

Bioelectrical impedance analysis estimation of water compartments in elderly diseased patients.

The Source study\*

Key words : fluid balance, total body water, extracellular water, bromide, oxygen18

Short title : Assessment of water spaces in the elderly

\*The Source study is a French multicentric study coordinated by Dr P Ritz (Human Nutrition Research Centre-Auvergne). Investigators were (by alphabetical order) :Dr S Acher (Paris, Bichat), Pr B Beaufrère (Clermont-Ferrand), F Blondé-Cynober (Paris, Hôtel-Dieu), Dr A Boulier (Paris, Bichat), Dr F Bouthier and Dr F Bouthier-Quintard (Limoges), Pr T Constans and Dr V Dardaine (Tours), Dr JC Desport (Limoges), Dr A Ghisolfi-Marque (Toulouse), Dr R Hermet (Clermont-Ferrand), Dr C Lambert (Paris, E. Roux), Pr B Vellas (Toulouse), Dr JP Vincent (Paris, E Roux), Dr MJ Arnaud (Perrier Vittel Water Institute). The study was supported by a grant from Perrier Vittel Water Institute, Vittel, France.

For correspondence :

Patrick Ritz MD, PhD

Service de Médecine B

Centre Hospitalier Universitaire

F-49033 ANGERS CEDEX 01

Tél : 00 (33) 241 354 499

Fax : 00 (33) 241 734 969

e-mail : [patrick.ritz@wanadoo.fr](mailto:patrick.ritz@wanadoo.fr)

## **ABSTRACT**

**OBJECTIVE :** To validate in geriatric patients bioelectrical impedance analysis (BIA) equations that had been derived to estimate total body water (TBW) and extracellular water (ECW) in healthy elderly subjects.

**SETTING AND DESIGN :** Multicentric trial, 6 geriatric wards.

**PARTICIPANTS :** 169 patients with varying degrees of hydration : dehydrated, euvolemic and overhydrated.

**METHODS :** BIA estimates of TBW and of ECW were compared to measurement of TBW with  $^{18}\text{O}$  dilution and of ECW with bromide dilution.

**RESULTS :** BIA estimated TBW with a mean difference of  $0.48 \pm 2.3$  litres (50 kHz,  $p = 0.01$ ) and  $0.69 \pm 2.2$  litres (100 kHz,  $p < 0.001$ ) compared with  $^{18}\text{O}$  dilution. The difference was not affected by the hydration status. Estimates of ECW with BIA were systematically biased compared with bromide dilution ;  $4.6 \pm 3.1$  litres (Segal et al equation,  $p < 0.001$ ) and  $3.4 \pm 2.9$  litres (Visser et al equation,  $p < 0.001$ ). A new equation is proposed, that is cross validated.

**CONCLUSION :** Body water spaces can be estimated accurately in geriatric patients with BIA.

## INTRODUCTION

Fluid imbalance is common amongst elderly patients. Dehydration is the most common fluid disorder responsible for an increased morbidity and mortality, and for a substantial hospital expenditure [1]. Its prevalence increases with age, from about 1 % of hospital admissions in 65 years old patients to above 5 % in seniors older than 85 [2]. Many age-related physiological and environmental disturbances are responsible for dehydration [1, 3-4]. Early diagnosis is sometimes difficult because the classical signs may be absent or misleading in an older patient [1]. At the other end of fluid imbalance is overhydration, which can result from many organ failures (heart, kidney, liver...), and from inappropriate fluid replacement therapy [5]. As for dehydration, there are numerous reports stressing the serious complications which can result from overhydration, and early diagnosis is also difficult.

Beyond gross fluid imbalance, a progressive cellular dehydration has been shown in critically ill patients [6], whereas between individual variation in cellular hydration can be quite substantial, even in healthy elderly people [7].

