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0021-972X/97/$03.00/0
Journal of Clinical Endocrinology and Metabolism
Vol. 82, No. 3
Printed in U.S.A.

Deuterium and Bromide Dilution, and Bioimpedance Spectrometry Independently Show That Growth Hormone-Deficient Adults Have an Enlarged Extracellular Water Compartment Related to Intracellular Water

WOUTER D. VAN MARKEN LICHTENBELT, YVONNE E. M. SNEL, ROBERT-JAN M. BRUMMER, AND HANS P. F. KOPPESCHAAR

Department of Human Biology, University of Maastricht (W.D.v.M.L.), and the Department of Internal Medicine, University Hospital Maastricht (R.-J.M.B.), Maastricht; and the Department of Endocrinology, University Hospital Utrecht (Y.E.M.S., H.P.F.K.), Utrecht, The Netherlands

ABSTRACT

GH has a strong influence on body composition. However, the effects of GH deficiency in adults on water compartments are not well understood. Therefore, extracellular water (ECW) and total body water were independently determined by deuterium and bromide dilution and by bioimpedance spectrometry in GH-deficient (GHD) adults and compared to those in controls, matched for age, sex, body weight, and height.

The results show that the percent body fat was significantly ($P < 0.05$) higher, and total body water and intracellular water (ICW) were significantly lower in GHD adults for males, females, and both sexes combined. ECW was not significantly different between the two groups. ECW/ICW in GHD adults (0.42 ± 0.03) was significantly ($P < 0.01$) higher than that in controls (0.39 ± 0.02). There was a significant positive relation between the ECW/ICW ratio and the percent body fat. These results were confirmed by the bioimpedance spectrometry measurements. (*J Clin Endocrinol Metab* **82:** 907–911, 1997)

GH HAS, APART from growth-stimulating effects, a strong influence on body composition (1). Recent studies have shown that adults with GH deficiency (GHD) have an increased body fat mass (BF) and a decreased fat-free mass (FFM) (2–5). Sustained high concentrations of GH, like those present in patients with acromegaly (6, 7), result in an relative increased amount of extracellular water (ECW). However, the effects of GHD in adults on water compartments are less well known. Because of the ability of GH to cause sodium retention (8, 9), a relative decrease in ECW is expected to occur when GH levels are reduced compared to that with normal GH levels. Indeed, it has been found that ECW is relatively low in GHD adults (10), and there is one study which indicates that in GHD adults ECW is reduced compared to total body water (TBW) (4). Other studies reveal no difference in relative ECW between GHD adults and controls (11, 12). However, the results of the studies mentioned could have been confounded because the methods used did not independently determine TBW and ECW, and control subjects were not matched for age, sex, body weight, and height.

The effect of GH administration on water compartments is also not unequivocal. GH treatment was shown to increase ECW in GHD adults (10, 12). However, as it is known that FFM increases with increased GH levels, it is essential to know whether the increase in ECW was relative to that in intracellular water (ICW). In another study in which ECW and ICW were determined independently, strong indications were found that GH treatment in elderly subjects results in an increase in ICW that was associated with a concomitant trend toward decreased ECW (13).

To elucidate the controversies concerning the water compartments in GHD adults, we performed a comparative study between GHD adults and controls, matched for age, sex, body weight, and height, in which TBW and ECW were determined independently using deuterium and bromide dilution as well as bioimpedance spectrometry (BIS) for predicting ECW and ICW.

Subjects and Methods

Subjects and hormone assays

Ten adult GHD patients, five men and five women, aged 28–63 yr, were included in the study. They were known to have GH deficiency for at least 12 months (mean, 9.3 yr; range, 2–30 yr). In nine patients, pituitary insufficiency was due to surgery and/or irradiation because of a pituitary tumor. In one patient (no. 1, Table 1), pituitary insufficiency was caused by irradiation for nasopharynx carcinoma.

