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Independent and Combined Effects of Testosterone and Growth Hormone on Extracellular Water in Hypopituitary Men

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Context: Symptoms of fluid retention in GH-deficient patients during GH replacement are greater in men than in women, suggesting that testosterone may augment or estradiol may attenuate the antinatriuretic actions of GH. The mechanisms underlying the sodium-retaining effects of GH are poorly understood.

Aim: The aim of this study was to investigate the effects of GH and testosterone, alone and in combination, on extracellular water (ECW) and the hormonal mechanisms involved.

Design: Two separate, open-label, randomized, two-period, crossover studies were performed; the first compared the effects of GH alone with those of GH and testosterone, and the second compared the effects of testosterone alone with those of GH and testosterone.

Participants: Twelve hypopituitary men with GH deficiency and hypogonadism were studied.

Intervention: During the weeks of intervention, GH (0.5 mg/d) and testosterone enanthate (250 mg) were administered by im injection.

Outcome Measures: The outcome measures were ECW, IGF-I, plasma renin activity (PRA), aldosterone (Aldo), and atrial natriuretic peptide (ANP).

Results: GH treatment significantly increased ($P < 0.05$) both IGF-I and ECW, and these changes were enhanced by cotreatment with testosterone ($P = 0.07$ for both). PRA, Aldo, and ANP levels did not change. Testosterone treatment alone did not change the IGF-I concentration, whereas cotreatment with GH induced a marked increase. Testosterone alone increased ($P < 0.05$) ECW, and the effect was augmented ($P < 0.01$) by cotreatment with GH. Although PRA and ANP did not change, plasma Aldo decreased after single and combined treatments.

Conclusion: GH and testosterone exerted independent and additive effects on ECW. The mechanisms of fluid retention for both hormones are likely to be exerted on the renal tubules. This is the first direct evidence that testosterone increases ECW. **(*J Clin Endocrinol Metab* 90: 3989–3994, 2005)**

GENDER-DEPENDENT CHANGES in body composition appear at the time of puberty (1), suggesting an important role of sex steroids. Total body water and fat-free mass are greater, whereas total body fat is lower, in men than in women. The proportion of fat-free mass accounted for by water and the proportion of body weight accounted for by extracellular water (ECW) are also greater in men (2). The water content of body cell mass and ECW is highly consistent between subjects and even between mammals, reflecting strong basic regulatory mechanisms controlled by hormones (3), including insulin, catecholamines, those of the renin-angiotensin-aldosterone system (RAAS), and natriuretic peptides.

The antinatriuretic effect of GH has been recognized for decades (4). The pathophysiological importance of this effect is evident from studies demonstrating reduced and increased ECW in adults with GH deficiency and acromegaly, respectively (4–6). Although the mechanistic processes underlying the antinatriuretic action of GH have not been fully elucidated, several have been proposed. The observations that GH increases serum and tissue levels of IGF-I, and that both GH and IGF-I receptors are expressed in renal tubules (7) have suggested that both hormones could play a role in the fluid retention (8). Evidence that the effect of GH is exerted indirectly is conflicting, with some (9–12), but not all (13), studies demonstrating activation of RAAS; some (14–16), but not all (13, 17), researchers have reported that GH may act through suppression of plasma atrial natriuretic peptide (ANP). Although the exact mechanisms remain unclear, these studies indicate that the GH/IGF-I axis plays an important role in sodium-fluid homeostasis.

The involvement of testosterone in sodium homeostasis is less clear. Some evidence comes from early clinical observations of edema developing during treatment with supraphysiological doses of testosterone for anemia secondary to hematological disorders and uremia (18). High doses of testosterone have been reported to increase, decrease, or exert no effect on plasma volume (19, 20) and to reduce urinary sodium excretion (18, 21). Furthermore, testosterone stimulates GH secretion (22) through an estrogen-dependent pathway (23, 24), making it possible that high doses of testosterone in GH-replete subjects may induce sodium and water retention indirectly through the actions of GH and IGF-I.

