Long-term effects of growth hormone (GH) on body fluid distribution in GH deficient adults: a four months double blind placebo controlled trial

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Abstract

Objective: Short-term growth hormone (GH) treatment normalises body fluid distribution in adult GH deficient patients, but the impact of long-term treatment on body fluid homeostasis has hitherto not been thoroughly examined in placebo controlled trials. To investigate if the water retaining effect of GH persists for a longer time we examined the impact of 4 months GH treatment on extracellular volume (ECV) and plasma volume (PV) in GH deficient adults.

Design: Twenty-four (18 male, 6 female) adult GH deficient patients aged 25–64 years were included and received either GH ($n=11$) or placebo ($n=13$) in a double blind parallel design.

Methods: Before and at the end of each 4 month period ECV and PV were assessed directly using ⁸²Br- and ¹²⁵I-albumin respectively, and blood samples were obtained.

Results: During GH treatment ECV increased significantly (before: 20.48 ± 0.99 l, 4 months: 23.77 ± 1.38 l ($P<0.01$)), but remained unchanged during placebo administration (before: 16.92 ± 1.01 l, 4 months: 17.60 ± 1.24 l ($P=0.37$)). The difference between the groups was significant ($P<0.05$). GH treatment also increased PV (before: 3.39 ± 0.27 l, 4 months: 3.71 ± 0.26 l ($P=0.01$)), although an insignificant increase in the placebo treated patients (before: 2.81 ± 0.18 l, 4 months: 2.89 ± 0.20 l ($P=0.37$)) resulted in an insignificant treatment effect ($P=0.07$). Serum insulin-like growth factor-I increased significantly during GH treatment and was not affected by placebo treatment. Plasma renin (mIU/l) increased during GH administration (before: 14.73 ± 2.16 , 4 months: 26.00 ± 6.22 ($P=0.03$)) and remained unchanged following placebo (before: 20.77 ± 5.13 , 4 months: 20.69 ± 6.67 ($P=0.99$)) leaving no significant treatment effect ($P=0.08$).

Conclusion: The long-term impact of GH treatment on body fluid distribution in adult GH deficient patients involves expansion of ECV and probably also PV. These data substantiate the role of GH as a regulator of fluid homeostasis in adult GH deficiency.

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Introduction

Growth hormone (GH) replacement therapy in adult GH deficient patients is associated with profound changes in body composition (1–3). These changes are characterised by an increase in lean body mass and a decrease in fat mass (4, 5). The contribution of sodium and water retention to the increase in lean body mass is still a matter of debate. In the first studies of GH substitution to adult GH deficient patients side effects ascribed to fluid retention were frequently reported and they were subsequently shown to be dose dependent (6, 7). Moreover, the volume expansion during GH administration is not transient in these patients, but remains expanded for weeks (8). Studies of the more long-term impact of GH on fluid distribution in adult GH deficient

patients have been scarce (3, 5, 9, 10) and uncontrolled (5, 9, 10). The methodologies employed can be criticised because the various fluid compartments have been indirectly assessed either with total body potassium (TBK) (3) or bioelectrical impedance (BIA) (5, 9). When using TBK to calculate lean body mass, the assumption that intracellular concentration of potassium and protein remain constant during GH treatment has to be fulfilled. This is not so during GH administration, since GH has protein sparing as well as potassium retaining effects (11). In line with this a recent report demonstrated TBK to be a poor predictor of fat mass and fat free mass using dual energy x-ray absorptiometry as reference technique (12). The bioelectrical estimates of body composition have been shown not to correlate well with dilutional methods in adult GH deficient patients

(10) and in normal subjects (13). Therefore, in a double blind placebo controlled study we have measured extracellular volume and plasma volume directly with ^{82}Br - and ^{125}I -albumin respectively in GH deficient patients before and after four months of treatment.

Materials and methods

Subjects

Twenty-four GH deficient adult patients, aged 25–64 (median 40) years, entered the study after informed consent and approval from the local ethical committee and the Danish National Board of Health. Further clinical characteristics of the patients are given in Table 1. None of the patients had received GH during adulthood. GH deficiency was defined as peak serum GH response less than $5\text{ }\mu\text{g/l}$ to two provocative tests.

