0021-972X/91/7204-0768$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 72, No. 4
*Printed in U.S.A.*

# Expansion of Extracellular Volume and Suppression of Atrial Natriuretic Peptide after Growth Hormone Administration in Normal Man

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**ABSTRACT.** Sodium retention and symptoms and signs of fluid retention are commonly recorded during GH administration in both GH-deficient patients and normal subjects. Most reports have however, been casuistic or uncontrolled. In a randomized double blind placebo-controlled cross-over study we therefore examined the effect of 14-day GH administration (12 IU sc at 2000 h) on plasma volume, extracellular volume (ECV), atrial natriuretic peptide (ANP), arginine vasopressin, and the renin angiotensin system in eight healthy adult men. A significant GH induced increase in serum insulin growth factor I was observed. GH caused a significant increase in ECV (L): 20.45 ± 0.45 (GH), 19.53 ± 0.48 (placebo) ($P < 0.01$), whereas plasma volume (L) remained unchanged 3.92 ± 0.16 (GH), 4.02 ± 0.13 (placebo). A significant decrease in plasma ANP (pmol/L) after GH administration was observed: 2.28 ± 0.54 (GH), 3.16 ± 0.53 (placebo) $P < 0.01$. Plasma aldosterone (pmol/L): 129 ± 14 (GH), 89 ± 17 (placebo), $P = 0.08$, and plasma angiotensin II (pmol/L) levels: 18 ± 12 (GH), 14 ± 7 (placebo), $P = 0.21$, were not significantly elevated. No changes in plasma arginine vasopressin occurred (1.86 ± 0.05 pmol/L *vs.* 1.90 ± 0.05, $P = 0.33$). Serum sodium and blood pressure remained unaffected. Moderate complaints, which could be ascribed to water retention, were recorded in four subjects [periorbital edema (n = 3), acral paraesthesia (n = 2) and light articular pain (n = 1)]. The symptoms were most pronounced after 2–3 days of treatment and diminished at the end of the period. In summary, 14 days of high dose GH administration caused a significant increase in ECV and a significant suppression of ANP. (*J Clin Endocrinol Metab* **72:** 768–772, 1991)

HYPERSECRETION of GH in acromegalic patients is known to be accompanied by expansion of extracellular volume (ECV) and plasma volume (PV), sodium retention, and increased total body water (1–3). Sodium retention and weight gain have also been reported during the initial phase of GH administration in GH-deficient patients (4–9) and in normal subjects (10–13). These reports indicate that GH may play a role in body fluid homeostasis, but much of the data is of a casuistic or an inadequately controlled nature.

Recently an acute GH induced activation of the renin angiotensin system in normal man was reported (11), suggesting this system as an effector of the antinatriuretic action of GH. In addition an impaired atrial natriuretic peptide (ANP) response to a saline load in acromegalic patients has been observed and in that study ANP was suggested to be the primary pathophysiological abnormality causing sodium retention in acromegaly (14).

Received July 31, 1990.
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We have conducted a controlled study on the effects of GH administration on ECV, PV, the renin angiotensin system, and ANP in normal subjects.

## Materials and Methods

### *Subjects*

Eight healthy males with a median age of 27.1 yr (range, 21–33 yr) and with normal body mass index participated in the study after informed consent. An oral glucose tolerance test was carried out before the study to exclude impaired glucose tolerance. The study was approved by the local ethical committee and the Danish National Board of Health. Apart from GH the subjects did not receive any mediation during the trial.

### *Design*

The study had a randomized, double-blind, placebo-controlled crossover design. The participants were treated with placebo and GH (12 IU/day; Norditropin, Novo-Nordisk, Denmark) during two separate 14-day periods. A six-week washout period was inserted between the two treatment periods. GH and placebo was dissolved in 1.5 ml sterile water and injected sc by the participants into an abdominal skinfold daily at 2000 h. The subjects were seen at the out-patient clinic after an

overnight fast at day 0, 3, 14, for blood sampling. At the end of each treatment period extracellular volume and plasma volume were determined, and blood samples were drawn for analysis of ANP, arginine vasopressin (AVP), aldosterone, and angiotensin II.

