

## Effect of equilibrated hydration changes on total body water estimates by bioelectrical impedance analysis

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The present study was performed to determine how equilibrated fluctuations in hydration affected the validity of bioelectrical impedance analysis (BIA) for body composition assessment. Total body water (TBW) expansion was induced by a 4 d endurance trial and the subsequent water loss was obtained over the recovery period. Twelve healthy men exercised on a cycle and treadmill alternately for 5 h/d over 4 d at moderate intensity. TBW, fat mass (FM) and fat-free mass (FFM) were assessed 3 d before the trial (control), and on the first and eighth day of recovery (R1 and R8 respectively). TBW was evaluated by <sup>2</sup>H dilution (TBW<sub>2H</sub>) as a reference method and by BIA (TBW<sub>BIA</sub>) at 100 kHz at the same time. TBW<sub>2H</sub> increased significantly between the control day and R1 by 1.87 (SD 1.11) litres ( $P=0.005$ ) and TBW<sub>BIA</sub> by 1.38 (SD 1.56) litres ( $P=0.009$ ). Both values returned to the control level on R8. For each period, TBW<sub>2H</sub> and TBW<sub>BIA</sub> did not differ significantly and were correlated ( $r^2$  0.85,  $P=0.0004$  for the control day;  $r^2$  0.63,  $P=0.03$  for R1;  $r^2$  0.75,  $P=0.02$  for R8). Plasma Na concentration and osmolality did not differ between the control day, R1 and R8. FFM gain (1208 (SD 1983) g) and FM loss (–1168 (SD 906) g) between the control day and R1 were followed by a FFM decrease (–624 (SD 1281) g) and a FM increase (860 (SD 1212) g) between R1 and R8. As expected, these FFM and FM changes were significantly correlated with TBW variations. The present results provide evidence that BIA may be a useful method for estimating TBW when fluid shifts are equilibrated and electrolyte concentrations are unchanged. However, it is not a valid technique for assessing FM and FFM under these conditions.

### <sup>2</sup>H dilution: Hyperhydration: Dehydration: Fat-free-mass hydration: Fat mass

Ultra-endurance exercises prolonged over several days or weeks may induce body composition changes mainly due to a loss of fat mass (FM) and to a lesser degree a loss of protein and carbohydrate mass (Ballor, 1996). A simple measurement of body mass (BM) cannot adequately quantify the FM loss. Total body water (TBW) is, in fact, known to increase transiently during such ultra-endurance events (Williams *et al.* 1979; Milledge *et al.* 1982; Fellmann *et al.* 1999) and could counterbalance FM loss, resulting in a lack of BM decrease and even, in some cases, in a BM increase.

Bioelectrical impedance analysis (BIA) has gained much attention because it is a rapid, inexpensive and non-invasive method of estimating TBW and body composition. Fat-free mass (FFM) assessed by BIA is dependent on the measurement of TBW and the use of a percentage of theoretical FFM hydration which is considered constant and equal to 0.732 (Baumgartner *et al.* 1990). The FM is subsequently calculated by subtracting FFM from BM. Consequently, body composition assessment using BIA

could be affected by two cumulative types of error. First, the TBW measurement is sensitive to confounding factors relative to the measurement conditions (time of recumbence before measurement, electrode placement, limb position) (Baumgartner *et al.* 1990). The TBW measurement is sensitive to factors dependent on the physiological state of the subject (body and skin temperatures, plasma osmolality and electrolyte concentrations) (Caton *et al.* 1988; Roos *et al.* 1992; Gudivaka *et al.* 1996; Rees *et al.* 1999). Second, since the BIA principle assumes a constant FFM hydration, the calculation of FFM from TBW is affected by body-water changes (Lukaski *et al.* 1985; Kushner *et al.* 1986). A TBW increase is thus perceived by BIA as an additional FFM resulting in an underestimation of FM values; conversely for a TBW decrease, FM will be overestimated.

