

Chronic Growth Hormone Excess Is Associated with Increased Aldosterone: A Study in Patients with Acromegaly and in Growth Hormone Transgenic Mice

MAXIMILIAN BIELOHUBY,* JOSEFINE ROEMMLER,* JENNY MANOLOPOULOU,* INGA JOHNSEN,*
MANDY SAWITZKY,† JOCHEN SCHOPHOHL,* MARTIN REINCKE,* ECKHARD WOLF,‡
ANDREAS HOEFELICH,† AND MARTIN BIDLINGMAIER*,¹

**Neuroendocrine Unit, Medizinische Klinik - Innenstadt, LMU, 80336 Munich, Germany; †Laboratory of Mouse Genetics, Research Unit Genetics and Biometry, Research Institute for the Biology of Farm Animals-FBN, 18196 Dummerstorf, Germany; and ‡Chair for Molecular Animal Breeding and Biotechnology, and Laboratory for Functional Genome Analysis (LAFUGA), Gene Center, LMU, 81337 Munich, Germany*

Acromegaly is a disease characterized by chronic growth hormone (GH) excess. Since hypertension is a common finding in patients with acromegaly, interactions between GH and the renin-angiotensin-aldosterone system (RAAS) are under controversial debate. We examined GH, IGF-I, aldosterone, and renin in a well-defined group of acromegalic patients before and after cure by surgery. In addition, we analyzed the impact of chronic GH excess on the RAAS in mouse models overexpressing GH alone (G) or in combination with insulin-like growth factor-binding protein-2 (IGFBP-2; GB). Normalization of GH secretion after cure by surgery was accompanied by significant decreases of serum aldosterone in acromegalic patients (pre-op: 96.5 ± 37.1 pg/mL, post-op: 41.3 ± 28.2 pg/mL; $P < 0.001$; $n = 13$), but renin concentrations were unaffected. In addition, aldosterone concentrations were positively correlated to GH levels (Spearman $r = 0.39$; $P = 0.025$; $n = 26$). To further study this association, we analysed two transgenic mouse models and found a similar relationship between GH and aldosterone in G mice, which showed about 3-fold elevated serum aldosterone levels in comparison to non-transgenic controls (males: 442 ± 331 pg/mL vs. 151 ± 84 pg/mL; $P = 0.002$; $n \geq 12$; females: 488 ± 161 pg/mL vs. 108 ± 125 pg/mL; P

$= 0.05$; $n \geq 4$). Expression of aldosterone synthase was similar in adrenal glands of C and G mice. Aldosterone levels in G and GB mice of both genders were not different, indicating that the elevated aldosterone was due to GH excess and not caused by elevated IGF-I, which is known to be blocked by IGFBP-2 overexpression. Also in the mouse models, changes in aldosterone were independent from renin. In summary, we show that chronic GH excess is associated with increased aldosterone in humans and mice. GH-induced increases of aldosterone potentially contribute to the increased cardiovascular risk in acromegalic patients. The underlying mechanism is likely to be independent of renin, excess IGF-I, or adrenal aldosterone synthase expression. *Exp Biol Med* 234:1002–1009, 2009

Key words: growth hormone; aldosterone; IGF-I; renin; acromegaly; adrenal gland

Introduction

Growth hormone (GH) is a major stimulus for postnatal growth (1, 2). A considerably high proportion of GH effects on growth is mediated by insulin-like growth factor-I (IGF-I) (3); however, different metabolic functions, such as for carbohydrate and lipid metabolism, can be influenced by direct GH effects (4, 5). GH has also been shown to interact with the hypothalamic-pituitary-adrenal axis (6–8). Effects of GH on glucocorticoids (9) have been studied in detail, but data on the interaction between GH and aldosterone are scarce and conclusions remain controversial.

Aldosterone is produced in the zona glomerulosa, the outmost zone of the adrenal gland. Its synthesis and secretion are mainly controlled by the renin-angiotensin-aldosterone system (RAAS) via angiotensin II and III (10) and by the potassium status. Furthermore, adrenocortico-

This study was funded in part by the German DFG-Research Training Unit 1029.

¹ To whom correspondence should be addressed at Medizinische Klinik – Innenstadt Klinikum der Universität, Ziemssenstr. 1, 80336 Munich, Germany. E-mail: martin.bidlingmaier@med.uni-muenchen.de

Received January 26, 2009.
Accepted April 8, 2009.

DOI: 10.3181/0901-RM-34
1535-3702/09/2348-1002\$15.00
Copyright © 2009 by the Society for Experimental Biology and Medicine

tropic hormone (ACTH) (11) and sodium homeostasis influence aldosterone secretion (12, 13). One of the key enzymes in aldosterone synthesis is aldosterone synthase (CYP 11B2). The classical action of aldosterone is the regulation of sodium and potassium homeostasis through modification of epithelial sodium channels in the kidney, thereby influencing fluid balance and blood pressure. The Framingham Offspring Study investigated the relation of baseline aldosterone levels to increases in blood pressure and the incidence of hypertension after four years in 1688 non-hypertensive participants. The results showed that elevated aldosterone levels, even within the physiological range, predispose the development of hypertension (14).

In humans, chronic excess of GH occurs in acromegaly, a disease characterized by autonomous GH secretion from mostly pituitary adenomas and subsequently elevated levels of IGF-I (15). Hypertension is frequent in acromegaly and patients suffer from an increased cardiovascular morbidity and mortality (16, 17).

A link between acromegaly and adrenal gland function was proposed 40 years ago (18, 19). However, previous studies on the RAAS in acromegalic patients have produced conflicting results. Therefore, we re-assessed the impact of chronic GH excess on the RAAS in a subpopulation of acromegalic patients selected to exclude interference from different regimes to treat acromegaly.

Subsequent to an association we found between GH and aldosterone in these patients and knowing the limitations of a retrospective patient study, we further explored the effect of chronic GH excess on the RAAS in a transgenic mouse model. In order to differentiate between direct and indirect (IGF-I mediated) effects of GH, we measured aldosterone and renin activity in GH transgenic mice (G) and in GH transgenic mice simultaneously overexpressing the IGF-inhibitory binding protein IGFBP-2 (GB).