For all of these reasons clinicians require a tool both for a discriminative diagnosis of fluid imbalance and for monitoring changes in water spaces in the elderly. A quantitative measurement of body water spaces is desirable. Total body water (TBW) increases with body weight and is negatively related to fatness. Therefore, a measured TBW value would only be informative about hydration status if it could be compared to a « standard » value. Time trends in TBW tell about changes in fluid balance. However, a preliminary step in the use of TBW measurements is to check the validity of the estimate. The best probes for water spaces are isotopic tracers of water ( $^2\text{H}_2\text{O}$ ,  $^3\text{H}_2\text{O}$ ,  $\text{H}_2^{18}\text{O}$ ) for TBW and bromide for extracellular water (ECW). In many clinical circumstances however, these methodologies might be impractical. They require expertise and expensive equipments. Furthermore, these dilution techniques impose a delay of 2-4 hours for the tracer to equilibrate within the pool before

sampling. This delay, plus the necessary time for measurements to be performed often contradicts the necessity for a swift therapeutic decision. Bioelectrical impedance analysis (BIA) is a quick, safe, non invasive technique to estimate TBW and ECW that does not require much cooperation from the subject [8]. It has been shown that accurate estimates of TBW and ECW can be obtained in healthy elderly subjects [8-10]. Precisions close to 1 litre (i.e. 3.8 %) can be obtained for estimates of TBW [8]. This technique is reproducible within subjects over a few hours (mean difference is 0.2 l [8]) and over a longer period of time (up to 28 days, the CV is about 3 % according to Olde Rikkert et al [11]). BIA might also be reliable to depict changes in body water [11-14]. Therefore, BIA might be this required tool for diagnosis and management of fluid imbalance. Apart from the work by Olde Rikkert et al [14] dealing with body fluid changes, no study has attempted to validate BIA in elderly patients with a wide range in hydration status. Therefore, the aim of the present study was to check the validity of equations derived by Vaché et al [8] in the healthy elderly, in a large group of diseased old patients, overhydrated, euvolemic, or dehydrated. The comparison between estimates of TBW and ECW with BIA was performed versus "gold standards", i.e. dilution of  $H_2^{18}O$  and Bromide.

## Subjects and methods

### **1- Subjects**

One hundred and sixty nine subjects living in six French institutions (see list on front page) were recruited to participate in the study that was approved by the Auvergne medical school ethical committee and by the French Ministry of Health. One ward was a step-down unit, others were long-stay units. All patients aged 60 years and over admitted to these wards were eligible for the study and were included if they had no exclusion criteria and signed written informed consent. Patients entered the hospital for various reasons (infection, acute organ failure, weight loss, hydration disorders and so on) and with varying medical conditions (heart failure, kidney failure, stroke aftermaths...). However, they were in clinically stable conditions, i.e. at least a week after the treatment had begun. No patient was excluded on the basis of his/her drug treatment. Exclusion criteria were : end of life, patients requiring intensive care (sepsis, surgery, acute organ failure...), ascites, artificial nutrition, any limb abnormality preventing BIA measurement. Physical characteristics of the volunteers are given in Table 1. Participants were varying in their hydration status, an estimate of which was performed by a senior geriatrician based on the following criteria : dehydrated if they had a lasting skinfold at the anterior side of the thigh and/or plasma sodium higher than  $142 \text{ mmol.l}^{-1}$ ; overhydrated if they had edema (ankles, arms or back sides) and/or plasma sodium lower than  $135 \text{ mmol.l}^{-1}$ ; euvoletic in all other cases. Table 2 shows water spaces, plasma sodium concentrations and osmolarity in the 3 groups. Wide ranges in plasma sodium ( $128\text{-}146 \text{ mmol/l}$ ) and in osmolarities ( $264\text{-}338 \text{ mmol}$ ) could be demonstrated in these patients.

### **2- Study design**

The protocol of the study consisted in the measurement of TBW by  $^{18}\text{O}$  dilution, and of ECW by bromide dilution, plus the determination of BIA and anthropometrical measurements.

### **2-1 Baseline samples**

After an overnight fast ( $\approx 12$  hours) volunteers gave a plasma and urine sample, for natural abundance determinations of  $^{18}\text{O}$  enrichments and bromide concentrations.

### **2-2 Doses**

A weighed amount of 2 %  $^{18}\text{O}$  enriched water ( $\approx 50$  g) was given orally to the 169 volunteers.

A weighed amount of  $\approx 20$  g potassium bromide syrup ( $\approx 1$  g Br) was given to half the volunteers. Flasks containing the doses were weighed after the doses were given and exact weights of doses taken were recorded. In the validation experiment on healthy elderly subjects [8], Visser's and Segal's equations were accurate. Therefore, we only dosed 84 patients with bromide.