It is now well documented that current BIA methodology may not provide valid estimates of TBW and consequently percentage fat measurement when acute alterations in hydration (for example, hypohydration after diuretic

**Abbreviations:** BM, body mass; BIA, bioelectrical impedance analysis; DXA, dual-energy X-ray absorptiometry; FFM, fat-free mass; FM, fat mass;  $\dot{V}O_2$ max, maximal O<sub>2</sub> uptake; R1, first day of recovery; R8, eighth day of recovery; TBW, total body water; TBW<sub>BIA</sub>, total body water as measured by bioelectrical impedance analysis.

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treatment, heat exposure and exercise, or hyperhydration using rehydration solutions) occur (O'Brien *et al.* 2002). These transient situations do not correspond to what has been observed after a 7 d endurance race (Fellmann *et al.* 1999). TBW expansion (+4.2 litres) and the subsequent water loss over the following 8 d were evaluated by BIA several hours after the cessation of the last exercise bout (more than 12 h) after the equilibrium between intra- and extracellular compartments through the membranes had occurred. Plasma osmolality and Na<sup>+</sup> concentration remained unchanged at each measurement period. To the authors' knowledge, no studies have investigated the effect of hydration changes on measurements of body composition using BIA when fluid shifts are stabilised without concomitant changes in electrolyte concentrations.

Therefore, the aim of the present study was to evaluate the effect of equilibrated fluid shifts on BIA measurements of TBW. The method used to induce this fluctuation in hydration was a 4 d period of endurance exercises which has been demonstrated to increase TBW and the subsequent water loss over the recovery period without concomitant plasma osmolality and electrolyte concentration changes (Fellmann *et al.* 1999). The amount of body water retention was evaluated by <sup>2</sup>H dilution, which was used as the reference method (Schoeller *et al.* 1980), and by BIA. The hypothesis was that BIA well tracks exercise-induced water retention changes under these conditions and, thus, is prone to errors in the estimation of FFM and FM.

## Materials and methods

### Subjects and study design

Twelve healthy men participated in the study, which was approved by the Ethics Committee on Human Research for Medical Sciences. The subjects were 28.3 ± 2.7 years old and weighed 73.4 ± 7.6 kg. All of them had received a medical examination and gave informed written consent. Two of them were well-trained triathletes and one was a cyclist (13 h training/week), and the others were recreationally active (2 to 4 h exercising/week).

The protocol was divided into three periods:

- (1) the control period undergone during the week before the endurance trial which included measurements of bioenergetics parameters, body composition and TBW;
- (2) the 4 d of exercise;
- (3) the first and the eighth day of recovery (R1 and R8 respectively) from the trial during which the same measurements as in the control period were performed with the exception of the bioenergetics measurements. During the recovery period between R1 and R8, the subjects were asked to refrain from heavy exercise.

### Bioenergetics measurements

At 4 d before beginning the trial, an infra-maximal test was performed on a treadmill (Super 2500; Gymrol, Roche La Molière, France). For the 5 min warming-up, the speed was 8.1 ± 0.2 km/h. Every 3 min, the speed was increased by 1.5 km/h until the heart rate was close to 80 % of the age-predicted maximal value. On the same day, 2 h later,

maximal O<sub>2</sub> uptake ( $\dot{V}O_{2\max}$ ) was determined on a cycle ergometer (Ergomeca, La Bayett, France). During this test, the pedalling velocity of 70 rpm was controlled using a revolution counter. For the 5 min warming-up, the workload was 102.1 ± 5.2 W. Every 3 min, the load was increased by 30 W. The exercise was stopped when the subjects were no longer able to sustain the work rate at the required pedalling. The highest  $\dot{V}O_2$  value obtained was defined as the  $\dot{V}O_{2\max}$ . During the two tests, an electrocardiogram was continuously recorded (Schiller, Wissembourg, France). The expired air volume was collected into a Douglas bag for 30 s at the end of each stage and was measured with a Tissot spirometer. CO<sub>2</sub> and O<sub>2</sub> fractions in the expired air were measured by CO<sub>2</sub> and O<sub>2</sub> analysers (CPX I D; Medical Graphics, St Paul, MA, USA). The running  $\dot{V}O_{2\max}$  was extrapolated from the relationship between  $\dot{V}O_2$  and heart rate of the submaximal test and the heart rate reached during the cycling maximal test. The relationships between  $\dot{V}O_2$  and power output, and  $\dot{V}O_2$  and running speed were also derived from these tests and used to adapt the exercise intensity on the cycle ergometer and treadmill during the exercise procedure.