Evidence that sex steroids influence GH action came from the observation that fluid retention is more marked in men

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Abbreviations: Aldo, Aldosterone; ANP, atrial natriuretic peptide; CV, coefficient of variation; ECW, extracellular water; PRA, plasma renin activity; RAAS, renin-angiotensin-aldosterone system.

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than in women during GH replacement therapy (25). The aim was to study the effects of GH and testosterone, alone and in combination, on ECW. To eliminate possible indirect actions of testosterone through the GH/IGF-I axis, subjects with combined hypogonadotropic hypogonadism and GH deficiency were studied.

Patients and Methods

Patients

Twelve hypopituitary men with GH deficiency and hypogonadotropic hypogonadism were recruited from the Endocrine Outpatient Clinic at St. Vincent's Hospital (Sydney, Australia). The clinical characteristics of these patients are shown in Table 1. Two studies were undertaken, the first compared the effects of GH alone with those of GH and testosterone in 10 men, and the second compared the effects of testosterone alone with those of GH and testosterone in nine men. Seven of the 12 men participated in both studies. GH deficiency was confirmed by a peak GH response to insulin-induced hypoglycemia of less than 3 ng/ml (26), and hypogonadotropic hypogonadism was defined as a serum testosterone level measured in a morning sample less than 4 nmol/liter accompanied by a low serum LH level. The duration of hypopituitarism was at least 1 yr. All subjects were receiving stable hormone replacement for other deficiencies throughout and in between the study periods, and they were not receiving any other treatment that could affect their body fluid homeostasis. All subjects gave their written informed consent to participate in the study before entering the protocol. The study design was approved by the research ethics committee of St. Vincent's Hospital.

Study design

Both studies were of open-label, randomized, crossover design, and together allowed comparison of the individual and combined effects of testosterone and GH while taking time-dependent effects into consideration. Before commencement of each study, subjects underwent a 6-wk run-in period when testosterone and GH were withdrawn. During this time and throughout the studies, they were instructed to follow their usual diet and habitual activities. In both studies, GH (Humatrope, Lilly Australia, Sydney, Australia) was administered at a dose of 1.5 IU/d (0.5 mg/d) daily, sc, by self-injection at 2000 h, and testosterone enanthate was administered at a dose of 250 mg, im, 2 wk before measurement.

The first study compared the effects of GH alone with those of combined GH and testosterone treatment in 10 subjects (Fig. 1). GH was administered daily for 6 wk. Testosterone was administered either at baseline (group A; n = 5) or wk 4 (group B; n = 5) of the study. Investigations were carried out at baseline, after 2 wk, and after 6 wk, so that the effects of testosterone were assessed 2 wk after administration. Thus, studies were carried out when subjects were not receiving GH or testosterone replacement, during replacement with GH alone, and during combined GH and testosterone replacement (Fig. 1).

The second study compared the effects of testosterone with those of combined testosterone and GH treatments in nine subjects (Fig. 1). Testosterone was administered at wk 2 and 6 of the study. GH was administered either during the first 4 wk (group A; n = 5) or the second 4 wk (group B; n = 4) of the study. Investigations were carried out at baseline, after 4 wk, and after 8 wk. Thus, studies were performed when subjects were not receiving GH or testosterone replacement, during replacement with testosterone alone, and during combined GH and testosterone replacement (Fig. 1).

At each visit and after an overnight fast, all subjects underwent measurements of plasma IGF-I, testosterone, plasma renin activity (PRA), aldosterone (Aldo), ANP, and ECW using the bromide dilution technique. For the latter, a carefully weighed amount of sodium bromide (1.8–2.0 g) was diluted in 60 ml sterile water, of which 10 ml were injected. Serum samples were collected before and 4 h after the injection. All serum/plasma samples were frozen after collection and assayed in a single run at the end of both study periods. Body weight was measured barefoot wearing indoor clothing to the nearest 0.1 kg, and systolic and diastolic blood pressures were measured after 5 min of supine rest using a sphygmomanometer.