Materials and Methods

Patients with Acromegaly. Out of the cohort of more than 200 patients with acromegaly seen in our outpatient clinic (Medizinische Klinik-Innenstadt, LMU, Munich, Germany) during the last decade, we selected 13 patients fulfilling the following criteria: a) GH-secreting pituitary adenoma, as documented by MRI and biochemically, b) no medical treatment of acromegaly before or after surgery (dopamine agonists, somatostatin analogues, or GH receptor antagonists), c) pre- and post-op normo-kalemia (>3.5 mmol/L), d) cure by trans-sphenoidal surgery alone, e) absence of concomitant diseases (including diabetes), and f) availability of plasma samples. Pre-op, all acromegalic patients had active disease, with a GH nadir >1 μ g/L after oral glucose tolerance test (OGTT) and elevated IGF-I. Within 3 months after surgery, all 13 patients were considered cured based on normalized IGF-I and GH nadir during OGTT <1 μ g/L. GH nadir during

Table 1. Characteristics of Acromegalic Patients

Parameter	Patients with acromegaly
Male/female patients	7/6
Age at diagnosis of acromegaly (years)	46.5 $\pm$ 8.9
Body weight	81.3 $\pm$ 17.1
Height (cm)	171.5 $\pm$ 11.1
First symptoms (anamnesic) until final diagnosis (years)	1.2 $\pm$ 6
Biochemical diagnosis until surgery ^a (months)	5.5 $\pm$ 4.1
Pre-op plasma K ⁺ (mmol/L)	4.1 $\pm$ 0.3
Post-op plasma K ⁺ (mmol/L)	4.1 $\pm$ 0.2
Pre-op plasma Na ⁺ (mmol/L)	141 $\pm$ 1.6
Post-op plasma Na ⁺ (mmol/L)	142 $\pm$ 3.6

^a One patient refused surgery for 3.5 years (not included in statistic).

OGTT, IGF-I, aldosterone, and renin concentrations were measured in the patients before (pre-op) and after (post-op) the surgical intervention. Blood samples for aldosterone and renin determinations were taken between 8:00 and 11:00 in the morning after 15 minutes of rest in a seated position. Seven patients were normotensive, whereas 6 of the 13 patients had a clinical diagnosis of hypertension. Before surgery, 3 of the 6 hypertensive patients were on beta blockers and 2 received angiotensin II receptor blockers. In addition, 3 of the hypertensive patients were on thiazide diuretics and 1 patient received an angiotensin converting enzyme inhibitor and a calcium channel blocker. After surgery, in 1 male patient, beta blocker therapy was stopped, but in another male patient, beta blocker therapy was added. Therefore, for the whole group, antihypertensive agents were the same pre- and post-op. Patients with TSH ($n = 1$) or ACTH deficiency ($n = 2$) were on stable doses of thyroxine and/or hydrocortisone throughout the observational period. Additional patient characteristics are shown in Table 1.

Animal Husbandry and Generation of Transgenic Mice. Mice were maintained under specified pathogen-free conditions in a closed barrier system and monitored as recommended (20). All mice had free access to a standard rodent pellet diet (V1534; Ssniff, Soest, Germany) and tap water *ad libitum*. The diet used contained 0.59% sodium chloride and 0.97% potassium, which is within the range of standard rodent pellet diets. At an age of 3 weeks, animals were weaned and separated according to gender.

Transgenic founder mice expressing bovine GH (bGH) under the control of the PEPCK promoter were originally generated on a C57BL/6 x SJL genetic background and the transgene was then transferred to a Naval Medical Research Institute (NMRI) outbred background (12 generations). Insulin-like growth factor-binding protein-2 (IGFBP-2) overexpressing mice originated from a B6D2F2 founder mouse (21) after backcrossing to C57BL/6N mice (>12

generations). For the generation of double transgenic mice, hemizygous PEPCK-bGH and CMV-IGFBP-2 transgenic mice have been crossed as described previously (22). Eleven-week-old male and female GH transgenic (G), GH and IGFBP-2 double-transgenic (GB), and non-transgenic littermate control mice (C) were analyzed.

Mice were weighed and anesthetized individually in a glass jar containing saturated ether vapour, and retro-orbital blood was collected with heparinized microcapillaries in clearly less than 1 min from initial handling within the cage. All blood samples were taken in the afternoon. After a blood sample was taken, the animals were sacrificed by cervical dislocation.

For weight analysis of the adrenal glands in male and female C, G, and GB mice (male: C: $n = 8$; G: $n = 8$; GB: $n = 8$; female: C: $n = 8$; G: $n = 6$; GB: $n = 6$), the abdominal cavity was opened, and the adrenal glands were recovered, carefully freed from adjacent tissues under a stereo dissecting microscope, and weighed individually to the nearest 0.1 mg. All animal experiments were carried out according to the German Animal Protection Law.

Hormone Measurements. Human Assays. GH concentrations during OGTT were taken from patients' files, but all measurements had been done in the same laboratory using either the Advantage GH assay (Nichols Diagnostics Institute, Bad Nauheim, Germany) or (after 2006), the Immulite 2000 GH assay (DPC Biermann, Bad Nauheim, Germany). Both assays had a functional sensitivity below 0.2 $\mu\text{g/L}$. Discrepancies between GH assay results are known, and in our hands, GH results as measured by the Immulite were slightly higher than those obtained by the Advantage at the time of the method change (correlation: Advantage = $0.927 \times \text{Immulite} + 0.105$). Nevertheless, patients were regarded as cured if GH nadir during OGTT was $<1.0 \mu\text{g/L}$, regardless of the assay used.

Concentrations of IGF-I were determined in one run using the Immulite 2000 IGF-I assay (DPC Biermann, Bad Nauheim, Germany). Intra- and inter-assay coefficients of variation were 7.9% and 8.3%, respectively. The results obtained were expressed as a multiple of the upper limit of the normal range ($\times\text{ULN}$), with reference to the published normative data for this method (23).

Aldosterone was measured in unextracted human serum samples by a commercially available RIA kit (Aldosterone Coat-A-Count, DPC Biermann). All samples were measured in duplicate. In our hands, intra- and inter-assay coefficients of variation were below 8% and 13%, respectively. Functional sensitivity was determined to be 35 pg/mL. After surgical cure, in 4 patients, aldosterone concentrations were below 35 pg/mL. These 4 values were set to 35 pg/mL for statistical analysis.

Plasma renin concentrations were determined using the Liaison active renin assay (Diasorin, Dietzenbach, Germany). This assay, based on monoclonal antibodies, only detects active renin molecules with no interference from pro-renin. Intra- and inter-assay coefficients of variation

were below 5.6% and 12.2%, respectively, and the functional sensitivity was $<2.0 \mu\text{U/mL}$.