GH deficiency was defined as a peak plasma GH concentration of 3 μg/L (9 mU/L) or less in response to insulin hypoglycemia (insulin tolerance test). In one patient (no. 10), an arginine infusion test was performed. In this patient, the peak plasma GH concentration was less than 0.1 μg/L. Deficiencies in pituitary functions and hormone replacement therapy are shown in Table 1.

The plasma GH concentration was determined using a RIA from Oris Industry Co. (Gif-sur-Yvette, France), which had a lower detection limit of 0.50 ± 0.04 mIU/L (0.25 μg/L); the intra- and interassay coefficients

Received August 8, 1996. Revision received November 20, 1996. Accepted November 28, 1996.

Address all correspondence and requests for reprints to: Dr. Wouter D. van Marken Lichtenbelt, Department of Human Biology, University of Maastricht, P.O. Box 616, 6200 MD Maastricht, The Netherlands.

TABLE 1. Deficiency in pituitary functions and hormone replacement therapy in the GHD patients

	Deficiency in pituitary functions	Hormone replacement therapy
Females		
1	A, T, G	Levothyroxine (100 μg), hydrocortisone (20 mg), estradiol (2 mg), levonorgestrel (150 μg)
2	A	Cortisone acetate (7.5 mg)
3	A, T, G	Hydrocortisone (25 mg), levothyroxine (137.5 μg), ethinyl estradiol (30 μg), levonorgestrel (150 μg)
4	T	Levothyroxine (100 μg)
5	A, G	Cortisone acetate (10 mg), ethinyl estradiol (30 μg), levonorgestrel (150 μg)
Males		
6	A, T, G	Cortisone acetate (12.5 mg), levothyroxine (125 μg), testosterone undeconoate (80 mg/day)
7	A, T, G	Levothyroxine (175 μg), cortisone acetate (5 mg), testosterone esters (250 mg every 3 wk, im)
8	A, T, G	Levothyroxine (125 μg), cortisone acetate (25 mg), testosterone esters (250 mg every 3 wk, im)
9	A, T, G	Cortisone acetate (25 mg), levothyroxine (200 μg), testosterone undeconoate (160 mg/day)
10	A, T, G	Cortisone acetate (10 mg), levothyroxine (50 μg), testosterone esters (250 mg every 3 wk, im)

A, ACTH; T, TSH; G, gonadotropins.

of variation were 7.7% and 11%, respectively. Insulin-like growth factor I was measured by RIA, using the antiserum from Underword and Van Kijk, distributed by the Hormone Distribution Program of the NIDDK. All samples were run in the same assay.

The control group consisted of 10 healthy adult volunteers, matched for age, sex, body weight, and height. They were recruited before any of the measurements in the controls were carried out. The subjects were studied as out-patients at the Department of Endocrinology. All measurements were performed after an overnight fast.

The aims and methods of the study were explained to all subjects, and informed consent was obtained. The study was approved by the ethics committee of the University Hospital of Utrecht.

TBW

TBW was determined by deuterium dilution, following the method described by Westerterp *et al.* (14). Subjects received an orally administered dose of deuterium (D_2O) of approximately 0.1 g/L estimated TBW. The appropriate amount of D_2O (99.8%; Akademie der Wissenschaften, Leipzig, Germany) was weighed out and diluted with tap water to 0.075 L for intake. D_2O enrichment in the body fluid was measured in urine. Before treatment, background urine samples were taken. The dose was given at 2200 h. Urine samples were taken after 10 h of treatment from the second voiding (first voiding at ~0700 h). Isotope abundance in urine was determined in duplicate with an isotope-ratio mass spectrometer (Aqua Sira, VG Isogas, Cheshire, UK). TBW was calculated as the D_2O dilution space divided by 1.04, correcting for exchange of the D_2O label with nonaqueous hydrogen of body solids.

The percent BF (%BF) was calculated as follows:

$$BF\% = (BM - TBW/0.72)/BM \times 100\%,$$

where BM is body mass (15).

