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ORIGINAL ARTICLE

Evaluation of air-displacement plethysmography and bioelectrical impedance analysis vs dual-energy X-ray absorptiometry for the assessment of fat-free mass in elderly subjects

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Objective: To evaluate air-displacement plethysmography (ADP) and bioelectrical impedance analysis (BIA) vs dual-energy X-ray absorptiometry (DXA) for the assessment of fat-free mass (FFM) in healthy elderly subjects.
Subjects: Forty-two women and twenty-six men aged 60–84 years.
Methods: FFM was measured by DXA and ADP. Body impedance (Z) was measured by four-polar BIA and the impedance index (ZI) was calculated as stature^2/Z. Selection of predictors (gender, age, weight and ZI at 5, 50 and 100 kHz) for BIA algorithms was carried out using bootstrapped stepwise linear regression on 1000 samples of 68 subjects. Limits of agreement were used as measures of interchangeability of ADP and BIA with DXA.
Results: The limits of agreement of ADP vs DXA were −11.0 to 2.4 kg in males and −4.8 to 2.2 kg in females. Gender, weight and ZI_{100} were selected as predictors of FFM by bootstrapped stepwise linear regression. In males, ZI_{100} (−12.2 to 12.2 kg) was much less accurate than weight (−6.0 to 6.0 kg) at predicting FFM and their combination did not improve the estimate (−6.0 to 6.0 kg). In females, ZI_{100} (−6.8 to 6.8 kg) was less accurate than weight (−5.6 to 5.6 kg) at predicting FFM and their combination improved the estimate only slightly (−5.0 to 5.0 kg).
Conclusions: In healthy elderly subjects, (1) ADP and DXA are not interchangeable for the assessment of FFM, especially in males; and (2) ZI_{100} is not superior to weight for the prediction of FFM and their combination is of little advantage and only in females.
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Introduction

Aging is associated with an increase in fat mass (FM) and a decrease in fat-free mass (FFM) (Roubenoff, 2000; Kyle *et al.*, 2001). While the health implications of FM expansion are known from many years, those of FFM depletion have started to be investigated only recently (Kennedy *et al.*, 2004; Jensen, 2005; Kyle *et al.*, 2005; Villareal *et al.*, 2005). For instance, obese elderly subjects with depleted FFM have more health problems than those without FFM depletion (Kyle *et al.*, 2005). Epidemiological studies are needed to test the functional relevance of the age-related loss of FFM. An accurate assessment of FFM requires sophisticated and expensive multicompartment models, which are not feasible for epidemiological studies (Pietrobelli *et al.*, 2001). Calibration of indirect techniques against direct methods provides the most common means of performing epidemiological studies of body composition (Heymsfield *et al.*, 2000).

Dual-energy X-ray absorptiometry (DXA) compares well with reference body composition methods and is

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increasingly used to evaluate body composition and to calibrate indirect techniques (Gallagher *et al*., 1997; Salamone *et al*., 2000; Malavolti *et al*., 2003). Air-displacement plethysmography (ADP) is a relatively new technique for the assessment of body composition (Fields and Hunter, 2004; Fields *et al*., 2005). ADP is less invasive than water-displacement plethysmography, which requires immersion into water and, contrarily to DXA, does not expose to ionizing radiations. ADP has thus a great potential for assessing body composition in the elderly, but few validation studies are available so far (Yee *et al*., 2001; Bosy-Westphal *et al*., 2003; Fields and Hunter, 2004; Alemán-Mateo *et al*., 2007). Bioelectrical impedance analysis (BIA) is presently regarded as the best option for the assessment of FFM in epidemiological studies (Heymsfield *et al*., 2000; Chumlea *et al*., 2002; Malavolti *et al*., 2003). BIA is in fact noninvasive, portable, rapid and inexpensive. However, BIA must be calibrated against direct methods before it can be employed for field studies (Guo *et al*., 1996).

The present study aimed at evaluating ADP and BIA vs DXA for the assessment of body composition in a convenience sample of healthy elderly subjects.