Mouse Assays. All serum samples were stored at -80°C until analysis. Concentrations of GH, IGFBP-2, and IGF-I in the different genetic groups of mice have been reported before (22). In brief, serum concentrations of GH were in the range of 2000 ng/mL, both in G and GB mice. Circulating IGF-I levels were two- to three-fold higher ($\sim 900 \text{ ng/mL}$) in G and GB compared to C mice. Serum IGFBP-2 levels were four- to nine-fold increased in GB mice ($\sim 2900 \text{ ng/mL}$) compared to C or G mice, respectively.

Aldosterone was measured (male mice: C, $n = 16$; G, $n = 12$; GB, $n = 8$; female mice: C, $n = 6$; G, $n = 4$; GB, $n = 4$) by a competitive time-resolved fluorescence immunoassay using a biotinylated aldosterone tracer, as recently described in detail (13).

Plasma renin activity (PRA) of the different genetic groups was determined in samples from female mice (C: $n = 6$; G: $n = 4$; GB: $n = 4$) using a commercially available radioimmunoassay kit (Angiotensin-I RIA, DiaSorin, Dietzenbach, Germany) with modifications similar to the ones previously described (24). Plasma was diluted 1:20 with maleate buffer containing maleic acid, Tris, EDTA, and neomycin sulphate. Then, 50 μL of the diluted sample were mixed on ice with 7.4 μL plasma of 5/6 nephrectomized rats (Charles River surgical services, Charles River, Sulzfeld, Germany), with 14.8 μL of maleate buffer and with 27.4 μL of assay buffers as provided by the manufacturer. Finally, 2 μL of the enzyme inhibitor phenylmethylsulfonyl-fluoride (PMFS) were added. The samples were then divided into 2 aliquots: one aliquot was incubated for 1.5 hours at 37°C ; the other aliquot remained at 0°C for 1.5 hours. Generated angiotensin-I was measured by RIA. PRA was calculated as the difference between the values obtained at 0°C and 37°C .

Expression of Aldosterone Synthase. Adrenal glands from six male mice per genetic group were homogenized in extraction buffer as described previously (7), and protein content was quantified using the bicinchoninic acid method. Protein (20 μg) was separated on 12% SDS-PAGE gels and transferred to polyvinylidene fluoride membranes (Millipore, Eschborn, Germany). Membranes were blocked (5% dry milk and 1% Tween 20 in Tris-buffered saline) and incubated with primary antibody (monoclonal mouse anti-aldosterone synthase, kindly provided by Dr. C. Gomez-Sanchez, Mississippi, USA) for 1.5 hours at room temperature at a dilution of 1:500. After three washings in Tris-buffered saline containing 1% Tween 20, membranes were incubated with horseradish peroxidase-coupled horse anti-mouse IgG (1:1000; Cell Signaling, Frankfurt/Main, Germany) for 1 hour at room temperature. Finally, membranes were developed on a Kodak Image Station using an enhanced chemiluminescence (ECL) detection kit (LumigenTM TMA-6 solution A and B; Lumigen, GE Healthcare, Germany). Band intensities were

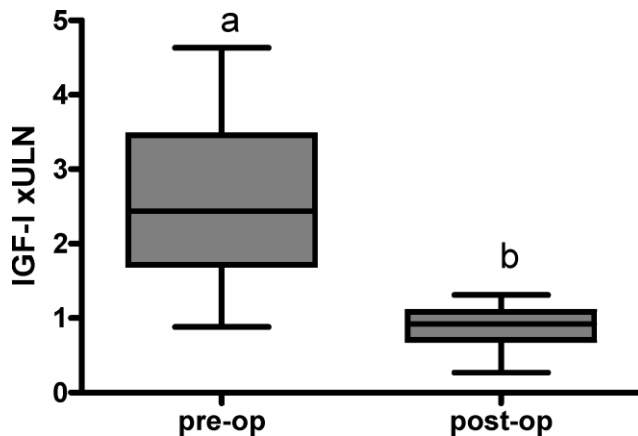


Figure 1. Serum IGF-I normalized for age and sex (xULN) in acromegalic patients before (pre-op) and after (post-op) successful transsphenoidal surgery (data are shown as medians $\pm$ SD, pre-op vs. post op; $P < 0.001$; $n = 13$). Medians labeled with different letters are significantly different.

quantified using the ImageQuant Software package (GE Healthcare). All signal intensities were normalized for the Coomassie blue signal.

Statistical Analysis. Statistical analysis was performed using the SPSS software package (SPSS Inc., version 15.0, Chicago, USA). Endocrine parameters in acromegalic patients before and after surgical cure were compared using the nonparametric Wilcoxon matched pairs test. For the statistical comparison of adrenal gland weights, endocrine hormones and aldosterone synthase expression in mice, one-way ANOVAs with subsequent Bonferroni post hoc tests were performed.

A non-parametric Spearman correlation was performed to determine the correlation between GH or IGF-I and aldosterone or renin in acromegalic patients. P values < 0.05 were considered significant. In the figures, means labeled with different letters are significantly different (“a” versus “b” or “a” versus “c”: indicates a statistically

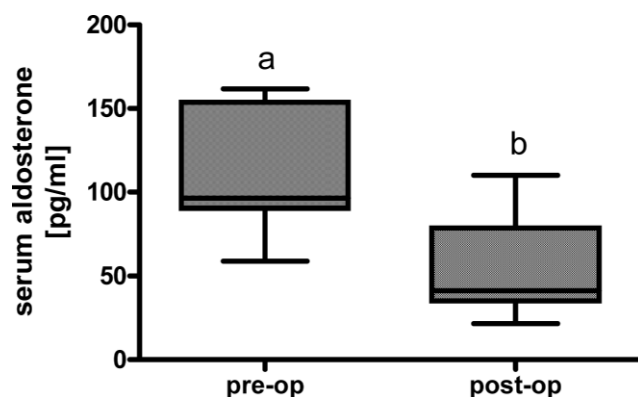


Figure 2. Serum aldosterone concentrations in acromegalic patients before (pre-op) and after (post-op) successful transsphenoidal surgery (data are shown as medians $\pm$ SD, pre-op vs. post op; $P < 0.001$; $n = 13$). Medians labeled with different letters are significantly different.