*ECV and PV*

The subjects were admitted to the endocrine unit at 0800 h after an overnight fast. They were placed in the supine position and iv catheters were inserted in the right and left cubital vein for blood sampling and infusion, respectively. At 1200 h the subjects received a standardized meal and 200 ml water.

For the measurement of extracellular volume 20 $\mu$Ci $Br^{82}$ dissolved in 20 ml NaCl was injected iv at 0900 h and blood samples were drawn 4 h later, as described by Leth and Binder (16). The subjects were allowed ambulatory activity from 0900–1200 h. Determination of PV was made at 1300 h by iv injection of 5 $\mu$Ci $I^{125}$-albumin in a 2 ml suspension, followed by blood sampling every 10 min for 50 min. PV was calculated according to the formula (15):

$$PV = A1/C0$$

A1 = the total of administered activity of $I^{125}$ albumin; C0 = the concentration of activity at the time t0, this value is calculated by extrapolating the values at t10, t20, t30 *etc.* Total extra-cellular volume was then calculated according to the formula (16).

$$ECV = 0.93((A - Te - Tu/Cpl) - PV) + PV$$

0.93: A fixed correction factor for protein concentration in plasma and the Gibbs Donnan effect; A: The amount of activity administered; Te: Loss of activity to erythrocytes, 4.7% of A; Tu: Loss of activity in the urine; Cpl: Plasma activity 4 h after injection of bromide. Samples for ANP, AVP, aldosterone, and angiotensin II were obtained at 0900 h.

*Assays*

Angiotensin II was determined by a slight modification of the method described by Kappelgaard *et al.* (17). RIA was performed after previous plasma extraction by means of a cation resin and subsequent elution from the resin with ethanol and ammonia/methanol. The coefficients of variation were 12% (interassay) and 8% (intraassay). Lower detectable limit: 2 pmol/L. Aldosterone was measured by a slight modification of a previously described method (18). RIA was performed on a residue from plasma prepared by extraction with dichlormethane and purification of silica gel columns. Paper chromatography was omitted due to the use of an antibody with high specificity (International CIS). The coefficients of variation were 13% (interassay) and 9% (intraassay). Lower detectable limit: 42 pmol/L.

AVP was measured as previously described (19, 20). RIA was performed after precipitation of plasma proteins with cold acetone and extraction of lipids with petroleum ether. The coefficients of variation were 13% (interassay) and 9% (intraassay). Lower detectable limit: 0.5 pmol/L.

ANP was determined by RIA as previously reported (21). ANP was extracted from plasma by means of Sep-pak C-18 cartridges (Water Associates) using 80% ethanol in 4% acetic acid. The coefficients of variation were 12% (interassay) and 10% (intraassay). Lower detectable limit: 1.1 pmol/L.

Sodium, potassium, and creatinine in plasma were measured by routine methods at the Department of Clinical Chemistry. Serum insulin like growth factor I (IGF-I) and serum insulin were measured at day 0, 3, and 14 by RIA as described previously (22). Serum C-peptide was assayed by a commercial kit (Immunonuclear, Stillwater, MN). All samples from each subject were run in the same assay. Before and at the end of each period of treatment, body weight and blood pressure were recorded.

*Statistics*

Student's *t* test for paired comparisons was used as statistical test. A $P$ value less than 0.05 (two-tailed) was considered to be significant. All results are expressed as mean ± SEM.

