CLINICAL STUDY

GH effect on enzyme activity of 11 β HSD in abdominal obesity is dependent on treatment duration

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Abstract

Objective: In the past years the interaction of GH and 11 β hydroxysteroid dehydrogenase (11 β HSD) in the pathogenesis of central obesity has been suggested.

Design: We studied the effects of 9 months of GH treatment on 11 β HSD activity and its relationship with body composition and insulin sensitivity in 30 men with abdominal obesity, aged 48–66 years, in a randomised, double-blind, placebo-controlled trial.

Methods: Urinary steroid profile was used to estimate 11 β HSD type 1 and 2 (11 β HSD1 and 11 β HSD2) activities. Abdominal s.c. and visceral adipose tissues were measured using computed tomography. Glucose disposal rate (GDR) obtained during a euglycaemic–hyperinsulinaemic glucose clamp was used to assess insulin sensitivity.

Results: In the GH-treated group the 11 β HSD1 activity decreased transiently after 6 weeks ($P < 0.01$) whereas 11 β HSD2 increased after 9 months of treatment ($P < 0.05$). Between 6 weeks and 9 months, GDR increased and visceral fat mass decreased. Changes in 11 β HSD1 correlated with changes in visceral fat mass between baseline and 6 weeks. There were no significant correlations between 11 β HSD1 and 11 β HSD 2 and changes in GDR.

Discussion: The study demonstrates that short- and long-term GH treatment has different effects on 11 β HSD1 and 11 β HSD2 activity. Moreover, the data do not support that long-term metabolic effects of GH are mediated through its action on 11 β HSD.

European Journal of Endocrinology 154 69–74

Introduction

11 β -Hydroxysteroid dehydrogenase (11 β HSD) is a fundamental regulator of glucocorticoid action. 11 β HSD type 1 (11 β HSD1) acts predominantly by generating cortisol from cortisone. This enzyme is primarily found in the liver but has also been found in adipose tissue (1–4). 11 β HSD type 2 (11 β HSD2) has the reverse action and is primarily found in the kidney (5). In past years 11 β HSD has been suggested to be a possible link in the pathogenesis of essential hypertension, obesity, glucose intolerance and the metabolic syndrome (3, 4, 6–10).

Using urinary steroid profiles the conversion of cortisone to cortisol has been found to be reduced in adults with abdominal adiposity, indicating reduced activity of 11 β HSD1 in central adiposity (9, 11). This is, however, not a consistent finding in all studies (12). Studies on hypopituitary patients receiving oral glucocorticoid replacement, acromegalic patients withdrawing from octreotide treatment and acromegalic patients treated surgically, indicate that growth hormone (GH) inhibits

11 β HSD1 and thereby decreases the conversion of cortisone to cortisol (increased urinary cortisol/cortisone ratio) (13–15). This is also supported by studies on rats, where GH and insulin have been found to suppress the conversion of cortisone to cortisol (15, 16). On the other hand, *in vitro* studies of human omental adipose cells demonstrate a dose-dependent inhibition of 11 β HSD1 by insulin-like growth factor-I (IGF-I) but not GH (15, 16). In a small study of hypopituitary patients this was explained as being an inhibitory effect of GH on 11 β HSD1 that was maximal at very low doses and not mediated indirectly by change in circulating insulin (17). In contrast to previous studies, this study found no correlation between the cortisol/cortisone ratio and serum IGF-I concentration. One study demonstrated paradoxically an increase in cortisol/cortisone ratio in adrenocorticotrophin (ACTH)-deficient hypopituitary patients treated with GH and hydrocortisone replacement therapy but not in hypopituitary patients with sufficient ACTH production (18). The study has been questioned as it depended on overnight urine samples in hypoadrenal

patients on conventional hydrocortisone replacement therapy, but it implies that the inhibition of 11β HSD1 by GH, with a reduced conversion of cortisone to cortisol, is compensated by a simultaneous inhibition of 11β HSD2, with a reduced conversion of cortisol to cortisone (18). If GH reduces the conversion of cortisol to cortisone, GH deficiency can add to the central adiposity seen in adult GH deficiency and additionally explain some of the lipolytic action of GH seen *in vivo*. In insulin-dependent diabetes mellitus, cortisol/cortisone ratio has been found to be reduced, supporting that glucose metabolism influences the activity of 11β HSD; but not all studies agree with this (19, 20).