ECW

The ECW compartment was determined by bromide dilution. A known amount of sodium bromide (60 mg bromide/L estimated TBW; Ph.Eur., Genfarma, Maarssen, The Netherlands) was mixed with the D_2O solution and thus administered simultaneously with the D_2O dose. Venous blood samples were obtained before intake and 10 h after ingestion of bromide (16). The bromide concentration in serum ultrafiltrate was determined with high performance liquid chromatography (17). Corrected bromide space was calculated according to the method of Miller *et al.* (17).

BIS

BIS measures the impedance of the body at different frequencies. It is possible to discriminate between the ECW compartment and the ICW compartment of the body, as tissue impedance at low frequencies is controlled by the electrical properties of ECW and at high frequencies by the TBW compartment (ECW plus ICW). A detailed description of the method was previously reported (18, 19). Complex BIS spectra were obtained at 50 logarithmically spaced frequencies from 5–500 kHz with a BIS analyzer (model 4000B, Xitron Technologies, San Diego, CA). We used a tetrapolar arrangement of gel electrodes placed on hand, wrist, ankle, and foot at the right side of the body (19). The measurements were carried out between 5–10 min after the subject lay supine on a bed. The impedance data were fitted to the Cole-Cole cell suspension model using nonlinear curve fitting, which revealed the ECW resistance and the intra-cellular water resistance (18, 19).

ECW and ICW volumes were predicted from a general mixture theory (18, 20). This implies that we used a formula that directly calculates the water compartments from resistance values, assuming specific resistances of ECW and ICW, rather than applying the common procedure in bioimpedance analyses of an empirically determined regression equation. The specific resistances (males: $\rho_e = 152.8$, $\rho_i = 979.1$; females: $\rho_e = 146.4$, $\rho_i = 947$) were obtained from an independent dataset at our laboratory.

Statistics

Wilcoxon's signed rank test was used to establish significance of any differences between GHD adults and matched controls. Spearman rank correlation coefficients were used to test relations between water compartments by different methods. The bias and limits of agreement between the different methods were calculated according to the method described by Bland and Altman (21). All data are expressed as the mean ± SD.

Results

Physical characteristics and %BF are presented in Table 2. %BF was significantly ($P < 0.05$) higher in GHD adults for males, females, and both sexes combined.

Water compartments by dilution

The absolute amounts of TBW and ICW were significantly lower in GHD adults compared to controls (by Wilcoxon signed rank, $P < 0.05$ and $P < 0.02$, respectively; Table 3), whereas ECW was not significantly different ($P > 0.1$). There were no gender differences in ECW/ICW in each group. ECW/ICW in GHD adults (0.42 ± 0.03) was significantly ($P < 0.01$) higher than in that in controls (0.39 ± 0.02). There was a significant positive relation between the ECW/ICW ratio and the %BF (ECW/ICW = 0.63%BF + 0.49; $r^2 = 0.49$; $P < 0.001$; Fig. 1).

Water compartments by BIS

In accordance with the dilution-based results, the volume estimations by BIS (Table 3) revealed that TBW and ICW were significantly lower in GHD adults than those in controls ($P < 0.05$ in both cases). There were no significant differences

TABLE 2. Physical characteristics of GHD adults and controls

Subject no.	GHD adults					Controls				
	Age (yr)	Ht (cm)	Wt (kg)	BMI (kg/m^2)	Body fat[b] (%)	Age (yr)	Ht (cm)	Wt (kg)	BMI (kg/m^2)	Body fat[b] (%)
Females										
1	30	157.5	65.1	26.2	41.9	30	170.1	69.2	23.9	33.6
2	45	173.1	90.1	30.1	42.6	43	172.0	96.1	32.5	37.3
3	44	170.9	61.2	21.0	38.0	46	170.6	60.5	20.8	24.3
4	56	167.3	63.5	22.7	36.2	55	170.4	68.9	23.7	31.9
5	53	164.1	72.3	26.8	38.2	67	162.1	61.3	23.3	35.8
Mean (SD)					39.4 (2.8)[a]					32.6 (5.1)[a]
Males										
6	28	174.5	61.0	20.0	30.4	32	178.3	66.0	20.8	12.7
7	46	179.2	77.1	24.0	29.4	44	185.0	72.9	21.3	25.5
8	54	166.9	61.4	22.0	27.3	55	171.1	62.4	21.3	22.1
9	63	176.4	71.5	23.0	22.9	67	179.3	80.1	24.9	12.0
10	58	178.4	79.5	25.0	26.4	60	182.6	77.3	23.2	24.0
Mean (SD)					27.3 (2.9)[a]					19.3 (6.4)[a]