### Exercise trial

The exercise period was performed in the laboratory at a moderate air temperature (22 ± 2°C). It consisted of 5 h daily exercise over 4 d, i.e. 50 min bouts three times in the morning and three times in the afternoon with a 15 min rest period in sitting position between each bout. A 2 h 15 min break was allowed for lunch. The 50 min bouts were made alternately on a cycle ergometer and a running treadmill. The cycle load and the treadmill speed, which were calculated from the data obtained during the control period, corresponded to a moderate mean exercise intensity which has been sustained in the field in a previous study and has been demonstrated to expand TBW (Fellmann *et al.* 1999). After cessation of the last exercise bout, subjects took a shower and their activities during the rest of the day consisted mainly of seated activities (reading, watching television).

### Diet

During the exercise period, food and beverages were available *ad libitum* but strictly measured. The quantity of fluid intake during meals and snacks was the sum of beverage drunk and the water content of food for 4 d.

Na mass was determined from dietary recording using GENI software with (MICRO6, Villers les Nancy, France) French (REGAL) and German (SOUCI) tables for food composition.

During the recovery period, diet was not monitored.

### Measurement conditions

The body composition measurements and the blood withdrawals were performed after an overnight fast of at least 12 h, in the same order as for the control period, R1 and R8. After voiding their bladders, subjects were weighed naked to ± 0.001 kg on a precision scale (ID1; Mettler Toledo, Columbus, OH, USA). Then a catheter was inserted

in an antecubital vein to obtain blood samples (5 ml) for biochemical analysis. Finally, TBW was measured by  $^2\text{H}$  dilution and by BIA ( $\text{TBW}_{\text{BIA}}$ ), and FM and FFM were assessed by BIA.

#### *Body composition: total body water*

**$^2\text{H}$  dilution.** After collection of a baseline urine sample, accurately weighed amount (0.15 g/kg BM) of  $^2\text{H}_2\text{O}$  (99.9 % enriched; Sigma Chemicals, Poole, UK) was taken orally by the volunteers (Ritz *et al.* 1994). Urine samples were collected again at 4, 5 and 6 h post-dose. Subjects remained fasted during the 6 h. Urine samples were kept at  $-20^\circ\text{C}$  until analysis. Before isotopic analysis, the samples were purified by vacuum sublimation and then were reduced to hydrogen at  $600^\circ\text{C}$  using the Zn technique reduction (Wong *et al.* 1987). The  $^2\text{H}:\text{H}$  ratio was measured on a dual-inlet mass spectrometer (GC-C-IRMS: MICROMASS, Manchester, UK). The mean enrichment (4–6 h post-dose) was used to calculate  $^2\text{H}$  dilution space with a 4 % correction for exchanges with non-aqueous compounds (Culebras & Moore, 1977).

#### *Bioelectrical impedance analysis*

Body impedance (resistance and reactance) were measured with an Analycor 3<sup>®</sup> impedancemeter with four electrodes at two frequencies (Eugédia, Paris, France). The last exercise period had occurred at least 15 h before the measurements. The subjects were dressed in underwear and had been in a relaxed supine position for 1 h in a temperature-controlled room ( $22^\circ\text{C}$ ) when the measurements were made on the limbs abducted from the body. The injector electrodes were placed just below the phalangeal-metacarpal joint in the middle of the dorsal side of the right hand and just below the transverse (metatarsal) arch on the upper side of the right foot. Detector electrodes were placed on the posterior side of the right wrist, midline with the prominent pisiform bone on the medial (fifth phalangeal) side and ventrally across the medial anklebone of the right ankle. The precision of the measurements was assessed on fifty healthy volunteers on whom the resistance was measured 8 h apart; the CV was shown to be 0.6 % (Vaché *et al.* 1998). The resistance and reactance were measured at 100 kHz three times.  $\text{TBW}_{\text{BIA}}$  was calculated using the specific Analycor equation (3S version 03-96, copyright A. BOULIER). The values reported in the results were the means of the three measurements.