TABLE 1. Clinical characteristics of the participating men with hypopituitarism

Subject no. (study 1 or 2)	Age (yr)	Cause of GHD	Treatment	Hormone replacement
1 (1&2)	66	Pituitary macroadenoma	S/X	A, T, G
2 (1&2)	61	Rathke's cyst	S	A, T, G, D
3 (1&2)	50	Pituitary macroadenoma	S	G
4 (2)	60	Pituitary macroadenoma	S	G
5 (2)	50	Craniopharyngioma	S/X	A, T, G
6 (1&2)	66	Pituitary macroadenoma	S	A, T, G
7 (1&2)	46	Pituitary macroadenoma	S	A, T, G
8 (1&2)	47	Pituitary macroadenoma	S	A, T, G
9 (1)	77	Pituitary macroadenoma	S	G
10 (1)	33	Craniopharyngioma	S/X	A; T, G
11 (1)	39	Pituitary macroadenoma	S	T, G, D
12 (1&2)	66	Pituitary macroadenoma	S	T, G

S, Surgery; X, irradiation; A, adrenal replacement; T, thyroid replacement; G, gonadal replacement; GHD, GH deficiency; D, deamino-8-D-arginine vasopressin.

Assays

Serum IGF-I was measured by RIA after acid-ethanol extraction as previously described (27), with intraassay coefficients of variation (CVs) of 9.4, 8.3, and 10.3% at 48, 254, and 1510 μg/liter, respectively. Testosterone was measured by a solid-phase chemiluminescent enzyme immunoassay (Immulite 2000, Diagnostic Products Corp., Los Angeles, CA) that had an intraassay CV of 9.6% at 6.7 nmol/liter. PRA was measured indirectly by RIA of angiotensin I (PerkinElmer Life Sciences, Los Angeles, CA). The level of endogenous angiotensin I was corrected by running an inhibited assay tube kept at 4 C while the 37 C generation of angiotensin I was occurring. Renin activity was maximized by preincubating the samples at pH 6.0. Plasma Aldo was measured by a solid phase immunoassay (Coat-A-Count TKAL2, Diagnostic Products Corp.) that has an interassay precision of 6.2%. Plasma samples for ANP measurement was stored at −70 C before assay using a solid-phase immunoradiometric assay (Shionogi & Co. Ltd., Osaka, Japan) with an intraassay CV of 6.3% at 18.9 ng/liter and a detection limit of 2.5 ng/liter.

Serum bromide was measured by HPLC. The serum samples were deproteinated by centrifugation through a filtration unit with cut-off size of 10 kDa (Amicon YM10, Millipore Corp., Bedford, MA). The protein-free ultrafiltrate was run through an anion exchange column (IC-Pak A, Waters Corp., Milford, MA) at a flow rate of 0.35 ml/min and a detection wavelength of 195 nm. The bromide concentration was determined using the area under the curve and comparing with known bromide standards. The intraassay CV for the bromide concentration determined by HPLC was 2.1%. ECW was calculated from the change in the serum bromide concentration 4 h after injection of a known amount of bromide using the formula reported by Miller *et al.* (2): ECW (liters) = $0.9 \times 0.95 \times$ Br dose (mmol)/Δ Br serum (mmol/liter), where Δ Br serum is the change in serum bromide concentration, 0.9 is the correction factor for nonextracellular distribution of bromide, and 0.95 is the correction factor for Donnan equilibrium. The mean day to day intrasubject CV for ECW based on four subjects studied on two occasions was 5.7%.

Statistical analysis

Data are presented as the mean ± SEM. The overall within-group treatment effect in each trial was determined using Friedman's ANOVA for repeated measurements. *Post hoc* analysis was performed using Wilcoxon's matched pairs, signed rank-sum test corrected for the number of measurements. The analysis of between-study levels was performed using the Mann-Whitney *U* test. An analysis of interactions between treatment and sequence was performed in which the direct and residual effects of treatment were measured separately to detect carryover effects. No such interaction was found. Statistical significance was considered at $P \leq 0.05$.