As central adiposity often is coincidental with insulin resistance and there is a strong inverse relationship between visceral fat mass and endogenous GH secretion, we found it requisite to study the cortisol metabolism in a population with central adiposity and insulin resistance. We have earlier shown that GH decreases visceral fat and has a biphasic effect on insulin resistance in such a group (21). By analysing 11β HSD activity under these circumstances we can study the relationship with central obesity, insulin/glucose metabolism and GH/IGF-I.

In the present study we define R_{new} a more precise ratio of cortisol/cortisone as a measure of 11β HSD1 activity, and Q , the quotient for urinary free cortisol and cortisone, as a measure of 11β HSD2 activity.

Patients and methods

Patients

Thirty men, aged 58.13 ± 0.86 (S.E.M.), with abdominal obesity, were recruited by advertisements in a local newspaper. The inclusion criteria for the study were, 50–65 years of age, body mass index (BMI) of 25–35 kg/m², low to normal serum IGF-I concentration, and a waist-to-hip ratio of more than 0.95. The exclusion criteria were overt diabetes mellitus, a previous cardiovascular event or heart disease.

Study design

The study was a 9-month, randomised, double-blind, placebo-controlled trial of the administration of recombinant human GH (Genotropin; Pharmacia, Sweden) (21).

The patients were studied as outpatients before, after 6 weeks, and after 9 months of treatment. Physical and laboratory examinations were performed on all visits. Body weight was measured in the morning to the nearest 0.1 kg wearing indoor clothing. Waist circumference was measured in the standing position with a flexible plastic tape midway between the lower rib margin and the iliac crest, and the hip girth was measured at the widest part of the hip. Blood tests and 24 h urine collection were obtained for cortisol

and cortisone metabolites in all participants at baseline, after 6 weeks and after 9 months.

The daily dose of GH was 9.5 µg/kg administered s.c. before bedtime. The dose was reduced by half in the event of side-effects. The average dose reduction during the 9 month study was 0.17 mg per day (range –1.7 to 0).

Ethics

Each participant received oral and written information of the study and signed an informed consent before being included into the study. The study was approved by the Ethics Committee at the University of Gothenburg and by the Swedish Medical Products Agency, Uppsala, Sweden.

Body composition

A five-scan computed tomography technique was used (Philips Tomoscan 350; Mahway, NJ, USA) to measure abdominal adipose tissue. Abdominal s.c. and visceral adipose tissue areas were determined at the level of L4–L5. The tissue areas and anatomical boundaries were determined as described previously (Cowdhury 1994 (22)).

Analytic methods

A euglycaemic–hyperinsulinaemic glucose clamp was performed after an overnight fast, as previously described (23). The glucose disposal rate (GDR) was measured for 20 min in steady-state conditions, which were reached after 100 min.

Blood samples were drawn in the morning after an overnight fast. The serum concentration of IGF-I was determined by a hydrochloric acid ethanol extraction RIA using authentic IGF-I for labelling (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA).

Serum insulin was determined by RIA (Phadebas, Pharmacia, Uppsala, Sweden) and blood glucose was measured by the glucose-6-phosphate dehydrogenase method (Kebo Lab, Stockholm, Sweden). Serum haemoglobin A1c (HbA1c) was determined by HPLC (Waters, Millipore AB, Sweden). Serum cortisol was measured by an IRMA (Orion Diagnostica, Orion Corporation Orion Diagnostica, Espoo, Finland).

Steroids in 24 h urine collections were analysed by using a simplified version of the method previously described (24). Briefly, steroids were extracted from 2.5 ml urine on a Sep-Pak C18 cartridge. Steroid glucuronides were then enzymatically hydrolysed, and following re-extraction on a Sep-Pak C18 cartridge, steroid sulphates were cleaved by solvolysis. Deconjugated steroids were purified on a column of the lipophilic anion exchanger, TEAP-LH-20, in bicarbonate form. The steroids were converted to *O*-methyloxime-trimethylsilyl ether derivatives and after removal of reagents on a