#### *Body composition: fat mass and fat-free mass*

FM and FFM were calculated from the same resistance and reactance as obtained for  $\text{TBW}_{\text{BIA}}$  measurement using the Analycor equation (3S version 03-96, copyright A. BOULIER).

#### *Biochemical parameters*

Plasma osmolality was determined by means of the freezing-point depression method (The Advanced<sup>™</sup> Osmometer 3D3; Advanced Instruments Inc., Norwood, MA, USA).

Automatic analysers were used for the measurements of plasma  $\text{Na}^+$ , and urea concentrations (Hitachi 911; Boehringer Mannheim, France).

#### *Statistical analysis*

All values were expressed as means and standard deviations. Statistical analyses (Statview software 5.0; SAS Institute, Inc.©, Norwood, MA, USA) were performed with a one-way repeated measurement ANOVA and when an overall difference was found, individual values were compared using a paired Student's *t* test. Values of the two groups were analysed with a Bonferroni test in order to compare the three well-trained subjects with the other moderately trained subjects. Z-Fischer test correlations were also calculated. The level of statistical significance was set at  $P < 0.05$  for all analyses.

#### **Results**

The subjects had a mean  $\dot{V}\text{O}_2\text{max}$  of 52.1 (SD 7.2) ml/min per kg BM (range 45.4–65.7 ml/min per kg BM) for cycling and a mean  $\dot{V}\text{O}_2\text{max}$  of 54.1 (SD 7.6) ml/min per kg BM (range 45.1–67.7 ml/min per kg BM) for running.

All the subjects achieved the 20 h of exercise expected during the 4 d. They ran at a mean speed of 8.8 (SD 0.1) km/h (corresponding to 62.5 (SD 4.5) % running  $\dot{V}\text{O}_2\text{max}$ ) and cycled at a mean external mechanical power of 132 (SD 2) W (corresponding to 57.3 (SD 4.7) % cycling  $\dot{V}\text{O}_2\text{max}$ ).

Because no significant differences were found between the well-trained subjects (two triathletes and one cyclist) and the other moderately trained subjects for the TBW and body composition values, all the subjects were included in the same group for statistical analyses.

#### *Diet*

Mean water and  $\text{Na}^+$  intakes (food and beverage) were of 7.36 (SD 1.61) litres and 8.49 (SD 0.93) g/d over the 4 d exercise.

#### *Biochemical parameters*

The biochemical parameters are displayed in Table 1.

The plasma osmolality and  $\text{Na}^+$  concentration did not significantly change between the control period, R1 and R8. The plasma urea concentration was significantly increased between the control day and R1 (+21.7 (SD 5.0) %) and returned to its basal value in R8.

#### *Body composition: total body water*

Mean TBW measured by  $^2\text{H}$  dilution increased by 1.87 (SD 1.11) litres (4.4 (SD 2.6) %) ( $P=0.005$ ) between the control day and R1, and returned to its basal value with a decrease of 1.13 (SD 0.83) litres ( $P=0.01$ ) between R1 and R8 (Table 2).