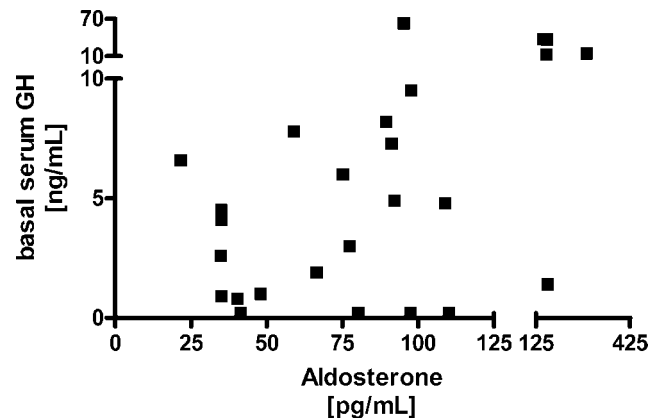


Figure 3. Basal serum GH and serum aldosterone in acromegalic patients before (pre-op) and after (post-op) successful transsphenoidal surgery. Non-parametric Spearman correlation revealed that basal serum GH levels are positively correlated to aldosterone concentrations ($P = 0.025$; Spearman $r = 0.39$; $n = 26$).

significant difference, “a” versus “a”: means no significant difference). Data from acromegalic patients are presented as medians $\pm$ SD, mouse data as means $\pm$ SD.

Results

Hormone Measurements in Acromegalic Patients Before and After Surgical Cure. Basal Serum GH and IGF-I Concentrations. After surgical removal of the pituitary adenoma (post-op) in acromegalic patients, all patients had an OGTT GH-nadir $< 1 \mu\text{g/L}$. Basal median GH concentrations were significantly higher before cure by surgery (pre-op: $8.2 \mu\text{g/L}$; post-op: $0.95 \mu\text{g/L}$; $P = 0.01$). Serum IGF-I levels decreased significantly (pre-op: $685 \pm 303.8 \text{ ng/mL}$, post-op: $208 \pm 82.6 \text{ ng/mL}$, $P < 0.001$; $n = 13$). When normalized for age and sex (xULN), IGF-I levels decreased accordingly (pre-op: 2.44 ± 1.2 , NT post-op: 0.92 ± 0.29 , $P < 0.001$, $n = 13$; Fig. 1).

Serum Aldosterone Concentrations. Surgical cure of acromegaly led to a significant decrease in serum aldosterone levels of patients (pre-op: $96.5 \pm 37.1 \text{ pg/mL}$, post-op: $41.3 \pm 28.2 \text{ pg/mL}$, $P < 0.001$, $n = 13$; Fig. 2). Pre-op aldosterone concentrations tended to be higher in normo- versus hypertensive patients, but the difference failed to reach statistical significance (pre-op: normotensive: $95 \pm 39 \text{ pg/mL}$, hypertensive: $128 \pm 76 \text{ pg/mL}$; $P = 0.45$). After cure by surgery, aldosterone levels declined significantly in normo- and hypertensive patients.

Serum Renin Concentrations. Serum renin concentrations in acromegalic patients tended to be lower after surgical cure, but the change failed to reach statistical significance (pre-op: $17.4 \pm 24 \mu\text{U/mL}$, post-op: $14.8 \pm 18.1 \mu\text{U/mL}$, $P = 0.47$, $n = 13$). Accordingly, the aldosterone to renin ratio declined post-op; however, this was not statistically significant (pre-op: 6.6 ± 7.7 ; post-op: 4.3 ± 8 ; $P = 0.13$; $n = 13$).

Correlation Between GH or IGF-I and Aldosterone. When analyzing pre-op and post-op samples in

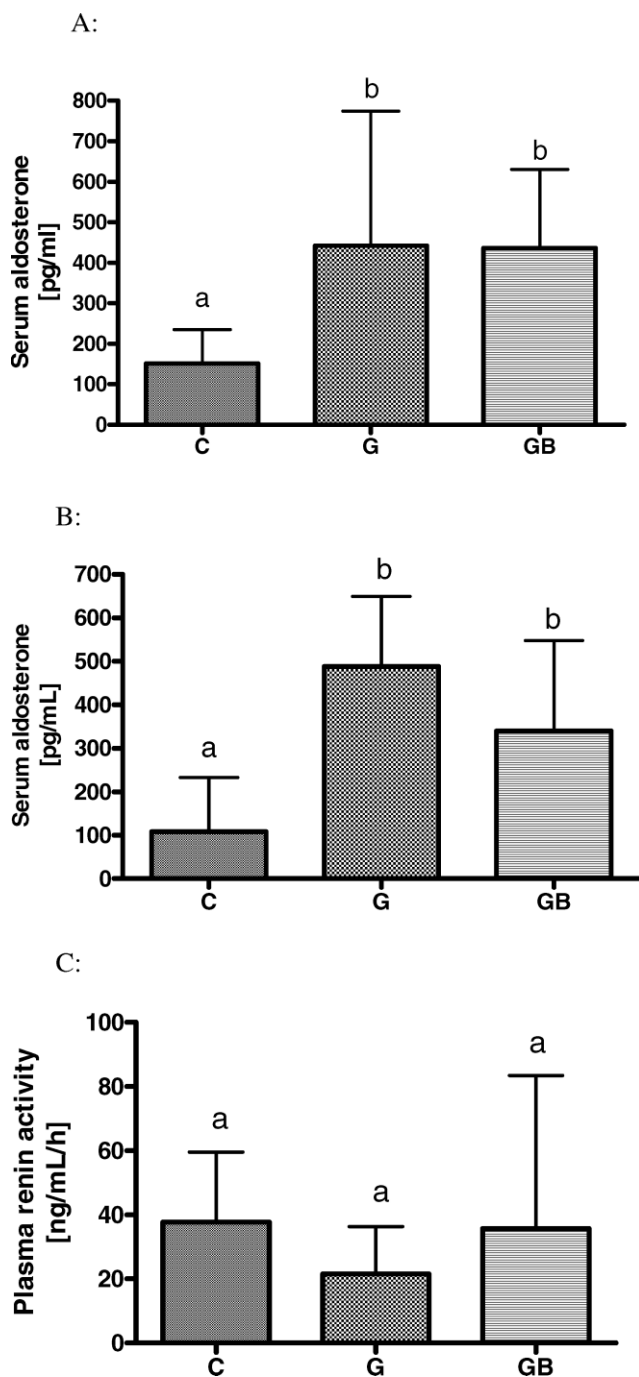


Figure 4A and 4B. Serum aldosterone concentrations in male (Fig. 4A) and female (Fig. 4B) non-transgenic controls (C), GH transgenic (G), and GH/IGFBP-2 double-transgenic (GB) mice. Data are shown as means $\pm$ SD. Means labeled with different letters are significantly different (males: C: $n=16$; G: $n=12$; GB: $n=8$; females: C: $n=6$; G: $n=4$; GB: $n=4$). **Figure 4C.** Plasma renin activity in female non-transgenic controls (C), GH transgenic (G), and GH/IGFBP-2 double-transgenic (GB) mice. Although plasma renin activity in G mice tended to be lower, no significant differences were present between the 3 genetic groups (data are shown as means $\pm$ SD. C: $n=6$; G: $n=4$; GB: $n=4$). Means labeled with the same letters are not significantly different.