## Results

Administration of GH for 14 days resulted in an increase in serum IGF-I from a value of 262 ± 13 $\mu$g/L to 541 ± 59 $\mu$g/L ($P < 0.01$); no changes occurred in IGF-I values during placebo treatment. Fasting serum insulin levels (pmol/L), measured in the morning 12 h after the last injection, was insignificantly increased after GH administration [171 ± 21 (GH), 142 ± 22 (placebo)]. Serum C-peptide (pmol/L) displayed a similar pattern (632 ± 248 (GH), 463 ± 139 (placebo)). GH induced a significant increase in plasma nonesterified fatty acids (data not shown).

*ECV (Fig. 1)*

All eight subjects exhibited an increase in ECV (L) after GH treatment [20.45 ± 0.45 (GH), 19.53 ± 0.48 (placebo), $P < 0.01$].

*PV (Fig. 1)*

GH administration did not significantly affect PV (L) (3.92 ± 0.45 (GH), 4.01 ± 0.36 (placebo), $P = 0.40$).

*ANP (Fig. 2)*

ANP values (pmol/L) were lower after GH treatment (2.28 ± 0.54 (GH), 3.16 ± 0.53 (placebo) $P = 0.013$).

*Renin angiotensin system*

Seven out of eight subjects showed elevation of aldosterone (picomoles per L) after GH (Fig. 2). Still the difference did not quite reach statistical significance (129 ± 14 (GH), 89 ± 17 (placebo) $P = 0.08$). Angiotensin II

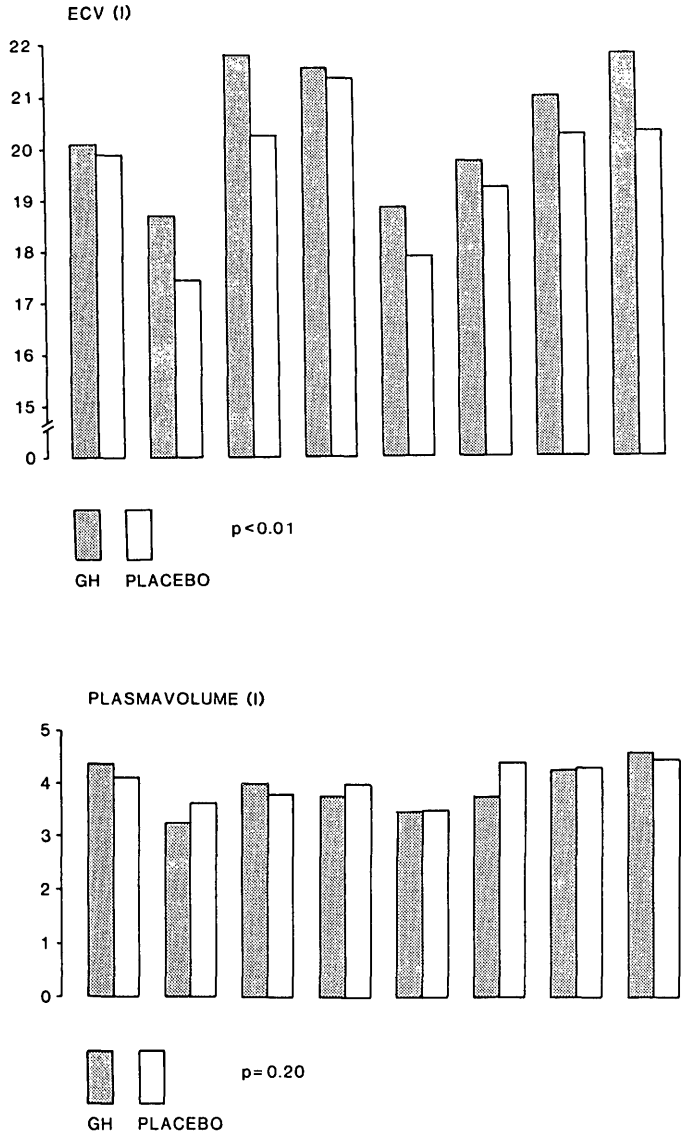


FIG. 1. Total ECV (*upper panel*) and total PV (*lower panel*) in the eight participating subjects after 14 days of GH (12 IU/day) administration and after 14 days of placebo administration.