There were no significant differences in physical characteristics between the groups, with the exception of percent body fat.
[a] Statistically significant difference between GHD patients and controls ($P < 0.05$).
[b] % BF = (BM − TBW/0.72)/BM × 100%.

TABLE 3. TBW, ECW, ICW, and ECW/ICW of GHD adults and controls

Subject no.	By dilution								By bioimpedance spectrometry							
	GHD adults				Matched controls				GHD adults				Matched controls			
	TBW	ECW	ICW	ECW/ICW	TBW	ECW	ICW	ECW/ICW	TBW	ECW	ICW	ECW/ICW	TBW	ECW	ICW	ECW/ICW
Females																
1	27.2	10.9	16.3	0.67	33.0	12.6	20.4	0.62	24.9	10.5	14.4	0.73	32.0	12.8	19.2	0.67
2	37.3	16.9	20.4	0.83	43.4	17.8	25.6	0.70	34.5	16.4	18.09	0.91	44.4	18.7	25.7	0.73
3	27.3	11.4	15.9	0.71	33.0	13.2	19.8	0.66	28.2	12.3	15.9	0.77	30.2	12.9	17.4	0.74
4	29.2	13.3	15.9	0.83	33.8	13.8	20.0	0.69	25.3	12.0	13.3	0.90	31.9	14.2	17.7	0.81
5	32.2	13.9	18.3	0.75	28.3	11.8	16.5	0.71	32.9	14.1	18.8	0.75	26.6	11.0	15.7	0.70
Mean				0.76[a]				0.68[a]				0.81[a]				0.73[a]
SD				0.07				0.04				0.09				0.05
Males																
6	30.6	11.2	19.3	0.58	41.5	15.3	26.2	0.58	29.5	12.0	17.5	0.68	37.8	15.3	22.5	0.68
7	39.2	16.4	22.8	0.72	39.1	15.6	23.5	0.66	38.6	17.8	20.8	0.86	37.4	16.3	21.1	0.77
8	32.1	13.8	18.3	0.75	35.0	13.7	21.3	0.65	29.3	13.3	16.1	0.83	35.7	14.8	20.9	0.71
9	39.7	15.0	24.7	0.61	50.8	17.9	32.9	0.54	38.4	15.5	22.9	0.68	46.6	18.3	28.3	0.65
10	42.1	17.1	25.0	0.68	42.3	15.7	26.6	0.59	40.3	18.2	22.1	0.82	41.8	18.0	23.8	0.76
Mean				0.67[a]				0.61[a]				0.77[a]				0.17[a]
SD				0.07				0.05				0.09				0.05
Total mean (SD)				0.71[b]				0.64[b]				0.79[b]				0.72[b]
				0.08				0.06				0.08				0.05

Results by dilution techniques and from bioimpedance spectrometry are presented separately. Body water compartments are in liters.
[a] Statistically significant difference between GHD patients and controls, $P < 0.05$.
[b] Statistically significant difference between GHD patients and controls, $P < 0.01$.

in ECW ($P > 0.1$) between GHD adults and controls. As with the dilution techniques, the BIS results showed that the ECW/ICW ratio was significantly higher in GHD adults than in the control group ($P < 0.01$).

Comparison of dilution techniques and BIS

The regression between TBW by D_2O dilution and TBW by BIS is highly significant ($r^2 = 0.94$; $P < 0.001$), with a standard error of estimate (SEE) of 1.6 L (Table 4). Also, ECW-dilution and ECW-BIS were significantly correlated ($r^2 = 0.91$; $P < 0.001$), with a SEE of 0.8 L. Bland Altman plots (21) reveal no significant relation between the amount of TBW and the difference between the two methods (Table 4). On the average, D_2O dilution revealed 1.5 ± 1.6 L higher TBW values than BIS. There was a weak positive relation between the differences in ECW by the two methods and the amount of ECW ($P < 0.05$; $r^2 = 0.26$). The mean difference was 0.3 L.