Lipidex 5000 column, they were analysed by gas chromatography on a Hewlett-Packard 5890 instrument equipped with a fused silica column. Stigmasterol added after hydrolysis was used as internal standard. Using this method essentially all urinary cortisol and cortisone metabolites could be quantified. Therefore, the ratio between the cortisol metabolites tetrahydrocortisol (THF), allotetrahydrocortisol (a-THF) and α -cortol and the cortisone metabolites tetrahydrocortisone (THE) and α -cortolone, R_{new} , was calculated as follows: $(\text{THF} + \text{a-THF} + \alpha\text{-cortol})/(\text{THE} + \alpha\text{-cortolone})$. The minor metabolites β -cortol and β -cortolone were not included, since they could not be quantified separately due to similar retention times on the GLC-column. Levels of urinary free cortisol and cortisone were determined by HPLC with UV detection (254 nm) following extraction and purification on a small octylsilane-bonded silica column. The quotient Q was calculated for urinary free cortisol/free cortisone.

Statistical methods

All the descriptive statistical results are presented as means $\pm$ S.E.M. Within- and between-group comparisons were performed using paired and unpaired Student's *t*-test. Correlations were sought by calculating Fisher's *r*. Before statistical analysis, logarithmic transformation of data with skewed distribution was performed. A two-tailed probability value less than 0.05 was considered significant.

Results

The GH and placebo groups were matched in terms of age, BMI (31.4 ± 0.7 vs 30.5 ± 0.8), and waist-to-hip ratio (1.01 ± 0.01 vs 1.03 ± 0.02) and did not differ significantly in terms of body composition and glucose metabolism at baseline.

Data on body composition and glucose metabolism have been reported (21). In summary, GH treatment reduced abdominal visceral and s.c. fat mass as

compared with placebo treatment without any change in body weight. Blood glucose and plasma insulin levels increased after 6 weeks and returned thereafter to baseline levels, whereas insulin sensitivity decreased after 6 weeks and was improved after 9 months in the GH-treated as compared with the placebo-treated group. The IGF-I levels did not change in the placebo group and increased to an average of 3.30 ± 0.35 S.D. above the predicted mean in the GH-treated group after 6 weeks and 1.89 ± 0.48 S.D. after 9 months.

Cortisol metabolism

Serum cortisol concentration decreased after 6 weeks of treatment followed by an increase after 9 months of treatment (Table 1). The enzyme activity of 11 β HSD1, expressed as R_{new} , followed the same pattern, with a significant decrease after 6 weeks of GH treatment as compared with placebo (Fig. 1). 11 β HSD2 activity, as quotient Q, was unchanged at 6 weeks, but decreased thereafter in the placebo group and increased in the GH-treated group, resulting in a significant between-group effect ($P = 0.0001$) after 9 months.

After 6 weeks of GH treatment, correlations were found between the change in 11 β HSD1 activity, expressed as R_{new} , and the change in visceral and s.c. abdominal fat (Table 2). After 9 months, the change in R_{new} correlated with the change in blood glucose, HbA1c levels and serum triglycerides (TGs). Correlations for R_{new} and major endpoints of the study are shown in Table 2.

After 6 weeks, the change in 11 β HSD2 activity, expressed as Q, correlated inversely with the change in blood glucose, HbA1c and serum TG levels. After 9 months, the change in Q showed a trend to correlate with the change in blood glucose, HbA1c and visceral and s.c. adipose fat mass. Between 6 weeks and 9 months, changes in Q correlated with changes in serum TG. Correlation calculations for Q are given in Table 3.

Table 1 Values for serum cortisol concentration, the calculated ratio of urinary cortisol/cortisone metabolites ($R_{\text{new}} = (\text{a-THF} + \text{THF} + \alpha\text{-cortol})/(\text{THE} + \alpha\text{-cortolone})$), the 11 β HSD1 activity) and the calculated quotient for urinary free cortisol/free cortisone (Q, the 11 β HSD2 activity) after GH treatment or placebo. All values are means $\pm$ S.E.M.