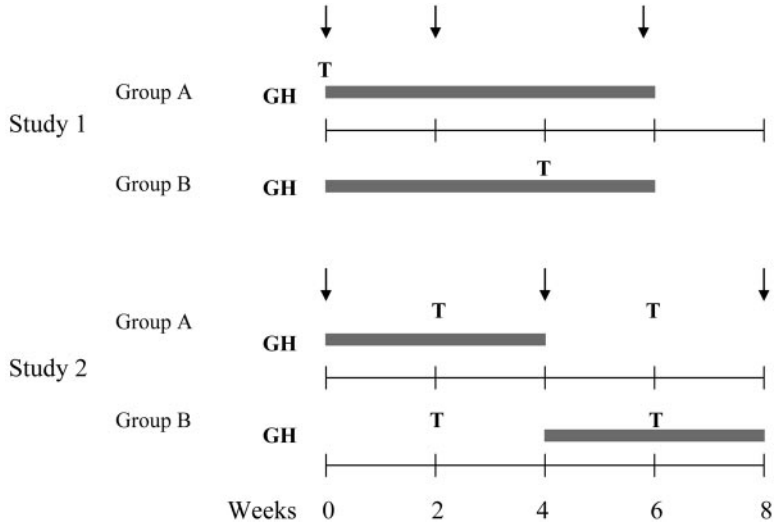

FIG. 1. Study 1 compares the effects of GH alone with combined treatment with GH and testosterone (T). Ten patients were randomized to either group A or group B. T was administered as 250 mg testosterone enanthate, im, at wk 0 in group A and wk 4 in group B. GH was administered daily at bedtime by sc injections at a dose of 1.5 IU for 6 wk. Study 2 compares T alone with combined treatment with T and GH. Nine patients were randomized to either group A or B. The same doses of GH and T were administered as in study 1. *Arrows* indicate the times of measurements. ■, Period of GH treatment. T, Time of testosterone administration.

Results

None of the patients reported side effects, and no clinical symptoms or signs associated with excess fluid retention were observed during treatment with the doses and regimen used for GH, testosterone, or their combination. No sequence effect was observed in either of the studies. Baseline values were comparable in the two studies, with the exception of IGF-I, which was slightly higher in study 2 (11.9 ± 1.1 *vs.* 8.4 ± 0.9 nmol/liter in study 1; $P < 0.05$).

GH and combined testosterone treatment (Table 2)

The serum IGF-I concentration increased in response to GH treatment alone and increased further when testosterone was added ($P = 0.07$), but the difference was not statistically significant. Serum testosterone concentrations were subnormal at baseline and during the GH treatment phase, and increased into the normal range during testosterone replacement therapy.

Mean body weight increased slightly during GH treatment and combined treatment, although the changes were not statistically significant. GH treatment alone increased ECW significantly by 2.4 ± 0.9 liters ($P < 0.05$), and combined treatment with GH and T induced an additional expansion of ECW by 1.2 ± 0.7 liters, which approached statistical significance ($P = 0.07$; Fig. 2A). Systolic and diastolic blood pressures were unaffected by single or combined treatments. No significant changes were seen in the plasma concentrations of Aldo, PRA, ANP, or the Aldo/PRA ratio.

Testosterone and combined GH treatment (Table 3)

Testosterone treatment alone did not change the mean serum IGF-I concentration, whereas adding GH to the treatment induced an increase into the normal range. Serum testosterone concentrations were low at baseline and increased into the normal range after the two treatment phases of testosterone alone and combined GH and testosterone.