(pmol/L) showed the same pattern: 18 ± 12 (GH), 14 pmol/L ± 7 (placebo).

*AVP, electrolytes, blood pressure, body weight*

AVP (pmol/L) remained unaffected by GH administration [1.86 ± 0.05 (GH), 1.90 ± 0.05 (placebo)]. Serum sodium (millimoles per L) was unchanged after GH administration [142.4 ± 1.6 (GH), 142.5 ± 1.9 (placebo)]. Likewise serum potassium and creatinine levels were similar in the two periods (data not shown). Blood pressure was unaltered during both periods (data not shown). During GH treatment there was a slight but nonsignificant increase in total body weight (kg) 79.1 ± 6.0 (GH), 78.7 ± 6.3 (placebo).

*Side effects*

During GH administration four subjects reported inconveniences, that could be related to fluid retention, *i.e.* moderate acral edema, light articular pains, and acral paraesthesia. The complaints were most pronounced from day 3 to 5 and then subsided. There were no complaints during placebo administration.

## Discussion

In the present study we observed expansion of ECV in all eight subjects after 14 days of GH therapy, whereas no changes in PV were recorded. Expansion of ECV was associated with a significant decrease in ANP. Plasma aldosterone was not significantly elevated and AVP and plasma angiotensin II remained unchanged. Serum insulin showed no significant elevation after GH administration.

There may have been alterations in the renin-angiotensin-aldosterone system (11), ANP, and serum insulin shortly after GH administration. Frequent blood sampling during the early phases of the study could have revealed any such changes. On the other hand our intention with the present study was to make the measurements during steady state hypersomatotropinemia.

To our knowledge, this is the first study, which in a controlled way shows the effect of GH on ECV and PV in normal man. It confirms previous observations of expansion of ECV in acromegaly (1–3) and in GH-deficient patients treated with GH (5, 7). Somewhat unexpectedly PV was unaffected by GH for 14 days. This is in contrast to studies of acromegaly where PV has been reported to be elevated (23). It is possible that GH excess for 14 days was too short to affect PV, when considering that acromegalic patients are exposed to GH excess of a much longer duration.

Suppression of ANP levels after GH administration has not been described previously. Theoretically a suppression of ANP release could contribute to expansion of ECV. Even minor changes in ANP levels have been shown to be able to induce substantial effects on venous capacitance and interstitial fluid volume (24). Conditions of volume expansion [*i.e.* saline loading (25), and heart failure (24)] are associated with a compensatory rise in the secretion of ANP, and thus natriuresis. In order to counterbalance an expansion of ECV, a rise in ANP would be expected. However, the observation of decreased levels of ANP after GH administration raises the possibility that GH directly inhibits ANP release from the atrias, perhaps by lowering atrial pressure. This may lend support from evidence of a GH-induced increased myocardial contractility in normal man (26) and improved myocardial performance in acromegalic rats (27). Impaired ANP response to a saline load in acro-