Variable	Baseline	6 weeks	P-value†	9 months	P-value‡#
Cortisol (nmol/l)					
GH	325.6 $\pm$ 19.8	257.8 $\pm$ 19.8	< 0.005	318.0 $\pm$ 40.3	—
Placebo	350.4 $\pm$ 29.0	306.3 $\pm$ 20.6	—	323.2 $\pm$ 29.5	—
R_{new}					
GH	0.74 $\pm$ 0.02	0.66 $\pm$ 0.02	< 0.01	0.71 $\pm$ 0.04	—
Placebo	0.87 $\pm$ 0.05	0.88 $\pm$ 0.05	—	0.87 $\pm$ 0.05	—
Q					
GH	0.22 $\pm$ 0.02	0.22 $\pm$ 0.01	—	0.25 $\pm$ 0.02	< 0.05
Placebo	0.38 $\pm$ 0.03	0.35 $\pm$ 0.04	—	0.32 $\pm$ 0.03	< 0.01

† Between baseline value and 6 weeks.

‡ Between baseline and 9 months.

Difference between groups with $P < 0.05$.

Figure 1 R_{new} is the ratio of urinary cortisol and cortisone metabolites (11 β HSD1 activity). Q is the quotient of urinary free cortisol/free cortisone (11 β HSD2 activity). The figure shows the change in R_{new} and Q (with S.E.M.) after 6 weeks and 9 months of GH or placebo treatment.

No correlation was found between IGF-I or GDR and measures of 11 β -HSD activity.

Discussion

In summary, GH treatment of men with abdominal obesity and insulin resistance reduced 11 β HSD1 activity significantly only after 6 weeks and increased 11 β HSD2 activity significantly only after 9 months. The changes observed in 11 β HSD correlated with changes in visceral fat mass, blood glucose and HbA1c, but not with measures of insulin sensitivity. These correlations were dependent on duration of GH treatment.

It has been suggested that Q better reflects 11 β HSD2 activity than R_{new} , which is used as an indicator of 11 β HSD1 activity (25, 26). Our data show that R_{new} and Q are affected differently, suggesting that both types of 11 β HSD respond to GH treatment. As R_{new} is a composite of activity of 11 β HSD1 and 11 β HSD2 it can only be considered to indicate specific changes in the former if the latter is unchanged. As changes in 11 β HSD2 were observed this might be a confounding factor. 11 β HSD1 activity was reduced after short-term treatment, leading to decreased conversion of cortisone to cortisol, and the increase in 11 β HSD2 activity became apparent only after more prolonged treatment, causing decreased conversion of cortisol to cortisone.

A previous study supports our finding of GH treatment influencing both isoenzymes of 11 β HSD (18), but this difference in timing of the effect on the different isoenzymes has not been observed before.

A previous study indicates that 11 β HSD1 may have a role in the pathogenesis of central obesity and another study found alterations in 11 β HSD1 to be associated with general obesity (26, 27). We found that abdominal fat mass and visceral fat mass continued to reduce with more prolonged GH treatment that was not accompanied with similar changes in 11 β HSD1 activity. The change in Q, however, showed correlations with the long-term change in central fat mass, suggesting that the change in adipose tissue is related to the change in the activity of 11 β HSD2. Although the overall regression analysis demonstrated varying correlations between 11 β HSD activity and central adiposity, our results imply that a stronger relationship exists between the change in 11 β HSD activity and central adiposity than between the change in 11 β HSD activity and insulin sensitivity (28). This is in line with a previous study that found adiposity to be correlated with 11 β HSD1 activity but not with insulin sensitivity (29). The associated change in Q and fat mass does not have to have a mechanistic link; it can simply be a parallel phenomenon.

GH/IGF-I-mediated inhibition of 11 β HSD1 provides a useful means of examining the hypothetical impact of

Table 2 Correlations with changes in 11 β HSD1 activity (R_{new}), in the GH-treated group. Correlations accord to the changes in respective parameter during the same period as R_{new} . Blood glucose = B-glucose. Visceral adipose tissue (ViAT45%) and s.c. adipose tissue (Sc45%) are measured as per cent of total adipose tissue at the level of L4–L5, indicating central visceral and s.c. fat mass.