A significant ($P < 0.05$) increase in body weight occurred after treatment with testosterone alone and combined treatment with GH. Mean body weight was slightly higher during combined treatment, but this did not reach statistical significance. ECW increased significantly by 2.0 ± 0.8 liter ($P < 0.05$) after testosterone treatment alone, and combined treatment with GH caused an additional increase of 2.3 ± 0.5 liters ($P = 0.006$; Fig. 2B). Blood pressure was unchanged throughout this part of the study. The plasma Aldo concentration decreased significantly ($P < 0.05$) in response to both testosterone alone and combined testosterone and GH treat-

TABLE 2. GH and GH plus testosterone (T) treatment in men with hypopituitarism (n = 10)

Measure	Baseline	GH	GH + T	*P*
IGF-I (nmol/liter)	8.4 ± 0.9	28.4 ± 3.2[a]	30.1 ± 2.9[a,b]	<0.001
T (mmol/liter)	2.6 ± 0.6	1.8 ± 0.7	12.2 ± 1.9[c]	0.01
Body weight (kg)	83.8 ± 5.3	84.3 ± 5.3	84.4 ± 5.3	0.3
ECW (kg)	15.6 ± 0.9	18.0 ± 1.2[a]	19.2 ± 1.4[a,b]	0.007
Systolic BP (mm Hg)	146 ± 9	144 ± 7	143 ± 7	0.5
Diastolic BP (mm Hg)	84 ± 4	85 ± 4	82 ± 3	0.9
PRA (fmol AI/liter·sec)	336 ± 83	391 ± 127	383 ± 106	0.2
Aldo (pmol/liter)	288 ± 64	258 ± 64	260 ± 76	0.7
Aldo/PRA ratio	0.90 ± 0.16	0.78 ± 0.16	0.73 ± 0.20	0.4
ANP (ng/liter)	4.80 ± 1.22	4.61 ± 1.91	4.39 ± 1.19	0.9

Significance was determined by Friedman ANOVA, followed by Wilcoxon matched pairs test. Normative values: Aldo, 80–1000 pmol/liter; PRA, 800–2100 fmol/liter·sec (divided by 214.2 = ng AI/liter·min); Aldo/PRA ratio, 0.4–1.6. AI, Angiotensin I; BP, blood pressure.

[a] $P < 0.05$ compared with baseline.

[b] $P < 0.07$ compared with GH alone.

[c] $P < 0.05$ compared with GH alone.

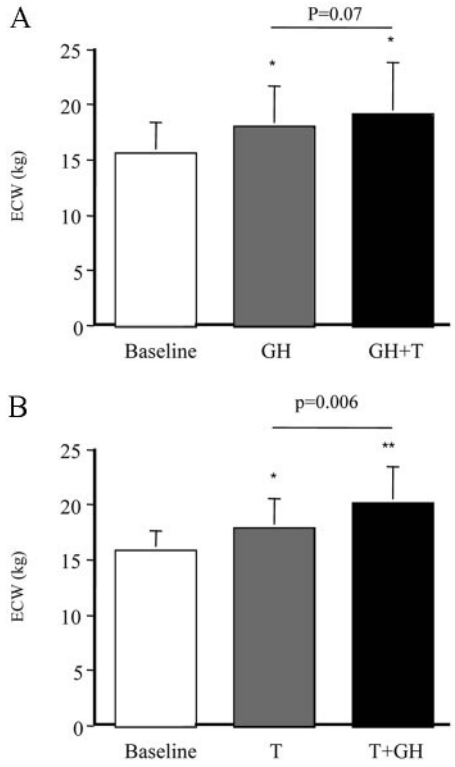

FIG. 2. Mean (±SE) ECW (kilograms) during study 1, comparing GH alone with combined treatment with GH and T (A), and during study 2, comparing T treatment alone with combined T and GH treatment (B). *, $P < 0.05$; and **, $P < 0.01$, compared with baseline values.

ment. No changes occurred in PRA, the Aldo/PRA ratio, or the serum ANP concentration.

Between-study comparisons

The average gain in body weight during testosterone treatment alone in study 2 exceeded the change in body weight during GH treatment alone in study 1 (1.5 ± 0.4 *vs.* 0.5 ± 0.3 kg; $P < 0.05$). There were no significant differences in the gain in ECW induced by GH and testosterone, nor were there differences between the gain in ECW induced by combined GH and testosterone treatment between the two studies. No significant correlations were found between changes in or levels of IGF-I and ECW during the different treatment periods.