patients, basal serum GH levels were positively correlated to aldosterone concentrations (Spearman $r=0.39$; $P=0.025$; $n=26$; Fig. 3). Also, IGF-I concentrations pre-op and post-op were positively correlated to aldosterone concentrations (Spearman $r=0.43$; $P=0.027$; $n=26$). In contrast, neither GH nor IGF-I levels were correlated to renin concentrations ($P=0.32$ and $P=0.81$, respectively).

Adrenal Gland Weights in Mice. The weight of both adrenal glands was significantly higher in male GH-overexpressing (G) mice when compared to C mice.

Simultaneous overexpression of IGFBP-2 in GH transgenic mice (GB) resulted in a significant reduction of adrenal gland weights when compared to only GH-overexpressing mice. (C: 3.55 ± 0.6 mg, G: 6.98 ± 0.4 mg, GB: 5.89 ± 0.4 mg; C vs. G and C vs. GB, $P < 0.001$; $n=8$ /genetic group). Also, in female mice, GH overexpression significantly increased adrenal gland weights, whereas co-overexpression of IGFBP-2 decreased adrenal weights when compared to only G (C: 7.53 ± 1.26 mg, G: 11.68 ± 1.36 mg, GB: 9.98 ± 0.88 mg; C vs. G and C vs. GB $P < 0.01$; G vs. GB, $P < 0.05$; $n \geq 6$ /genetic group).

Hormone Analysis in Mice. Male GH-overexpressing mice (G) showed significantly higher serum aldosterone concentrations compared to non-transgenic controls (442 ± 331 pg/mL vs. 151 ± 84 pg/mL; $P=0.002$, $n \geq 12$; Fig. 4A). Similarly, male GH transgenic mice simultaneously overexpressing IGFBP-2 (GB) also had significantly increased serum aldosterone levels when compared to controls (GB: 436 ± 194 pg/mL, $P < 0.001$, $n=8$; Fig. 4A). No differences in aldosterone concentrations were present between male G and GB mice ($P=0.96$; Fig. 4A).

Significantly increased serum aldosterone levels were also observed in female G and GB mice (C: 108 ± 125 pg/mL; G: 488 ± 161 pg/mL; GB: 340 ± 207 pg/mL; C vs. G, $P < 0.01$; C vs. GB, $P=0.05$; G vs. GB, $P=0.3$; $n \geq 4$; Fig. 4B). There were no significant differences comparing the aldosterone concentrations of male and female mice within the same genotype.

In contrast, overexpression of GH alone or in combination with IGFBP-2 had no significant effect on plasma renin activity when compared to female control mice (C: 37.7 ± 21.8 ng/mL/h vs. G: 21.5 ± 14.8 ng/mL/h, $P=0.25$; GB: 35.7 ± 47.7 ng/mL/h, C vs. GB $P=0.94$; G vs. GB $P=0.59$; C: $n=6$; G: $n=4$; GB: $n=4$; Fig. 4C).

Expression of Aldosterone Synthase in Adrenal Glands. Expression of aldosterone synthase (CYP 11B2) in adrenal gland homogenisates of male mice was not different between C and G mice and was lower in GB mice (aldosterone synthase expression (% of controls): C: $100 \pm 43.9\%$, G: $93.1 \pm 43.3\%$, GB: $35.7 \pm 27.7\%$; C vs. GB $P < 0.05$; $n=6$ /genetic group; Fig. 5).

Discussion

The main finding of our investigation is that aldosterone was increased under conditions of chronic GH excess in

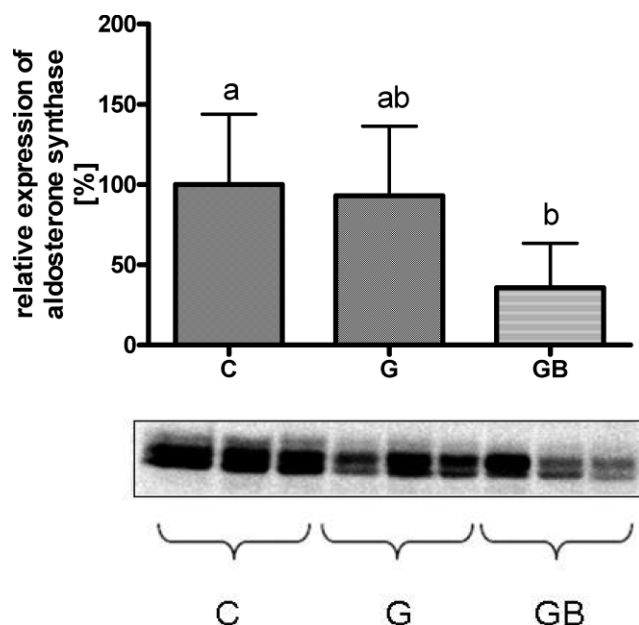


Figure 5. Expression of aldosterone synthase in adrenal gland homogenisates of C, G, and GB mice (male mice, $n = 6$ /genetic group). Data are presented as means $\pm$ SD and are expressed relative to the expression level of controls, which has been set to 100%. Means labeled with different letters are significantly different, whereas “a” vs. “a” or “ab” is not statistically significant. ANOVA analysis and subsequent Bonferroni testing did not reveal significant differences between the genetic groups.

both humans and mice. After successful surgery, aldosterone levels decreased in our group of acromegalic patients in parallel to the normalization of GH secretory status. In male and female GH-overexpressing mice, which also show chronically increased serum GH levels, aldosterone concentrations were manifestly increased compared to control animals. Although we could not identify a precise mechanism by which chronic excess GH increases aldosterone, we were able to exclude a number of likely causes in mice: effects of renin, excess IGF-I, and increased adrenal aldosterone synthase (CYP 11B2) expression.