The ECW/ICW ratios of the dilution and BIS measurements were also significantly correlated ($r^2 = 0.69$; $P < 0.001$; SEE = 0.04). Finally, there was a significant correlation between the difference in ECW/ICW between GHD adults and their matched controls ($r^2 = 0.67$; $P < 0.005$; SEE = 0.03; Fig. 2) by BIS and dilution methods.

Discussion

Relative size water compartments in GHD adults

This study reveals that GHD adults compared to their matched controls have an enlarged ECW compartment relative to the ICW compartment, as shown by two independent methods. The difference is evident among all pairs studied.

Although the GHD adults had a significantly higher %BF than the controls, the total amount of ECW was not different between GHD adults and controls, whereas ICW was significantly lower in GHD. This indicates that the increased ECW/ICW ratio in GHD adults is due to a relative decrease in ICW, rather than an increase in ECW. As suggested by Snel *et al.* (11), it is conceivable that in chronic GHD, the amount of ECW is in the normal range due to sodium- and water-regulating mechanisms other than GH, such as the renin-aldosterone system and/or atrial natriuretic peptide.

It is often reported that chronic GHD results in an enlarged %BF, as assessed by various methods (2–4, 12). In our study, %BF indeed is significantly increased comparied with that in the control subjects. We also found a significant positive relation between the ECW/ICW ratio and the %BF. It is well established that obese subjects have a relatively large ECW/ICW ratio (0.63 in nonobese *vs.* 0.81 in obese) (22, 23). This can in part be attributed to the water component of adipose tissue, which consists of 78% ECW (24). Thus, in GHD adults two compensating processes may occur: low GH concentrations result in a low ICW, and a relative expansion of body fat results in a relative expansion of the ECW. Furthermore, Waki *et al.* (23) suggest obesity-related edema and hormonal responses related to adipose tissue.

Finally, the decreased FFM, as shown in GHD adults, may be the result of cell shrinking (25), which, in turn, results in a decrease in ICW. This brings us to the results reported by Thompson *et al.* (13), who showed that GH treatment in elderly women increased the IWC compartment more than can be accounted for by the increase in FFM. This could be attributed to swelling of muscle cells.

Thus, a decreased FFM in combination with a relatively stable ECW could result in the observed increased ECW/ICW ratio.

Comparison between dilution techniques and BIS

BIS measures the impedance of the body at a whole range of frequencies instead of the classical bioimpedance analysis that uses a single frequency (mostly 50 kHz). BIS is a relatively new method that takes into account the fact that electrical resistance is frequency dependent on the electrical properties of the cell membrane. Consequently, with BIS it is possible to discriminate between ECW and ICW. At the same time, direct body fluid calculations are relatively new (18). This method has the advantage that it is not depending on empirically derived prediction formulas. However, the coefficients used for the specific resistances for ECW and ICW reflect, on the one hand, the actual specific resistances of the water compartments and, on the other hand, are dependent on the methods used to determine ECW and TBW (or ICW). In this study, bromide and D_2O dilution was applied with an equilibration time of 10 h. Therefore, resistance coefficients were determined at our laboratory beforehand and independently in another group of subjects. It is important to realize that these coefficients do not affect the correlation coefficients