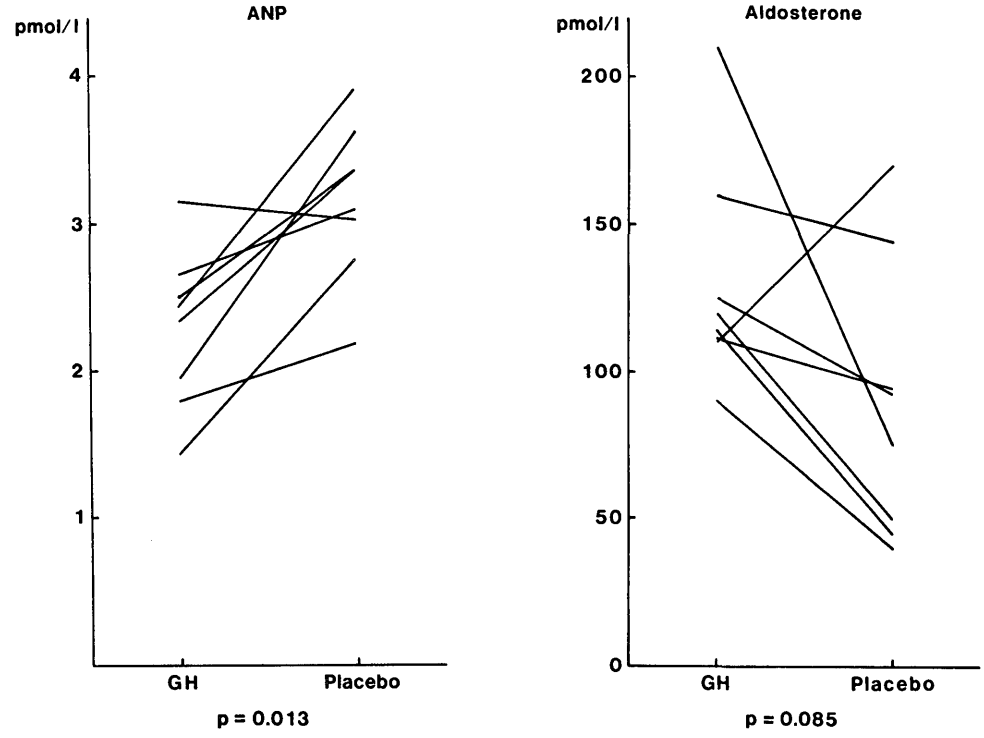


FIG. 2. ANP in eight subjects after GH administration (12 IU/day) for 14 days and after placebo administration (*left panel*) and aldosterone (*right panel*) after GH administration (12 IU/day for 14 days) and after the placebo period.

megalic patients has recently been reported (14). Basal ANP levels were within normal range in these patients, but it was proposed that the lack of increase in ANP constituted a primary pathophysiological abnormality causing sodium retention.

Recently a 7-fold increase in aldosterone after 5 days of GH administration in normal adults (0.2 U/kg/day) was reported (11), and previously a GH-induced stimulation of aldosterone biosynthesis *in vitro* has been observed (28). This could indicate an influence of GH on the renin-angiotensin-aldosterone system. Others have, however, not been able to observe any changes in the renin aldosterone system during GH infusion (29, 30), or in acromegalic patients (31). In the present study elevated levels of aldosterone after GH administration were observed in seven out of eight subjects. It is interesting to speculate that the elevation, although not significant, could be caused by a lack of ANP suppression of aldosterone production, since there is agreement that the atrial peptides have a direct inhibitory effect on aldosterone production (32).

Insulin has been reported to promote renal tubular sodium resorption and increase volume resorption at the level of the proximal tubule (33). Acromegalic patients are normally hyperinsulinaemic (34) and GH is known to induce insulin secretion (35). A likely explanation for the expansion of ECV seen in the present study could therefore be GH-induced hyperinsulinemia. However, serum insulin levels were not significantly elevated during the trial, probably because 12 h elapsed from the time of GH administration to the time of blood sampling.

In summary, the present study favors suppression of ANP as a possible mechanism behind GH-induced expansion of ECV. Whether this is due to decreased atrial pressure or to a classic hormonal inhibition remains unknown. Future studies on these issues should be designed in a way that enables evaluation of more dynamic changes, especially during the initial phases of GH exposure.

## Acknowledgment

We are indebted to Elin Bang, Joan Hansen, Lisbeth Mikkelsen, Kirsten T. Jensen, J. Sørensen, and Anette Mengel for their invaluable assistance. A special thank to Jørgen Marqversen, Department of Nuclear Medicine, Aarhus Kommunehospital who most kindly provided isotopes for us.

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