Literature reports about the effects of GH on aldosterone or on renin plasma levels are highly discrepant. On the one hand, GH administration did not affect the RAAS (25–27). On the other hand, GH administration stimulated the RAAS and aldosterone secretion in healthy subjects (8–30), in children with idiopathic short stature (31), and in hypopituitary adults (32).

There are several explanations for the controversial reports: Very likely, short-term GH administration and chronic GH excess have different effects on the RAAS. Furthermore, GH excess in acromegaly and normalization of GH levels in substitution therapy might differentially affect the RAAS. Finally, and may be most relevant, effects of treatment regimes for both acromegaly and hypertension could have masked the effects of GH on the RAAS. Consequently, to exclude potentially confounding factors and to study directly the impact of GH on aldosterone

concentrations, we investigated a well-characterized, selected group of acromegalic patients. The retrospective design of our study allowed us to select acromegalic patients cured by surgery alone and thus to exclude any effects from medical treatment of acromegaly. However, due to this setting, it was not possible to exclude all effects of antihypertensive medication. Although we excluded patients on mineralocorticoid receptor antagonists, and overall the antihypertensive drugs in the group were unchanged before and after surgery, it might be possible that the antihypertensive drugs influenced aldosterone and renin concentrations in the six patients receiving antihypertensive medication. Investigating this in a prospective setting after a washout period for antihypertensive drugs remains a task for future studies.

Both pre- and post-op aldosterone concentrations were within the method-specific normal range and therefore would not have been considered suspicious in routine clinical diagnostics. Nevertheless, the decrease in aldosterone in cured acromegalic patients might be clinically relevant, as the results from the Framingham Offspring Study show that higher aldosterone levels, even within the physiological range, predispose the development of hypertension (14). Among the many systemic complications of acromegaly in humans (17), hypertension is a common finding. It has been suggested that development of hypertension in acromegalic patients may be, at least in part, a consequence of the chronic exposure to GH and IGF-I excess (33). Accordingly, successful surgical treatment of acromegaly induced a significant reduction of cardiovascular risk factors, including a reduction of the mean 24-hour blood pressure (34). This has also recently been proven in a multicentric observational study in hypertensive acromegalic patients, where control of GH secretory status after successful therapy was associated with marked improvements in blood pressure control (35). Therefore, it might be speculated that reduction of aldosterone post-op could positively influence blood pressure control in cured patients with acromegaly. Furthermore, it should be investigated whether treatment of hypertension with mineralocorticoid antagonists in patients with active excess GH could be of particular benefit. Normalization of GH secretion is thought to ameliorate blood pressure status by reducing GH-induced fluid retention. However, the GH-induced fluid retention should physiologically lead to a suppression of the RAAS, which in turn would lead to lower aldosterone values (27). In contrast, we observed that aldosterone concentrations were significantly higher in acromegalic patients before surgical cure. Therefore, the observed effects of GH on aldosterone concentrations seem to be uncoupled from plasma volume. In our study, GH-induced increases in aldosterone levels were independent of renin concentrations, as renin was not affected by GH excess. This, however, must be interpreted with caution, as variation of renin concentrations was large and our study group was relatively small.

Mice chronically overexpressing GH showed significantly elevated aldosterone concentrations, whereas in non-

transgenic littermates, aldosterone was in the same magnitude as previously published for mice of the same age and genetic background (13). Also, GH transgenic mice, simultaneously overexpressing IGFBP-2 (GB), showed increased serum aldosterone concentrations and aldosterone levels were not different in mice overexpressing GH only. Only very few rodent studies directly investigated the interaction between growth hormone and the RAAS. In one study, GH was injected in a strain of genetically GH-deficient Lewis rats (36), and a stimulatory effect on the renin-angiotensin system was observed, but plasma aldosterone levels were unaffected. In contrast to our transgenic mouse model, in which mice are characterized by supra-physiological GH and IGF-I levels, these GH-injected dwarf rats still had lower IGF-I levels when compared to wild-type Lewis rats. Therefore, it might be speculated that aldosterone is only increased through GH excess above a certain threshold. IGFBP-2 is a presumed inhibitor of IGF-I action *in vitro* (37) and *in vivo* (21, 38) and the *in vivo* inhibition of IGF-I in GB mice resulted in reduced adrenal gland weights, reduced cortical volumes, lower corticosterone concentrations (7), and a smaller volume of the zona glomerulosa (39). However, aldosterone was not affected in GB mice, which indicates that the sole reduction of adrenal gland size or zona glomerulosa, respectively, in GB versus G mice did not lead to reductions in serum aldosterone in our mouse models of GH excess. Furthermore, the increased aldosterone levels seem to be mediated by GH alone and not by IGF-I. Although we analyzed plasma renin activity in female mice only, our results indicate that the increase in aldosterone in G and GB mice is not related to changes in plasma renin activity. A previous study showed an increased mean arterial blood pressure in mice overexpressing GH (40). Although the authors proposed that the hypertensive phenotype is mainly maintained by a narrowing of the resistance vasculature, one might speculate that the increase in aldosterone, which was not measured in that study, could contribute to the hypertensive phenotype. Furthermore, in a rat model of GH excess, it was found that ENaC-mediated sodium transport was increased in the aldosterone-sensitive part of the distal nephron, which could contribute to the sodium retention and thereby also to blood pressure control (41).

Recently, the pathogenesis of hypertension was linked to the RAAS, since polymorphisms of the aldosterone synthase (CYP 11B2) gene were found in acromegalic patients (42). To analyze the interaction between GH and this key enzyme of aldosterone synthesis, we studied protein expression of aldosterone synthase in the adrenal glands of transgenic and non-transgenic mice. However, expression of aldosterone synthase was not different between controls and GH transgenic mice and the low expression in GB mice deserves further investigation. This was unexpected, since it has recently been shown that acute regulation of aldosterone synthesis is accompanied by fast transcriptional modulation of steroidogenic enzymes, including aldosterone synthase (43). On the one hand, we might speculate that excess GH

could influence preceding enzymes of the aldosterone synthesis pathway like steroidogenic acute regulatory protein (StAR) or P450 side chain cleavage enzyme. In addition, the chronicity of excess GH might override the acute regulatory axis of aldosterone synthesis, potentially explaining why we did not find changes in aldosterone synthase expression. On the other hand, aldosterone synthase expression has also been found in a number of extra-adrenal tissues (44, 45); thus, excess GH could possibly affect extra-adrenal aldosterone production.

