUC after 12 months GH replacement tends to be slightly higher than the corresponding pre-therapy AUC, however, this difference does not reach statistical significance.

alter the pharmacokinetics of orally administered CS. Whereas the total serum CS levels remained essentially stable, the serum levels of the bioactive fCS tended to be slightly higher on hGH replacement compared with pre-therapy concentrations. Although not statistically significant, these findings are consistent with the observed approximately one-third decline in CBG levels, confirming previous results (1). This is not due to influencing metabolic factors, since levels of insulin, thyroid hormones and free fatty acids are considered to be intra-individually essentially stable after overnight fasting and drug restriction. The fCS levels on hGH therapy remained within the normal range for all patients, indicating that CS dose adjustment may not be required upon initiation of hGH replacement therapy.

Contrary to the CBG levels, the plasma concentrations of thyroxine-binding globulin and sex-hormone binding globulin were not affected by hGH replacement. Thus, hGH replacement should not alter the transport of these hormones. The bioavailability, however, can be changed, for example by increased fT4 to fT3 conversion.

hGH replacement therapy in adults has been associated with sodium and water retention (15, 16) which has been attributed, at least in part, to activation of the renin-angiotensin system (17, 18). It has also been speculated that increased CS bioavailability might contribute to sodium retention (19). This effect is not due to an enhanced renal conversion of hormonally inactive cortisone to active cortisol (4). Rather, inhibition of 11βHSD1 and a consecutive increase in the clearance of cortisol was speculated to precipitate secondary adrenal failure after commencement of hGH replacement therapy (4). Hypothetical local tissue-specific alterations in cortisol metabolism not accompanied by a change in circulating cortisol concentration cannot be ruled out. Given that hGH replacement had only a marginal effect on fCS levels in our patients, we conclude that the latter mechanism may not be of clinical relevance in the setting of physiological hGH substitution therapy. However, in rare instances initiation of hGH replacement therapy may unmask a reduced ACTH reserve due to an inhibition of 11βHSD1 in patients without glucocorticoid replacement.

Acknowledgements

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