Design

The patients were randomised to receive either GH ($n=11$) 2 IU/m^2 daily (Norditropin, Novo Nordisk, Gentofte, Denmark) or placebo ($n=13$) for four months. Before and after 4 months treatment, extracellular volume (ECV) and plasma volume (PV) were determined as follows. The patients arrived at the clinical research unit at 1900 h. The next morning at 0800 h a cannula was inserted into a cubital vein for blood sampling and another cannula was inserted into an antebrachial vein on the contralateral arm for

isotope infusion. Extracellular volume and plasma volume were determined using Br^{82} and I^{125} -albumin respectively (14, 15). Br^{82} was injected at 0900 h and blood samples were obtained 4 h later. Plasma volume was determined between 1300 and 1400. Blood samples were drawn after 30 min rest in the supine position. The subjects were instructed not to change dietary habits and fluid intake during the study period. Twenty-four-hour ambulatory blood pressure was measured every 20 min in the day time (0600–2400 h) and every hour during the night using a portable automatic monitor (SpaceLabs model 90202, Redmond, WA, USA) (16). All participating females were substituted with sex hormones and were examined at mid-cycle.

Assays

Serum insulin-like growth factor-I (IGF-I) was measured by radioimmunoassay as described previously (17). The mean $\pm$ S.E. value of IGF-I for this assay obtained in 27 age matched healthy subjects was $177 \pm 10\text{ }\mu\text{g/l}$ (18). Plasma renin concentrations were determined using an antibody-trapping method based on conversion of exogenous human renin substrate to angiotensin I (ANG I) in the presence of antiserum against this peptide. The amount of ANG I formed was determined in a subsequent RIA step adding ^{125}I -ANG I and incubating overnight in the same tubes used for the enzymatic reaction. The renin concentration was calculated from the activity of a standard renin preparation incubated under identical conditions. This standard

Table 1 Clinical data of the patients. Besides being GH deficient, some patients had insufficiencies of the pituitary–thyroid, pituitary–gonadal and/or pituitary–adrenal axes (1, 2 or 3). Some of these patients received vasopressin (ADH).

Sex/age (years)	Insufficient pituitary axes	Treatment/dose reduction	Side-effects	Diagnosis
F/30	1, ADH	Placebo	—	Prolactinoma
M/37	3	Placebo	—	Adenoma op.
M/28	3	Placebo	—	Adenoma op.
M/39	0	Placebo/50%	Sleeplessness, myalgia	Adenoma
M/36	3, ADH	GH/20%	Arthralgia	Cranio-pharyngeoma op.
F/31	3, ADH	GH	—	a. carotis int. aneur. op.
M/40	3	Placebo	—	Adenoma op.
M/43	3	GH	—	Adenoma op.
M/29	3, ADH	Placebo	—	Cranio-pharyngeoma op.
M/49	3	Placebo	—	Cranio-pharyngeoma op.
M/56	3	GH/50%	Edema	Adenoma op.
M/25	3, ADH	GH	—	Germinoma
M/30	3, ADH	GH/40%	Edema	Adenoma op.
M/26	3, ADH	Placebo	—	Cranio-pharyngeoma op.
F/26	2	Placebo	—	Idiopathic
M/28	2	GH	—	mb. cushing
M/40	3, ADH	GH/33%	Myalgia, arthralgia	Cranio-pharyngeoma op.
M/37	0	Placebo	—	Idiopathic
M/64	3	Placebo	—	Cranio-pharyngeoma op.
F/34	1	Placebo	—	Idiopathic
M/53	3	GH/20%	Edema	Cranio-pharyngeoma op.
F/31	1	Placebo	—	Adenoma

Op, transsphenoidal operation at least once; a. carotis int aneur., arteria interna aneurysm; mb. cushing, morbus cushing.

renin preparation 68/356 was obtained from The National Institute for Biological Standards and Control, Potters Bar, Herts, UK. All samples from each subject were run in the same assay.