In conclusion, we have shown that in both humans and mice, chronic GH excess is associated with increased aldosterone levels, which is likely to be independent of systemic renin secretion. The clear association between GH secretion and aldosterone might have been detected in our study because potentially confounding factors have been minimized by the very strict inclusion criteria. In mice, this effect seems to be mediated by GH and not through IGF-I. Although the increased serum GH levels in GH overexpressing mice have a different aetiology than those seen in the acromegalic patients, the transgenic mice might be a helpful model to study the relation between GH excess and serum aldosterone and to investigate the underlying mechanisms.

The impact of chronic GH excess upon aldosterone secretion potentially contributes to the development of hypertension in acromegaly. Furthermore, given the direct effects of aldosterone on the heart, this might also aggravate cardiovascular risk profile in these patients. By reducing circulating aldosterone, rapid cure in acromegaly might help to protect the patients from cardiovascular complications. In turn, it should be investigated if patients with acromegaly might particularly benefit from mineralocorticoid antagonist treatment.

1. Palmiter RD, Norstedt G, Gelinas RE, Hammer RE, Brinster RL. Metallothionein-human GH fusion genes stimulate growth of mice. *Science* 222:809–814, 1983.
2. Lichanska AM, Waters MJ. How growth hormone controls growth, obesity and sexual dimorphism. *Trends Genet* 24:41–47, 2008.
3. Lupu F, Terwilliger JD, Lee K, Segre GV, Efstratiadis A. Roles of growth hormone and insulin-like growth factor 1 in mouse postnatal growth. *Dev Biol* 229:141–162, 2001.
4. Davidson MB. Effect of growth hormone on carbohydrate and lipid metabolism. *Endocr Rev* 8:115–131, 1987.
5. Butler AA, Le Roith D. Control of growth by the somatotropic axis: Growth hormone and the insulin-like growth factors have related and independent roles. *Annu Rev Physiol* 63:141–164, 2001.
6. Weber MM, Fottner C, Schmidt P, Brodowski KM, Gittner K, Lahm H, Engelhardt D, Wolf E. Postnatal overexpression of insulin-like growth factor II in transgenic mice is associated with adrenocortical hyperplasia and enhanced steroidogenesis. *Endocrinology* 140:1537–1543, 1999.
7. Hoefflich A, Weber MM, Fisch T, Nedbal S, Fottner C, Elmlinger MW, Wanke R, Wolf E. Insulin-like growth factor binding protein 2 (IGFBP-2) separates hypertrophic and hyperplastic effects of growth hormone (GH)/IGF-I excess on adrenocortical cells *in vivo*. *FASEB J* 16:1721–1731, 2002.
8. Coulter CL, Goldsmith PC, Mesiano S, Voytek CC, Martin MC, Han