The resistance and reactance at 100 kHz used in the specific equation for  $\text{TBW}_{\text{BIA}}$  calculation are reported in Table 2.  $\text{TBW}_{\text{BIA}}$  increased significantly by 1.38 (SD 1.56) litres (i.e. +3.2 (SD 3.4) %;  $P=0.009$ ) between the

**Table 1.** Plasma osmolality, sodium and urea on the control day and on the first (R1) and the eighth (R8) day of recovery†  
(Mean values and standard deviations)

	Control		R1		R8		ANOVA
	Mean	SD	Mean	SD	Mean	SD	
Osmolality (mosmol/kg water)	287.6	1.6	288.7	3.7	289.3	5.4	NS
Na (mmol/l)	139.9	1.6	139.0	1.3	140.0	1.6	NS
Urea (mmol/l)	6.0	0.2	7.3*	0.3	6.8	0.5	<i>P</i> <0.05

\* Mean value is significantly different from that for the control day (*P*<0.05).  
† For details of procedures, see pp. 154–155.

control period and R1 and then decreased between R1 and R8 (−0.70 (SD 0.46) litres). The R8 value was not significantly different from the control value.

The mean TBW values calculated using the two techniques did not differ significantly and were highly correlated for the control day (*r*<sup>2</sup> 0.85; *P*=0.0004), R1 (*r*<sup>2</sup> 0.63; *P*=0.03) and R8 (*r*<sup>2</sup> 0.75; *P*=0.016).

A normal power calculation was performed assuming a normal distribution two-sample equal variance for TBW<sub>BIA</sub>. It was found that for a sample size of twelve subjects, the probability that the null hypothesis would be true is 0.1321.

*Body composition: fat mass and fat-free mass*

BM did not change significantly between the control period, R1 and R8 (ANOVA; *P*>0.05) (Table 3).

FFM increased significantly between the control day and R1 (+1208 (SD 1983) g, i.e. 2.0 (SD 2.4) %; *P*=0.02) and

decreased significantly between R1 and R8 (−624 (SD 1281) g, i.e. −1.0 (SD 2.0) %; *P*=0.01). The R8 value was not significantly different from the control value. FM decreased significantly between the control day and R1 (−1168 (SD 906) g, i.e. −19.8 (SD 24.7) %; *P*<0.001). Between R1 and R8, FM increased significantly (860 (SD 1212) g, i.e. +11.6 (SD 13.8) %; *P*=0.04). FM measured on R8 was not significantly different from the control value.

As expected, the FFM and FM changes were highly correlated with the variations of TBW<sub>BIA</sub> between the control day and R1 and between R1 and R8 (Table 4).

**Discussion**

BIA remains a useful method for estimating TBW if both the stabilisation of hydration status after fluctuations have been reached, and plasma osmolality and electrolyte concentrations are unchanged. However, BIA assuming a

**Table 2.** Reactance at 100kHz (Xc 100), resistance at 100kHz (R 100) and total body water assessed by <sup>2</sup>H dilution (TBW<sub>2H</sub>) and bioimpedance analysis (TBW<sub>BIA</sub>) on the control day and on the first day (R1) and eighth day (R8) of recovery‡  
(Mean values and standard deviations)

	Xc 100 (Ω)		R 100 (Ω)		TBW <sub>BIA</sub> (litres)		TBW <sub>2H</sub> (litres)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	36	7	462	69	46.64	6.14	47.31	1.02
R1	32**	5	447*	55	48.02**	5.55	49.18*	1.07
R8	37††	8	456†	66	47.30†	5.54	48.05†	1.71

Mean value is significantly different from that for the control day: \**P*<0.05, \*\**P*<0.01.  
Mean value is significantly different from that for R1: †*P*<0.05, ††*P*<0.01.  
‡ For details of procedures, see p. 155.

**Table 3.** Body mass (BM), fat mass (FM) and fat-free mass (FFM) assessed by bioimpedance analysis on the control day and on the first (R1) and the eighth (R8) day of recovery‡  
(Mean values and standard deviations)

	Control		R1		R8		ANOVA
	Mean	SD	Mean	SD	Mean	SD	
BM (kg)	73.39	7.56	73.43	6.87	73.03	7.02	NS
FM (kg)	8.10	2.99	6.93**	3.54	7.45†	3.13	<i>P</i> <0.01
FM (%)	10.92	3.92	9.34	4.40	10.05	3.98	
FFM (kg)	65.29	6.49	66.49*	5.83	65.87†	5.18	<i>P</i> <0.05

Mean value is significantly different from that for the control day: \**P*<0.05, \*\**P*<0.01.  
Mean value is significantly different from that for R1: †*P*<0.05.  
‡ For details of procedures, see p. 155.