Statistical analyses

SAS statistical software was employed. Student's paired *t*-test was used for within group comparisons. Simple unpaired *t*-test was used to compare the differences in delta values (i.e. baseline vs 4 months) between the two treatment groups. A *P* value less than 0.05 was considered significant. All results are expressed as means $\pm$ S.E.M.

Figure 1 (a) Changes in ECV before and after 4 months treatment with GH or placebo. Within group comparisons: $P < 0.01$ (GH), $P = 0.37$ (placebo); between group comparison $P < 0.05$. (b) Changes in PV before and after 4 months treatment with GH or placebo. Within group comparisons: $P = 0.01$ (GH), $P = 0.37$ (placebo); between group comparison $P = 0.07$.

Results

Two out of eleven patients receiving GH (1 female, 1 male) were excluded due to failure with compliance. Five GH treated patients underwent dose reductions between 20 and 50%, whereas one placebo treated patient had the dose reduced by 50%. The mean peak GH response to an insulin tolerance test, a clonidine stimulation test or an arginine infusion was $0.92 \pm 0.13 \mu\text{g/l}$. Further data are given in Table 1.

Body composition

Due to technical problems ECV measurements were not performed in two subjects (1 female, 1 male) receiving GH and placebo respectively. Extracellular volume (Fig. 1a) increased significantly in the GH treated patients, and remained unchanged in the placebo treated patients (Table 2). Plasma volume (Fig. 1b) increased in all 9 subjects receiving GH and in 8 out of 13 placebo treated patients (Table 2). Body height was significantly greater in the GH treated patients (GH ($n=9$): 176.3 ± 2.9 cm, placebo ($n=13$): 167.4 ± 3.4 cm) ($P < 0.01$). Body weight was not changed in the GH treated patients but decreased significantly in the placebo treated patients (before GH: 95.5 ± 6.0 kg, after GH: 94.3 ± 6.4 kg; before placebo: 76.2 ± 5.6 kg, after placebo: 75.7 ± 7.5 kg) ($P < 0.05$).

Plasma renin and blood pressure

Plasma renin (Fig. 2) increased during GH treatment and remained unchanged during placebo administration (Table 2). Twenty-four-hour mean ambulatory systolic as well as diastolic blood pressure (mm Hg) was unaffected by GH (before GH: 122 ± 6 and 75 ± 4 respectively, after GH: 121 ± 5 and 73 ± 4 respectively)

Figure 2 Changes in plasma renin levels before and after 4 months treatment with GH or placebo. Within group comparisons: $P = 0.03$ (GH), $P = 0.99$ (placebo); between group comparison $P = 0.08$.

Table 2 Results of measurements of ECV, PV, renin and IGF-I in patients before and after 4 months treatment with GH or placebo.

	Treatment	Before treatment	After 4 months treatment	P value*
ECV (l) (n = 8)	GH	20.48 ± 0.99	23.77 ± 1.38	0.03
ECV (l) (n = 12)	Placebo	16.92 ± 1.01	17.60 ± 1.24	0.03
PV (l) (n = 9)	GH	3.39 ± 0.27	3.71 ± 0.30	0.07
PV (l) (n = 13)	Placebo	2.81 ± 0.18	2.89 ± 0.20	0.07
Renin (mU/l)	GH	12.67 ± 2.04	27.33 ± 7.45	0.08
Renin (mU/l)	Placebo	20.77 ± 5.13	20.69 ± 6.67	0.08
IGF-I (µg/l)	GH	106 ± 19	327 ± 36	<0.01
IGF-I (µg/l)	Placebo	110 ± 17	103 ± 18	<0.01

* P values are the results of between group comparisons.

Figure 3 Mean serum IGF-I levels before and after 4 months treatment with GH or placebo. $P < 0.01$, between group comparison.

and placebo (before placebo: 124 ± 4 and 77 ± 3 respectively, after placebo: 126 ± 3 and 79 ± 3 respectively) (not significant).

IGF-I

Mean serum IGF-I (Fig. 3) was increased by GH without being affected during placebo administration (Table 2).