### **2-3 Post-dose samples**

A plasma and a urine sample were collected 4 and 5 hours after the dose. In the mean time volunteers were allowed to have their usual breakfast but were limited to a consumption of 250 ml of water (this is 1 SD for the measurement of TBW with  $^{18}\text{O}$  diluted water, (8)). Plasma and urine samples were immediately frozen and kept at  $-20^\circ$  until analysis.

### **2-4 Anthropometric measurements**

Body weight was measured in light clothing, and to the nearest 0.1 kg with SECA scales (SECA, Les Mureaux, France). Height was measured (in volunteers that could stand) to the nearest 0.2 cm with a height gauge. In all volunteers, the knee-to-heel length was measured to the nearest 0.2 cm with a SECA toise according to the technique described by Chumlea et al [15]. Chumlea's equations [15] were used to estimate height from knee-to-heel length, in 43 patients. Anthropometric measurements were performed during the 5<sup>th</sup> hour following the dose of tracers.

## **2-5 Bioelectrical impedance analysis (BIA)**

BIA measurements were performed with an Analycor-3 analyzer (Spengler, France). All the investigators had been equipped with analyzers from the same series and had attended a course where the complete procedure was taught. Measurements were performed after a rest of at least 30 minutes, during the 5<sup>th</sup> hour post-dose, in a temperature controlled room. Four surface electrodes (Sentry Silver EKG electrodes) were placed on a clean and degreased skin at the limb ends in a well standardized manner. The current detector electrodes were located in the distal end of the third metacarpal bone and of the second metatarsal bone. The current detector electrodes were located between the styloid processes of the radius and ulna, and between the two maleoli of the ankle. Measurements were performed both on the left and on the right side of the body. Three frequencies were used 5, 50 and 100 kHz, at a current of 400  $\mu$ Amp. Electronic precision of the instrument is better than 1  $\Omega$ , and the response is linear between 100 and 2500  $\Omega$ . Reproducibility with Sentry electrodes is better than 2  $\Omega$ .

## **2-6 Analytical methods**

<sup>18</sup>O enrichments were measured with the CO<sub>2</sub>-H<sub>2</sub>O equilibration technique, adapted for use with vacutainers on a continuous-flow gas chromatography-isotope ratio mass spectrometer ( $\mu$ gas, Micromass, UK) as already described [8]. Plasma bromide concentrations were measured by means of HPLC as described by Miller and Cappon [16] using a diode array detector (Partisil 10 SAX column, Whatman International Ltd, UK). Protein-free plasma samples were obtained after centrifugation using a MPS1 micropartition system (Amicon, France).

## **2-7 Calculations and statistical methods**

<sup>18</sup>O dilution spaces were calculated from increases between mean enrichment at times 4 and 5 hours post-dose and baseline values. TBW was considered 1 % smaller than <sup>18</sup>O dilution space to account for exchanges with non-aqueous compounds [17]. ECW was calculated from

mean concentration in plasma bromide at times 4 and 5 hours post-dose, according to Miller and Cappon [16]. The equation that gives ECW is :

$$ECW = 0.90 * 0.95 * Br \text{ dose}/\Delta (\text{Br plasma})$$

where “Br dose” is the dose given, “delta (Br plasma)” is the difference between mean plasma concentration after dose, and baseline concentration. Correction factor 0.95 is for Donnan equilibrium and 0.90 corrects for the distribution of Br in non extracellular sites.

TBW was calculated from impedances measured at 50 and 100 kHz with equations derived by Vaché [8]:

$$TBW (\text{litres}) = 2.896 + 0.366 Ht^2/R100 + 0.137 wt + 2.485G$$

$$TBW (\text{litres}) = 3.026 + 0.358 Ht^2/R50 + 0.149 wt + 2.924G$$

ECW was calculated from impedance measured at 5 kHz with equations derived by Segal et al [18] and Visser et al [9]. These equations were used because they were accurate in healthy elderly subjects, with the same BIA analyser and the same tracer method.

$$ECW (\text{Segal, litres}) = - 6.1 + 0.284 Ht^2/R5 + 0.112 wt$$

$$ECW (\text{Visser, men, litres}) = 4.8 + 0.225 Ht^2/R5$$

$$ECW (\text{Visser, women, litres}) = 1.7 + 0.2 Ht^2/R5 + 0.057 wt$$

In all of these equations Ht is height in cm, R is impedance, wt is weight in kg and G is gender (0 for women, and 1 for men).