**Table 4.** Correlations between variations of total body water (TBW), and fat mass (FM) and fat-free mass (FFM) changes assessed by bioimpedance analysis between the control day and the first day of recovery (R1–Control), and between the first and the eighth day of recovery (R8–R1)\*

	R1–Control		R8–R1	
	$r^2$	$P$	$r^2$	$P$
TBW–FFM	0.98	<0.0001	0.99	<0.0001
TBW–FM	0.66	0.006	0.71	0.001

\* For details of procedures see p. 155.

constant FFM hydration was inappropriate for body composition estimates under these experimental conditions.

As expected, our experimental design induced a TBW expansion assessed by BIA immediately after the 4 d endurance trial (+1.38 litres) and a subsequent water loss over the 8 d of the recovery period (–0.70 litres). The reference method ( $^2\text{H}$  dilution) also provided similar results.

In theory, hyperhydration would induce a decrease in resistance compared with euhydration because relatively more fluid is available to conduct the current and, conversely, hypohydration should increase resistance. Our data are consistent with the expected results in both situations; the resistance decreased at the end of the endurance trial and increased during the recovery period (Table 2). Because a great variety of methodological and physiological factors, other than body composition, may affect the validity of BIA to assess TBW, the present study complied with optimal test conditions. Measurements were made at rest, 15 h after the last bout of exercise, on fasted subjects lying for 1 h, lightly dressed, in a room with controlled ambient temperature (22°C). Additionally, body and skin temperature drifts generally observed after exercise could represent other factors for BIA measurement errors since a decrease in resistance and FM values has been described after intense elevation of body and skin temperatures (Caton *et al.* 1988; Gudivaka *et al.* 1996). In the present study, despite the lack of such measurements, it is reasonable to postulate that skin and body temperatures had returned to their basal level 15 h after the cessation of the last exercise bout.

Conflicting results on the BIA variability when hydration status varies are reported in the literature and have been recently examined by O'Brien *et al.* (2002). Regardless of the methods used for inducing either hyperhydration and rehydration, or hypohydration, alteration of resistance either did not fit with the theory or did not correlate with change in body weight. In conclusion, these authors emphasised the importance of a constant electrolyte concentration associated with stabilised fluid shifts to accurately detect changes in water volume by BIA. These both conditions rarely exist when hydration status is altered. The main discrepancy between the present study and the previous ones would probably be explained by the difference in the hydration change procedures. In the present study, the hyperhydration (between the control day and R1) was the result of an ultra-endurance exercise while the most common strategies consist in infusion or ingestion of fluids of various compositions (O'Brien *et al.*

2002). Conversely, the loss of water (between R1 and R8) occurred over the recovery period following the exercise event whereas hypohydration status has usually been obtained after heat exposure, exercise or their combination, after dialysis or diuretic treatment (O'Brien *et al.* 2002). Since the BIA measurements were made in both situations at least 15 h after the last exercise bout, it can be hypothesised that water flux between the intra- and extracellular pools were balanced. The lack of changes in plasma osmolality and  $\text{Na}^+$  concentration gives evidence that electrolyte concentrations were stabilised.

Therefore, when changes in body water are well equilibrated and optimal measurements conditions are respected, the BIA method provides an accurate estimate of TBW. This is supported by the lack of significant difference between the mean TBW values obtained by BIA and the reference method ( $^2\text{H}$  dilution), and their significant relationships on the control day, R1 and R8.