- VK, Jaffe RB. Functional maturation of the primate fetal adrenal in vivo: I. Role of insulin-like growth factors (IGFs), IGF-I receptor, and IGF binding proteins in growth regulation. *Endocrinology* 137:4487–4498, 1996.
9. Agha A, Monson JP. Modulation of glucocorticoid metabolism by the growth hormone–IGF-I axis. *Clin Endocrinol (Oxf)* 66:459–465, 2007.
10. Williams GH. Aldosterone biosynthesis, regulation, and classical mechanism of action. *Heart Fail Rev* 10:7–13, 2005.
11. Seely EW, Conlin PR, Brent GA, Dluhy RG. Adrenocorticotropin stimulation of aldosterone: Prolonged continuous versus pulsatile infusion. *J Clin Endocrinol Metab* 69:1028–1032, 1989.
12. Williams GH. Cardiovascular benefits of aldosterone receptor antagonists: What about potassium? *Hypertension* 46:265–266, 2005.
13. Manolopoulou J, Bielohuby M, Caton SJ, Gomez-Sanchez CE, Renner-Mueller I, Wolf E, Lichtenauer UD, Beuschlein F, Hoefflich A, Bidlingmaier M. A highly sensitive immunofluorometric assay for the measurement of aldosterone in small sample volumes: Validation in mouse serum. *J Endocrinol* 196:215–224, 2008.
14. Vasan RS, Evans JC, Larson MG, Wilson PW, Meigs JB, Rifai N, Benjamin EJ, Levy D. Serum aldosterone and the incidence of hypertension in nonhypertensive persons. *N Engl J Med* 351:33–41, 2004.
15. Melmed S. Acromegaly. *N Engl J Med* 322:966–977, 1990.
16. Lombardi G, Galdiero M, Auriemma RS, Pivonello R, Colao A. Acromegaly and the cardiovascular system. *Neuroendocrinology* 83: 211–217, 2006.
17. Colao A, Ferone D, Marzullo P, Lombardi G. Systemic complications of acromegaly: Epidemiology, pathogenesis, and management. *Endocr Rev* 25:102–152, 2004.
18. Strauch G, Vallotton MB, Touitou Y, Bricaire H. The renin-angiotensin-aldosterone system in normotensive and hypertensive patients with acromegaly. *N Engl J Med* 287:795–799, 1972.
19. Lim NY, Dingman JF. Androgenic adrenal hyperfunction in acromegaly. *N Engl J Med* 271:1189–1194, 1964.
20. Nicklas W, Baneux P, Boot R, Decelle T, Deeny AA, Fumanelli M, Illgen-Wilcke B. Recommendations for the health monitoring of rodent and rabbit colonies in breeding and experimental units. *Lab Anim* 36: 20–42, 2002.
21. Hoefflich A, Wu M, Mohan S, Foll J, Wanke R, Froehlich T, Arnold GJ, Lahm H, Kolb HJ, Wolf E. Overexpression of insulin-like growth factor-binding protein-2 in transgenic mice reduces postnatal body weight gain. *Endocrinology* 140:5488–5496, 1999.
22. Hoefflich A, Nedbal S, Blum WF, Erhard M, Lahm H, Brem G, Kolb HJ, Wanke R, Wolf E. Growth inhibition in giant growth hormone transgenic mice by overexpression of insulin-like growth factor-binding protein-2. *Endocrinology* 142:1889–1898, 2001.
23. Elmlinger MW, Kuhnel W, Weber MM, Ranke MB. Reference ranges for two automated chemiluminescent assays for serum insulin-like growth factor I (IGF-I) and IGF-binding protein 3 (IGFBP-3). *Clin Chem Lab Med* 42:654–664, 2004.
24. Castrop H, Lorenz JN, Hansen PB, Friis U, Mizel D, Oppermann M, Jensen BL, Briggs J, Skott O, Schnermann J. Contribution of the basolateral isoform of the Na-K-2Cl⁻ cotransporter (NKCC1/BSC2) to renin secretion. *Am J Physiol Renal Physiol* 289:F1185–F1192, 2005.
25. Moller J, Jensen MB, Frandsen E, Moller N, Kissmeyer P, Laurberg S. Growth hormone treatment improves body fluid distribution in patients undergoing elective abdominal surgery. *Clin Endocrinol (Oxf)* 49:597–602, 1998.
26. Hoffman DM, Crampton L, Sernia C, Nguyen TV, Ho KK. Short-term growth hormone (GH) treatment of GH-deficient adults increases body sodium and extracellular water, but not blood pressure. *J Clin Endocrinol Metab* 81:1123–1128, 1996.
27. Ekman B, Ohman PK, Arnqvist HJ, Lindstrom T, Nystrom FH. Individualized growth hormone substitution with normalized IGF-I levels does not stimulate the renin-angiotensin-aldosterone system. *Clin Endocrinol (Oxf)* 57:473–479, 2002.
28. Lampit M, Nave T, Hochberg Z. Water and sodium retention during short-term administration of growth hormone to short normal children. *Horm Res* 50:83–88, 1998.
29. Ho KY, Weissberger AJ. The antinatriuretic action of biosynthetic human growth hormone in man involves activation of the renin-angiotensin system. *Metabolism* 39:133–137, 1990.
30. Hansen TK, Moller J, Thomsen K, Frandsen E, Dall R, Jorgensen JO, Christiansen JS. Effects of growth hormone on renal tubular handling of sodium in healthy humans. *Am J Physiol Endocrinol Metab* 281: E1326–E1332, 2001.
31. Hanukoglu A, Belutserkovsky O, Phillip M. Growth hormone activates renin-aldosterone system in children with idiopathic short stature and in a pseudohypoadosteronism patient with a mutation in epithelial sodium channel alpha subunit. *J Steroid Biochem Mol Biol* 77:49–57, 2001.
32. Weaver JU, Thaventhiran L, Noonan K, Burrin JM, Taylor NF, Norman MR, Monson JP. The effect of growth hormone replacement on cortisol metabolism and glucocorticoid sensitivity in hypopituitary adults. *Clin Endocrinol (Oxf)* 41:639–648, 1994.
33. Bondanelli M, Ambrosio MR, degli Uberti EC. Pathogenesis and prevalence of hypertension in acromegaly. *Pituitary* 4:239–249, 2001.
34. Jaffrain-Rea ML, Minniti G, Moroni C, Esposito V, Ferretti E, Santoro A, Infusino T, Tamburrano G, Cantore G, Cassone R. Impact of successful transsphenoidal surgery on cardiovascular risk factors in acromegaly. *Eur J Endocrinol* 148:193–201, 2003.
35. Colao A, Terzolo M, Bondanelli M, Galderisi M, Vitale G, Reimondo G, Ambrosio MR, Pivonello R, Lombardi G, Angeli A, Degli Uberti EC. GH and IGF-I excess control contributes to blood pressure control: Results of an observational, retrospective, multicentre study in 105 hypertensive acromegalic patients on hypertensive treatment. *Clin Endocrinol (Oxf)*, 2008.
36. Wyse B, Waters M, Sernia C. Stimulation of the renin-angiotensin system by growth hormone in Lewis dwarf rats. *Am J Physiol* 265(2 Pt 1):E332–E339, 1993.
37. Wolf E, Lahm H, Wu M, Wanke R, Hoefflich A. Effects of IGFBP-2 overexpression in vitro and in vivo. *Pediatr Nephrol* 14:572–578, 2000.
38. Wolf E, Jehle PM, Weber MM, Sauerwein H, Daxenberger A, Breier BH, Besenfelder U, Frenyo L, Brem G. Human insulin-like growth factor I (IGF-I) produced in the mammary glands of transgenic rabbits: Yield, receptor binding, mitogenic activity, and effects on IGF-binding proteins. *Endocrinology* 138:307–313, 1997.
39. Fisch T. Effects of insulin-like growth factor binding protein-2 (IGFBP-2) overexpression on adrenal and renal growth processes and functions: Findings in transgenic mouse models. Doctoral thesis, Institute of Veterinary Pathology. Ludwig-Maximilians University, Munich, 2004.
40. Bohlooly YM, Carlson L, Olsson B, Gustafsson H, Andersson IJ, Tornell J, Bergstrom G. Vascular function and blood pressure in GH transgenic mice. *Endocrinology* 142:3317–3323, 2001.
41. Kamenicky P, Viengchareun S, Blanchard A, Meduri G, Zizzari P, Imbert-Teboul M, Doucet A, Chanson P, Lommes M. Epithelial sodium channel is a key mediator of growth hormone-induced sodium retention in acromegaly. *Endocrinology* 149:3294–3305, 2008.
42. Mulatero P, Veglio F, Maffei P, Bondanelli M, Bovio S, Daffara F, Leotta G, Angeli A, Calvo C, Martini C, Degli Uberti EC, Terzolo M. CYP11B2 –344T/C gene polymorphism and blood pressure in patients with acromegaly. *J Clin Endocrinol Metab* 91:5008–5012, 2006.
43. Spyroglou A, Manolopoulou J, Wagner S, Bidlingmaier M, Reincke M, Beuschlein F. Short term regulation of aldosterone secretion after stimulation and suppression experiments in mice. *J Mol Endocrinol*, 2009.
44. Davies E, MacKenzie SM. Extra-adrenal production of corticosteroids. *Clin Exp Pharmacol Physiol* 30:437–445, 2003.
45. Ye P, Kenyon CJ, Mackenzie SM, Nichol K, Seckl JR, Fraser R, Connell JM, Davies E. Effects of ACTH, dexamethasone, and adrenalectomy on 11 β -hydroxylase (CYP11B1) and aldosterone synthase (CYP11B2) gene expression in the rat central nervous system. *J Endocrinol* 196:305–311, 2008.