Variable	Correlation with	$\Delta B-6$ weeks		$\Delta B-9$ months		$\Delta 6$ weeks–9 months	
		Fisher's <i>r</i>	<i>P</i> -value	Fisher's <i>r</i>	<i>P</i> -value	Fisher's <i>r</i>	<i>P</i> -value
R_{new}	B-glucose	0.36	0.2	0.62	0.009	0.21	0.5
	HbA1c	0.12	0.7	0.58	0.02	–0.07	0.8
	Triglycerides	0.28	0.3	0.63	0.007	0.21	0.4
	ViAT45%	0.55	0.03	–0.08	0.8	0.37	0.2
	Sc45%	–0.55	0.03	0.08	0.8	–0.37	0.2

The correlation are with changes in R_{new} after GH treatment during the period between: baseline and 6 weeks = $\Delta B-6$ weeks; baseline and 9 months = $\Delta B-9$ months; 6 weeks and 9 months = $\Delta 6$ weeks–9 months.

Table 3 Correlations with changes in 11 β HSD2 activity (Q) in the GH-treated group. Correlations accord to the changes in respective parameter during the same period as Q. Blood glucose = B-glucose.

Variable	Correlation with	Δ B–6 weeks		Δ B–9 months		Δ 6 weeks–9 months	
		Fisher's <i>r</i>	<i>P</i> -value	Fisher's <i>r</i>	<i>P</i> -value	Fisher's <i>r</i>	<i>P</i> -value
Q	B-glucose	–0.53	0.04	–0.48	0.06	0.29	0.3
	HbA1c	–0.60	0.01	–0.44	0.09	0.02	1.0
	Triglycerides	0.57	0.02	–0.09	0.8	0.57	0.02

The correlations are with changes in Q after GH treatment during the period between: baseline and 6 weeks = Δ B–6 weeks; baseline and 9 months = Δ B–9 months; 6 weeks and 9 months = Δ 6 weeks–9 months.

abnormal tissue-specific glucocorticoid metabolism in determining visceral adiposity. One of the major findings of the present study may be that such a mechanism is quantitatively minor in viscerally adipose but otherwise normal subjects. The change in 11 β HSD1 activity, expressed as R_{new} , correlated with visceral fat mass after 6 weeks of treatment. The decrease in serum cortisol and R_{new} after 6 weeks was only transient, while visceral fat mass was found to decrease continuously from the beginning of GH treatment. This suggests that the long-term effect of GH on visceral fat mass is not mediated through its action on glucocorticoid metabolism. The possibility remains, however, that the reduction in R_{new} in the initial phase of treatment augments the lipolytic action of GH and counteracts the diabetogenic action of GH during more prolonged treatment, as indicated in the regression analysis. The transient change in R_{new} and sustained change in visceral fat mass in our study is, however, contradictory to the findings in acromegaly, where a major and sustained reduction in 11 β HSD1 activity has been observed along with sustained reduction in visceral fat and no change in 11 β HSD2 activity (Q) (14, 25, 30). Our results may be explained by the duration of treatment that is much longer than the 6–8 week treatment previously reported (14, 30).

It has been argued that changes in cortisol-binding globulin might explain changes in circulating cortisol levels after GH replacement therapy, but results have been inconsistent (31, 32).

Tomlinson *et al.* showed that GH in a dose of 0.4 mg daily, inhibited 11 β HSD1 without affecting fat mass in men and women with simple obesity (33). Our dose was higher and the metabolic effects of treatment more marked, in a study containing only men with abdominal obesity. The difference in effect can thus be dose dependent as well as gender dependent, as we both find the type 1 and 2 of the 11 β HSD to be affected by GH treatment.

We conclude that the effect of GH on 11 β HSD is different after short-term and long-term treatment. This may help to explain some paradoxical results from previous studies on GH. We could also demonstrate that GH has an effect on both 11 β HSD1 and 11 β HSD2. We were not able to show parallel statistically significant changes between 11 β HSD activity,

insulin sensitivity and visceral fat mass during long-term GH treatment. We therefore find it unlikely that the key metabolic changes of GH are mediated through changes in glucocorticoid metabolism. It is still tempting to speculate that the short-term changes seen in 11 β HSD1 may help to mediate some of the beneficial effects of GH in fat mass and fat distribution.

Acknowledgements

The personnel at the Medical Research Centre, the Clinical Metabolic Laboratory and the Research Centre for Endocrinology and Metabolism are gratefully acknowledged for their skilful technical support. The placebo/rhGH preparations were kindly provided by Pharmacia & Upjohn, Stockholm, Sweden.

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Received 12 August 2005

Accepted 26 September 2005