Results are expressed as mean  $\pm$  SD unless stated otherwise. Comparisons of means were performed by ANOVA or Student t-test where applicable. Agreement between measurements was assessed with the technique described by Bland and Altman [19]. Multiple regression models were calculated with stepwise backward regressions (F to enter = 4, F to exit = 3.96). Statistical computations were performed on Statview 4.0 statistical package (Abacus Concept, US). Significance was accepted at the 5 % level.

## RESULTS

### 1- Physical characteristics

Physical characteristics for the patients are given in Table 1. Forty four patients were dehydrated, 58 were overhydrated while 67 were considered as having a normal hydration status.

### 2- Bioelectrical Impedance Analysis

Impedance did not differ significantly between the right and the left side of the body, at current frequencies of 5, 50 and 100 kHz (data not shown). Therefore, impedances measured on both sides were averaged for each individual.

Total body water calculated with the equation using impedance at 50 kHz differed from TBW measured by  $^{18}\text{O}$  dilution by  $0.48 \pm 2.3$  litres ( $P = 0.01$ ). This difference was neither affected by the hydration status nor by the gender of the patients. It was  $0.25 \pm 2.4$  litre in dehydrated patients,  $0.42 \pm 2.4$  litre in overhydrated patients, and  $0.69 \pm 2.2$  litre in euvoletic patients (Anova  $F = 0.47$ ,  $P = 0.63$ )

Total body water calculated with the equation using impedance at 100 kHz differed from TBW measured by  $^{18}\text{O}$  dilution by  $0.62 \pm 2.4$  litres ( $P = 0.001$ ). This difference was not affected by the hydration status of the patients,  $0.39 \pm 2.5$  litre in dehydrated patients and  $0.57 \pm 2.41$  litre in overhydrated patients, and  $0.82 \pm 2.4$  litre in euvoletic patients (Anova  $F = 0.41$ ,  $p = 0.66$ ). Figure 1 displays the Bland and Altman plot for TBW measurements ( $^{18}\text{O}$  dilution) and estimates (50 and 100 kHz). For the 126 patients in whom both height and knee height were available, TBW calculated from impedance at 50 kHz and either measured height or height derived from knee height differed by 0.2 litre ( $P = 0.04$ ).

Extracellular water calculated with equations using impedance at 5 kHz differed from ECW measured by bromide dilution. The difference was  $4.6 \pm 3.1$  litres with Segal et al's [18] equation ( $P < 0.001$ ) and  $3.4 \pm 2.9$  litres with Visser et al's equation ( $P < 0.001$ , [9]), and was

not affected by gender. The subjects having had ECW measured with bromide were split in 2 groups by randomization. A model specific for the first group (Table 3) was set by multiple linear regression of variables that were correlated to ECW in this group. Only  $Ht^2/R5$  turned out to be an independent variable. When applied to the second group, this model created no bias (Table 2). The reverse procedure was applied (model established on the second group and applied to the first group), produced a very similar equation without bias. Therefore, data from the two groups were pooled, and subjected to the multiple regression analysis procedure. Only  $Ht^2/R5$  came out as a significant and independent variable (Table 3).

Table 1 : Physical characteristics of the patients

	Men (n = 60)	Women (n = 109)
Age (yrs)	79.9 ± 9.3	82.9 ± 7.4 <sup>1</sup>
Weight (kg)	65.9 ± 12.5	58.3 ± 13.6 <sup>1</sup>
Height (cm)	165.9 ± 7.5	152.6 ± 6.7 <sup>1</sup>
Knee height (cm)	52.4 ± 2.9	48.1 ± 2.6 <sup>1</sup>
BMI (kg.m <sup>-2</sup> )	23.9 ± 4.0	24.9 ± 4.8
TBW (l) <sup>2</sup>	34.0 ± 5.5	26.3 ± 4.6 <sup>1</sup>
ECW (l) <sup>3</sup>	19.9 ± 4.3	14.9 ± 2.9 <sup>1</sup>