Discussion

In a placebo controlled, double blind parallel study we examined the impact of 4 months GH treatment on body fluid distribution in adult GH deficient patients. Our main finding is a significant increase in ECV in the GH treated group compared with the placebo group in whom ECV remained unchanged. Plasma volume was also significantly increased in the GH treated group, but due to a small PV increase in the placebo group also the treatment

effect just failed to reach statistical significance. In line with previous reports plasma renin was stimulated by GH.

Untreated adult GH deficiency has been shown to be associated with decreased ECV in several studies (8, 19, 20) and GH substitution has been shown to normalise ECV in these patients compared with an appropriate control group (8). These observations have recently been challenged in two studies in which ECV was reported to be relatively enlarged in untreated patients (21, 22). In both studies, however, females were examined irrespective of the stage of their menstrual cycles which, due to the water retaining effect of sex hormones (23), may have influenced the results. Moreover, the first study included a control group where body mass index differed significantly from the patients. In the present study 5 premenopausal female subjects were examined during sex hormone substitution, thereby minimising the confounding effects of sex hormones. Recently, GH replacement for one year was shown to increase directly assessed ECV (10) in an uncontrolled study. Bengtsson *et al.* previously demonstrated that 26 weeks of GH therapy increased ECV (3). In that study the four compartment model was used which is dependent on a constant potassium/nitrogen ratio and a constant protein content of body cell mass of 25%. There is evidence to suggest that these assumptions may not be valid in GH deficient patients (11), and measurement of total body potassium has actually been shown to be a poor predictor of lean body mass in GH deficient patients (12). Even so, the data obtained are comparable to results using more direct assays (6). By and large it seems appropriate to conclude that GH substitution in GH deficient adults is associated with a sustained increase in ECV, which probably represents a normalisation compared with the untreated state.

Plasma volume increased significantly in 9 out of 9 GH treated patients. Due to increments in the placebo treated patients the treatment effect was, however, not significant. However, in view of several previous studies in which GH induced increments in PV have been reported (8, 24, 25) we favour the interpretation that the lack of statistical significance represents a type 2 error. In line with several previous studies plasma renin

levels were elevated by GH but did not reach statistical significance. This finding indicates that the renin–angiotensin–aldosterone system is responsible for the antinatriuretic effect of GH, because otherwise one would anticipate feed-back suppression of the system by the GH induced volume expansion. To further support this notion we reported previously that healthy subjects receiving GH in combination with angiotensin converting enzyme inhibitors or aldosterone antagonists failed to increase their ECV compared with subjects receiving GH administration alone (26). Renin is released from the juxtaglomerular apparatus in response to decreased presentation of fluid volume in the afferent arteriole. Hence renin levels should be increased in states of volume deficiency such as GH deficiency. By contrast, plasma renin has been reported to be decreased in one patient with GH deficiency in whom GH treatment subsequently increased renin (27). Altogether the data indicate that GH deficient patients may exhibit an impaired function of the renin–angiotensin–aldosterone system, and that GH substitution acts to restore this together with normalisation of body fluid distribution.

In line with other studies (7) no changes in either diurnal systolic or diastolic blood pressure were observed.

The patients received an initial GH dose of 2 IU/m², and subsequent dose reductions were made in 5 GH treated patients and in 1 placebo treated patient. The mean IGF-I levels in the GH treated group were significantly higher compared with a matched group of healthy subjects, which confirms that the dosages were too high. The degree to which a more physiological replacement dose would have influenced our findings is not known, although the clinical experience is that even very low GH doses translate into substantial changes in body composition. By coincidence the gender distribution was uneven between the GH and the placebo groups. In the GH treated group only males underwent ECV measurements and only one female completed PV measurements. Male patients are more responsive to GH substitution in terms of IGF-I generation and changes in body composition (28), and it is therefore likely that this gender specificity also applies to ECV and PV. Future studies are needed to address this issue.

In conclusion ECV remains elevated for at least 4 months in adult GH deficient patients treated with GH. This implies that fluid retention or repletion often seen during the initial phases of GH substitution to adult GH deficient patients is not just a transient phenomenon but probably reflects a physiological normalisation of body composition. This effect of GH seems to be mediated through an activation of the renin–angiotensin–aldosterone system.

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