Discussion

These two randomized, controlled trials have examined the individual and combined effects of GH and testosterone on ECW. The principal novel findings were that testosterone alone significantly increased ECW and amplified the GH-induced increase in ECW. By limiting the study to male subjects with combined severe GH deficiency and hypogonadotropic hypogonadism, the potentially confounding influence of stimulation of pituitary GH release by testosterone was avoided.

The GH-induced increase in ECW has been consistently reported and probably explains the dose-dependent symptoms related to fluid retention that commonly accompany GH replacement in adults (28, 29). Only one study has quantified changes in ECW using doses considered appropriate for replacement therapy in adults (16). After 1 wk of treatment, PRA, but not Aldo, levels increased, whereas after 12 months of treatment, the levels of both were not different from baseline. In the present study, 2–6 wk of GH treatment increased ECW without significantly affecting PRA or the concentration of Aldo. These findings contrast with those of previous shorter trials, which found stimulation of the RAAS in normal subjects after treatment with supraphysiological dose of GH, approximating two to three times that used in the present study (11, 12). The collective observations suggest that during physiological GH replacement, the sustained increase in ECW is unlikely to be mediated through the RAAS (16). This does not, however, rule out a role of RAAS in the early sodium-retaining effect of GH that peaks 1–3 d after commencing GH treatment (30).

This is the first controlled study demonstrating that testosterone increases ECW. Previous data concerning the effects of testosterone on plasma volume (19, 20) and urinary sodium excretion (18, 21) are limited and conflicting. The underlying mechanism is unknown, but several possibilities exist. Testosterone could act directly on the kidney, because androgen receptors are expressed in renal tubules (31). There is evidence that androgens stimulate the expression of the angiotensinogen gene in the kidney (32, 33). Therefore, androgens could activate the local renal RAAS to stimulate

TABLE 3. Testosterone (T) and combined T and GH treatment in men with hypopituitarism (n = 9)

Measure	Baseline	T	T + GH	*P*
IGF-I (nmol/liter)	11.9 ± 1.1	11.9 ± 1.1	37.5 ± 4.3[a,b]	<0.001
Testosterone (mmol/liter)	2.0 ± 0.5	14.8 ± 1.9[a]	14.0 ± 2.9[a]	0.002
Body weight (kg)	89.0 ± 5.7	90.5 ± 5.7[a]	90.8 ± 5.9[a]	0.01
ECW (kg)	15.9 ± 0.6	17.9 ± 0.9[a]	20.2 ± 1.1[a,b]	<0.001
Systolic BP (mm Hg)	139 ± 8	136 ± 5	139 ± 6	0.8
Diastolic BP (mm Hg)	87 ± 4	86 ± 4	86 ± 4	0.8
PRA (fmol AI/liter·sec)	334 ± 70	281 ± 86	339 ± 133	0.5
Aldosterone (pmol/liter)	313 ± 63	226 ± 58[a]	226 ± 59[a]	0.02
Aldo/PRA ratio	0.93 ± 0.20	0.86 ± 0.25	0.71 ± 0.19	0.5
ANP (ng/liter)	6.03 ± 1.40	6.18 ± 1.65	4.35 ± 1.52	0.9

Significance was determined by Friedman ANOVA, followed by Wilcoxon matched pairs test. Normative values: Aldo, 80–1000 pmol/liter; PRA, 800–2100 fmol/liter·sec (divided by 214.2 = ng AI/liter·min); Aldo/PRA ratio, 0.4–1.6. AI, Angiotensin I; BP, blood pressure.

[a] $P < 0.05$ compared with baseline.

[b] $P < 0.05$ compared with T alone.

sodium and water retention through an autocrine or paracrine mechanism (34). The epithelial sodium channel plays an important role in the sodium balance, as demonstrated by genetic abnormalities in its activity, such as in Liddle's syndrome (35). It has recently been reported that androgens increase mRNA expression of the α-subunit of the epithelial sodium channel in a human renal cell line (36), providing a potential mechanism of sodium and water retention by testosterone.