<sup>1</sup> significantly different between men and women

<sup>2</sup> Data obtained by <sup>18</sup>O dilution

<sup>3</sup> Data are limited to 84 values, 47 women and 37 men and were obtained by bromide dilution

Table 2: Hydration parameters in the study groups

	Dehydrated (n=44)	Euvolemic (n=67)	Overhydrated (n=58)
TBW (L)	29.7±6.6	28.6±5.8	29.6±6.3
ECW (L)*	17.9±4.6	16.2±4.0	17.7±4.5
ICW (L)*	12.2±4.3	13.6±4.3	11.8±3.8
Na (mmol.l <sup>-1</sup> ) <sup>1</sup>	141.1±3.6	138.7±1.7	137.0±4.5
Osmolarity (mmol) <sup>2</sup>	294.2±10	296.0±13	288.4±10.4

See methods section for the description of the hydration categories. \* number of patients limited to 84.

<sup>1</sup>P<0.001, <sup>2</sup>P<0.01; ANOVA. Osmolarity was calculated as [2(Na+K)+urea+glycemia].

Table 3 : Regression models established to predict ECW from impedance measured at 5 kHz

Model established on	Equation	R <sup>2</sup>	SEE	Mean difference on the other group
Group 1 (n=42)	$2.82 \pm 0.36 \text{ Ht}^2/\text{R5}$	0.61	2.6	$- 0.18 \pm 2.44$ (P = 0.70)
Group 2 (n=42)	$5.74 + 0.27 \text{ Ht}^2/\text{R5}$	0.43	2.4	$0.4 \pm 2.69$ (P = 0.44)
Group 1 + Group 2 (n=84)	$3.66 + 0.33 \text{ Ht}^2/\text{R5}$	0.55	2.5	

## DISCUSSION

The present study aimed at testing in geriatric patients the validity of BIA equations that were derived in healthy elderly volunteers. The main result of this multicentric trial is that regardless the hydration status of the patients BIA can be used as a bed-site technique for estimating TBW.

BIA relies on a very simple principle. Cells are envisaged as floating in a water and electrolytes milieu (TBW) contained in a cylinder (the body). The reciprocal of the impedance opposed to a light alternative current is proportional to TBW (for a current frequency higher than or equal to 50 kHz) or to ECW (for frequencies below 5 kHz, [20]). This impedance then needs to be converted into TBW or ECW by means of equations, that are said to be age-, disease- and population specific [8].

Very few equations relate to TBW estimates in healthy elderly subjects [8, 9, 21, 22], and even less are pertinent to geriatric patients [14]. We chose Vaché et al's [8] equations since they were acquired with the same bioelectrical impedance analyzer (Analycor 3, Spengler, France) and since the reference method ( $^{18}\text{O}$  dilution) was the same.  $^{18}\text{O}$  dilution has several advantages over  $^2\text{H}$  dilution to measure TBW [8, 23]. The net result is that TBW estimates with  $^{18}\text{O}$  are very precise with a between-day, within-subject CV of 0.7 % for repeated measurements [8], and are very accurate.

Since not all BIA users have machines delivering a 100 kHz current, we investigated equations derived for 50 and 100 kHz frequencies. For the group of patients, estimated TBW differed from reference measurements by 0.48 litre (50 kHz) and 0.62 litre (100 kHz). Although these differences are significant in statistical terms (probably as the result of the large number of degrees of freedom) we believe that they are acceptable in clinical practice. The 95 % limits of confidence were 0.11 - 0.84 litre (50 kHz) and 0.24 - 1.00 litre (100 kHz). We also consider that height calculated from knee-height [15] can be used since the difference