As a consequence, during endurance events prolonged over several days which may simultaneously induce water retention and fat loss, and in some cases protein loss, BIA may not be a reliable estimator for FM. Because BIA assumes a fixed hydration constant, the changes in hydration induce errors in the estimated FFM and hence FM which is evaluated by difference between BM and FFM. Indeed, the FFM gain evaluated by BIA at the end of the 4 d of exercise (+1208 g) questions the validity of BIA because a highly catabolic hormonal environment has often been described during such endurance events (Lemon & Proctor, 1991). During a similar trial (Fellmann *et al.* 1992), it has previously been shown that plasma cortisol concentration was continuously maintained at high levels. This favours the breakdown of protein. In the present study, the significant increase of plasma urea (+22 %) (Table 1) provides indirect evidence for proteolysis. Furthermore, a decrease in protein synthesis has also been described during endurance events (Dohm *et al.* 1987; Lemon & Proctor, 1991). Therefore a FFM gain at the end of the 4 d of exercise estimated by BIA may mainly be the consequence of water retention but did not allow information on protein mass changes. This is supported by the highly significant correlations found between TBW and FFM gains during the exercise trial and loss during the recovery period (Table 4). Consequently, BIA is also prone to errors in estimating FM. The TBW variation explained 66 % of FM variation assessed by BIA between the control day and R1 and 71 % between R1 and R8. Similarly, the FFM loss over the 7 d of recovery (–624 g) seems unlikely.

At present, only a few methods are sufficiently accurate and validated for quantifying body components in the research laboratory when the hydration status varies concomitantly with fat and protein mass as occurs during endurance events prolonged over several days. The chemical analysis of carcasses is limited for most studies, and multi-compartment models are costly, laborious and require active subject involvement. Among other available body composition methods, a relatively new method, the dual-energy X-ray absorptiometry (DXA) method has been rapidly adopted for the assessment of whole-body composition. DXA has the advantage of being a three-com-

partment model that quantifies fat, soft lean tissue and bone mineral. However, recent validation studies (Tylavsky *et al.* 2003; Van der Ploeg *et al.* 2003) underline that FFM and FM deviate from the criterion four-component model. Moreover there are still important concerns about the influence of FFM hydration variation on DXA soft tissue components estimates. Some reports consider the DXA method to be unaffected by fluid-balance changes (Going *et al.* 1993; Pietrobelli *et al.* 1996; De Lorenzo *et al.* 1998; Ritz, 1998) whereas other studies suggest the opposite (Horber *et al.* 1992; Formica *et al.* 1993; Pietrobelli *et al.* 1998; Speakman *et al.* 2001). In this context, FM and FFM for the subjects in the present study have also been estimated by DXA using a Hologic QDR 4500 A (whole body 5.55 software version; Hologic, Inc., Zaventem, Belgium). Unlike for BIA, no correlation could be found between TBW and FM changes. These findings are consistent with previous *in vitro* and *in vivo* studies. Going *et al.* (1993) also reported that, in human subjects, FM results assessed by DXA did not change significantly after both oral water ingestion and dehydration of 1.5 litres while 98 % of FFM variation was explained by water change. For the same magnitude of TBW variation as in the present study, Pietrobelli *et al.* (1996) did not find a significant change of FM results in subtracting 1 kg of extracellular fluid to the phantom (reference man). Our results are also in line with a previous study using a DXA hydration model (Pietrobelli *et al.* 1998). These authors demonstrated that the magnitude of DXA fat errors was small (< 1 %) with hydration changes of 1–5 % which are similar to the present experimental conditions (the range of water variation observed was –2.5 to +6.5 %). These preliminary results indicate that DXA could be less influenced by hydration status changes than BIA for FFM and FM assessment. However this hypothesis needs further investigation using comparison with the criterion four-compartment model before validating this method for body composition assessment under these conditions.

In conclusion, the present study has practical implications because the results demonstrate that BIA may be a useful method for estimating TBW variation if equilibrium between the fluid compartments has been reached and electrolyte concentrations are unchanged. However BIA is not a valid technique for assessing body composition when hydration status, FFM and FM do simultaneously vary.

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