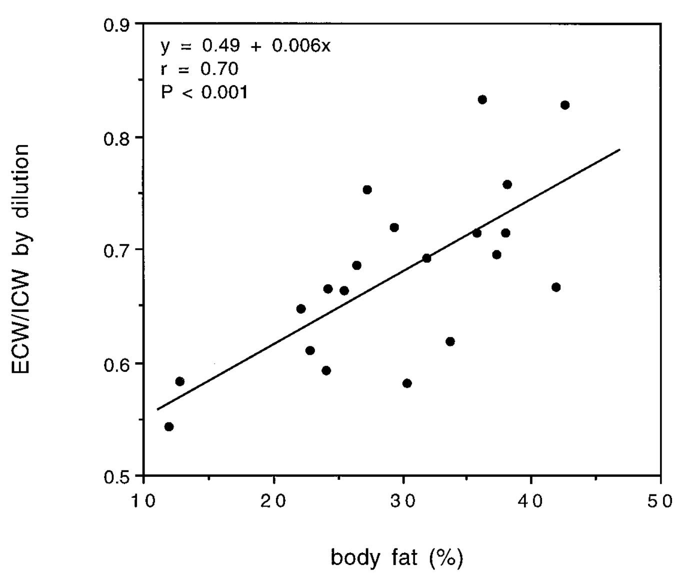

FIG. 1. The relation between ECW/ICW ratio, determined by dilution techniques, and %BF (△, GHD adults; ●, controls).

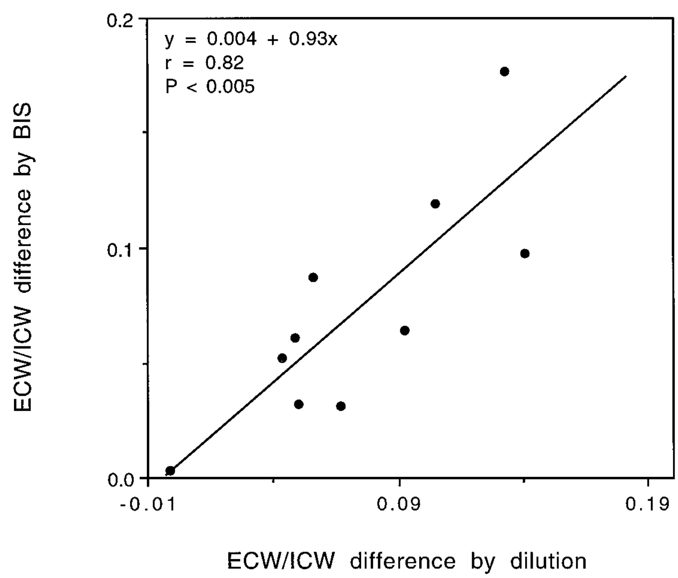

FIG. 2. The difference in the ECW/ICW ratio of GHD adults and controls by BIS plotted against the difference in the ECW/ICW ratio of GHD adults and controls by dilution.

TABLE 4. Regression analysis (least squares method) between results from dilution techniques and BIS

	Compartments			Comparison between the difference against the mean				95% CI	
	r^2	*P*	SEE	r^2	*P*	Bias	Error		
TBW	0.94	0.0001	1.60		NS	−1.5	1.58	−2.3	−0.8
ECW	0.91	0.0001	0.80	0.26	0.02				
ECW/IBW	0.69	0.0001	0.066		NS	0.079	0.045	0.058	0.10

Data are given of the square of the correlation coefficient, *P* value, and SEE. Square correlation coefficient, *P* value, bias and error (SD), and 95% confidence interval (CI = bias ± 2 × ($SD/n^{0.5}$), according to the Bland and Altman procedure.

and SEEs derived from comparisons between the water compartments by BIS and the dilution techniques.

The outcome of the comparison between the methods is promising. Despite the low number of subjects studied, correlations of the BIS results with the dilution techniques revealed high squared correlation coefficients ($r^2 \geq 0.91$) and low SEEs (TBW, 1.6 L; ECW, 0.8 L), indicating good accuracy on an individual level. The bias between the methods was small. Clearly, a larger dataset is needed to investigate whether the same resistance indexes can be used in different patient groups. Very interesting for a clinical setting is the good fit between the differences in ECW/ICW ratios between GHD adults and controls. This indicates that changes in water compartments and distribution of water compartments can reliably be detected by BIS. Longitudinal intervention studies are especially necessary to further elucidate the clinical applicability of this noninvasive fast technique.

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