it induces (in comparison to measured height) is minimal (0.2 litre). Therefore, BIA can be used as a discriminative tool for TBW measurement. It is also important that this applies to situations with varying degrees of hydration, precisely when clinicians require an estimate of TBW. The present study concerned subjects with only a mild degree of dehydration. This is because written informed consent was required for participating in the study. Although dehydrated patients are numerous, dehydration impairs cognitive functions and prevents an informed consent. The high prevalence of fluid imbalance makes BIA an attractive tool. Even in those patients where estimated TBW was different from measured TBW, it could be hypothesized that BIA is a good tool for monitoring changes in fluid balance. Support for this comes from a study by Olde Rikkert et al [11]. In their study (on healthy elderly subjects), it was shown that over 28 days, within-subject between-day CV for repeated measurements was 3 %, five times smaller than the between-subject variation. This means that repeated measurements were within 1 litre for those subjects. Furthermore, Olde Rikkert et al [14] showed that the weight and water loss induced by a furosemide administration were correctly monitored by BIA. Individual differences shown in figure 1 might appear to be large. However, the reproducibility of BIA estimates in the short term [8] and over longer periods (28d, 11) suggests that a volunteer with a large residual in figure 1, might remain so on later assessments. BIA could therefore be useful in monitoring changes in TBW.

In contrast, BIA with published equations leads to systematic biases in estimating ECW when applied to the geriatric patients of the present study. Visser et al's equations [9], derived on healthy elderly subjects had proved accurate in our group of healthy subjects (mean difference  $0.0 \pm 2.5$  litre). The same was true for Segal et al's equation [18], although derived in adults (mean difference  $0.0 \pm 3.0$  litre). The bias observed in the present geriatric patients could either come from an inaccurate measurement of ECW with bromide, from altered electrical properties of cell membranes, or from changes in fluid repartition. It is unlikely that

the bromide measurements are erroneous. Indeed, the same technique was used for healthy subjects [8] and in the present study, the mean CV for plateau concentration in bromide was 1.5 % (data not shown). Furthermore, Finn et al [6] in critically ill patients and Kim et al [24] in AIDS patients have shown that intracellular penetrance of the bromide tracer is not changed appreciably. Bromide distribution is therefore confined to ECW, provided a 10 % correction is made [16]. It could also be the case that the repartition between intracellular and extracellular water is altered by age and/or disease. Steen [25] showed that the ratio of ECW to TBW increased with age. If the extracellular fluid expansion was mostly in the limbs, impedance at 5 kHz would be underestimated and ECW artificially increased. Furthermore, limbs represent the largest component of the impedance of the body.

ECW was not correlated to plasma sodium, osmolality or classical protein markers of malnutrition (data not shown). We have derived an equation to calculate ECW in these specific patients that remains to be evaluated in other diseased patients.

In conclusion, BIA with specific equations for elderly subjects could be used as a bed-side tool for discriminative diagnosis and for monitoring changes in fluid balance in geriatric patients. This validity applies to TBW accross the range of hydration disorders.

**Acknowlegments:** Authors thank Line Godiveau for secretarial assistance, Miriam Ryan for correcting the english, and Perrier Vittel Water Institute for funding the study.

## REFERENCES

- 1 - Weinberg AD, Minaker KL, and the council of scientific affairs, American Medical Association. Dehydration. Evaluation and management in older adults. *JAMA* 1995;**274**:1552-6.
- 2 - Molaschi M, Ponzetto M, Massaia M, Scarafiotti C, Ferrario E. Hypernatremic dehydration in the elderly on admission to hospital. *Age & Nutrition* 1998;**9**:39-43.
- 3 - Leaf A. Dehydration in the elderly. *N Engl J Med* 1984;**311**:791-2.
- 4 - Mooradian AD. Water balance in the elderly. In Endocrinology and metabolism in the elderly. JE Morley, SG Korenman eds. *Blackwell Scientific Publications Boston* 1991:pp 124-136.
- 5 - Shizgal HM, Soloman S, Gutelius JR. Body water distribution after operation. *Surg Gynecol and Obstet* 1977;**144**:35-41.
- 6 - Finn PJ, Plank LD, Clark MA, Connolly AB, Hill GL. Progressive cellular dehydration and proteolysis in critically ill patients. *Lancet* 1996;**347**:654-6.
- 7 - Clasey JL, Kanaley JA, Wideman L, Heymsfield SB, Teastes CD, Gutgessel ME, Thorner MO, Hartman ML, Weltman A. Validity of methods of body composition assessment in young and older men and women. *J Appl Physiol* 1999;**86** : 1728-38.
- 8 - Vaché C, Rousset P, Gachon P, Gachon AM, Morio B, Boulier A, Coudert J, Beaufrère B, Ritz P. Bioelectrical impedance analysis measurements of total body water and extracellular water in healthy elderly subjects. *Int J Obesity* 1998;**22**:537-43.
- 9 - Visser M, Deurenberg P, Van Staveren WA. Multi-frequency bioelectrical impedance for assessing total body water and extracellular water in elderly subjects. *Eur J Clin Nutr* 1995;**49**:256-66.