Adults with GH deficiency have reduced ECW whether adjusted for lean body mass or expressed as ECW/total body water ratio (13, 37). After 4 wk of GH treatment of adult GH deficiency patients, the level of ECW and the ECW/total body water ratio was normalized without any significant change in intracellular water (37). With continuing therapy, no additional increase was noted in ECW, whereas there was an increase in intracellular water, suggesting a preferential increase in body cell mass (37). These data, based on bromide, deuterium, and sodium dilution techniques, indicate that most of the initial changes in lean body mass in response to GH are related to changes in ECW.

Plasma Aldo levels fell significantly during testosterone treatment, whereas a modest fall, which failed to reach significance, occurred during GH treatment. During combined treatments, a significant fall in Aldo was also observed. The uniform trend toward a fall in Aldo levels observed with single and combined treatments suggests an adaptive response to ECW expansion. The observation that the fall in Aldo was greater in the presence of testosterone suggests that additional androgen-mediated mechanisms are probably involved. Androgen receptors have been identified in human adrenocortical cells and appear to exert an inhibitory influence. *In vitro* studies have demonstrated that testosterone reduced the proliferation of human adrenal adenoma and adrenocortical cancer cell lines (38). It is possible that testosterone directly suppresses Aldo biosynthesis or secretion, but this remains to be demonstrated.

The effects of testosterone on the volume and distribution of ECW could theoretically occur secondary to aromatization to estrogen in peripheral tissues. Estrogen may cause fluid retention through reduction of the plasma antidiuretic hormone (arginine vasopressin)-plasma osmolality set point (39, 40) or stimulating the synthesis of hepatic angiotensinogen (41), enhancing the overall activity of RAAS and leading to sodium retention. However, this postulate is not supported by the observation that urinary sodium excretion is increased during oral contraceptive use (42) or that the plasma renin concentration is reduced in women receiving estrogen treatment (43). Moreover, estrogen reduces the plasma renin concentration, the activity of angiotensin-converting enzyme, and the Aldo response to angiotensin II (44, 45). These actions of estrogen putatively generated from aromatization of androgens could explain the slight reduction in plasma Aldo levels in response to testosterone in our study.

In hypophysectomized, castrated, male rats, testosterone administration does not increase serum levels of IGF-I or IGF-I gene expression in the liver (46). However, androgen-dependent body hair growth is increased during GH replacement in men receiving stable testosterone treatment (47), suggesting that tissue sensitivity to testosterone in humans is enhanced by GH action. This is supported by the present findings that testosterone enhanced the effect of GH on IGF-I production. Because IGF-I itself stimulates sodium retention (48), it is possible that the greater increase in ECW during combined treatment compared with GH alone may have been IGF-I mediated. However, this cannot explain the increase in ECW in response to testosterone alone, because there was no change in the serum IGF-I concentration. This observation provides additional support for the idea that testosterone may exert direct effects on the renal tubules to induce a sodium-sparing effect.

Few data exist concerning the influence of testosterone on ANP and brain natriuretic peptide. These peptides are characterized by natriuretic, diuretic, and vasodilatory activities (49) and are stimulated by conditions of intravascular volume expansion. Short-term testosterone administration to elderly men increased the plasma concentration of ANP (50), possibly as a compensatory mechanism to testosterone-induced fluid load or a direct effect of testosterone. In the present study, ANP was not affected by either testosterone or GH, indicating that it is unlikely to be a major mediator of fluid retention induced by these two hormones.

In conclusion, we provided the first evidence that testosterone increased ECW volume, adding to the fluid-retaining effects of GH during combined administration. Other endocrine systems known to regulate ECW were unaffected by testosterone, suggesting a direct effect of testosterone on renal tubular function. The positive interaction between testosterone and GH in sodium and fluid homeostasis highlights the greater susceptibility to the development of edema and calls for caution in the initiation of GH replacement therapy in men.

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