- 10 - Bussolotto M, Ceccon A, Sergi G, Giantin V, Beninca P, Enzi G. Assessment of body composition in elderly : accuracy of bioelectrical impedance analysis. *Gerontology* 1999;**45**:39-43.
- 11 - Olde Rikkert MGM, Deurenberg P, Jansen RWMM, van't Hof MA, Hoefnagels WHL. Validation of multifrequency bioelectrical impedance analysis in monitoring fluid balance in healthy elderly subjects. *J Gerontol* 1997;**52A**:M137-M141.
- 12 - Deurenberg P, Schouten FJM. Loss of total body water and extracellular water assessed by multifrequency impedance. *Eur J Clin Nutr* 1992;**46**:247-55.
- 13 - De Lorenzo A, Barra PFA, Sasso GF, Battistini NC, Deurenberg P. Body impedance measurement during dialysis. *Eur J Clin Nutr* 1991;**45**:321-5.
- 14 - Olde Rikkert MGM, Deurenberg P, Jansen RWMM, van't Hof MA, Hoefnagels WHL. Validation of multifrequency bioelectrical impedance analysis in detecting changes in fluid balance of geriatric patients. *J Am Geriatric Soc* **1997**;45:1345-51.
- 15 - Chumlea WC, Roche AF, Steinbaugh ML. Estimating stature from knee height for persons 60 to 90 years of age. *J Am Geriat Soc* 1985; **33**:116-20.
- 16 - Miller ME, Cappon CJ. Anion exchange chromatographic determination of bromide in serum. *Clin Chim* 1984;**30**:781-3.
- 17 - Ritz P, Johnson PG, Coward WA. Measurement of <sup>2</sup>H and <sup>18</sup>O in body water : analytical considerations and physiological implications. *Br J Nutr* 1994; **2**:1-10.
- 18 - Segal KR, Burastero S, Chun A, Coronel P, Pierson RN Jr, Wang J. Estimation of extracellular and total body water by multiple-frequency bioelectrical impedance measurement. *Am J Clin Nutr* 1991;**54**:26-9.
- 19 - Bland JM, Altman DG. Statistical method for assessing agreement between two methods of clinical measurements. *Lancet* 1986;**1**:307-10.

- 20 - Lukaski HC. Methods for the assessment of human body composition : traditional and new. *Am J Clin Nutr* 1987;**46**:537-56.
- 21 - Deurenberg P, VanderKooy K, Evers P, Hulshof T. Assessment of body composition by bioelectrical impedance in a population aged > 60 y. *Am J Clin Nutr* 1990;**51**:3-6.
- 22 - Svendsen OL, Haarbo J, Heitmann BL, Godfredsen A, Christiansen C. Measurement of body fat in elderly subjects by dual-energy x-ray absorptiometry bioelectrical impedance and anthropometry. *Am J Clin Nutr* 1991;**53**:1117-23.
- 23 - Schoeller DA, Van Santen E, Peterson DW, Dietz W, Jaspán J, Klein PD. Total body water measurements in humans with <sup>18</sup>O and <sup>2</sup>H labelled water. *Am J Clin Nutr* 1980;**33**:2286-93.
- 24 - Kim J, Wang ZM, Gallagher D, Kotler DP, Ma K, Heymsfield SB Extracellular water : sodium bromide dilution estimates compared with other markers in patients with acquired immunodeficiency syndrome. *JPEN* 1999;**23**:61-6.
- 25 - Steen B. Body composition and aging. *Nutr Rev* 1988;**46**:45-51.

### **Figure legend**

Agreement between TBW measured by  $^{18}\text{O}$  dilution and estimated by BIA at 50 kHz (Panel A) or 100 kHz (Panel B), according to Bland and Altman [19]. The y-axis is the difference between the two measurements and the x-axis is the mean of measured and estimated values.