Materials and methods

Subjects

Eligible for the study were white Caucasian subjects of both genders fulfilling the following criteria: (1) age $\geqslant$60 years, (2) body mass index (BMI) $\geqslant$18.5 and $\leqslant$40 kg/m^2, (3) absence of acute and chronic disease, as determined by clinical history and physical examination. A total of 68 subjects (42 females and 26 males) were recruited through advertisements on local newspapers. The study procedures had been approved by the local ethical committee and all subjects gave informed consent. All measurements were carried out after an overnight fast.

Anthropometry. Body weight was measured using the balance incorporated into the ADP unit (see below) and stature using a stadiometer. BMI was calculated as weight (kg)/stature (m)^2.

BIA

Whole-body impedance (Z) was measured on the left side of the body with a four-polar impedance meter (Human IM Scan, DS-Medica, Milano, Italy) at frequencies of 5, 50 and 100 kHz following international guidelines (Deurenberg, 1994). The coefficient of variation (CV) for BIA measurements in our laboratory is 2.0%, as determined by three repeated weekly measurements of three adult subjects with daily body weight variations $\leqslant$1.0%. The impedance index (ZI) was calculated as stature (cm)^2/Z (Ω).

DXA

FFM (lean tissue mass + bone mineral content) and FM were measured using a Lunar DPX-L densitometer and adult software version 3.6 (Lunar Corporation, Madison, WI, USA). The CV for FFM and FM measurements in our laboratory is 3.0%, as determined by three repeated weekly measurements of three adult subjects with daily body weight variations $\leqslant$1.0%.

ADP

Body weight, body volume and thoracic gas volume were measured using a BOD POD unit (Life Measurement Inc., Concorde, CA, USA). FFM and FM were obtained from body density (BD) using Siri's formula. Subjects wore Lycra swimsuits and caps during measurements, as suggested by the manufacturer. The CV for BD in our laboratory is 2.0%, as determined by three repeated weekly measurements of three adult subjects with daily body weight variations $\leqslant$1.0%.

Statistical analysis

Continuous variables are given as means and standard deviations (s.d.). Selection of variables for FFM prediction algorithms was carried out by stepwise bootstrapped linear regression on 1000 random samples of 68 subjects (Harrell, 2001). Candidate predictors were gender, age, weight and ZI at frequencies of 5, 50 and 100 kHz (Bedogni *et al*., 2003). Predictors identified at bootstrap analysis were entered into multiple regression models with standard errors of coefficients calculated by bootstrap analysis on 1000 random samples of 68 subjects (Gonçalves and White, 2005). Bland and Altman method was used to calculate the limits of agreement between ADP and DXA for the assessment of FFM and Pitman test was used to evaluate proportional bias (Ludbrook, 2002). Bias was defined as the difference between FFM measured by ADP or estimated by BIA and FFM measured by DXA. Statistical significance was set to a two-tailed P-value <0.05. Statistical analysis was carried out using STATA 9.2 (StataCorp, College Station, TX, USA).

Results

Table 1 gives the characteristics of the 68 subjects, 42 females and 26 males, who were studied.

The subjects were aged from 60 to 84 years and had a great variability in BMI (18.5–39.5 kg/m^2) and FM as determined by DXA (23.5–34.9%). Males were heavier ($P<0.0001$) and taller ($P<0.0001$) and had higher values of BMI ($P=0.0029$) and lower values of Z ($P\leqslant0.0186$) and percent FM ($P<0.0001$) than females. Twelve subjects (six males and six females) were obese.

The mean (s.d.) difference between body mass (FM + FFM) measured by DXA and body weight measured by ADP was

Table 1 Measurements of the study subjects

	Females (n = *42*)	*Males* (n = *26*)	P-*value*
Age (years)	68 (5) [60–84]	67 (4) [60–78]	0.6079
Weight (kg)	64.5 (10.4) [45.5–91.0]	86.9 (13.4) [63.1–113.9]	<0.0001
Stature (m)	1.58 (0.06) [1.43–1.75]	1.73 (0.06) [1.60–1.86]	<0.0001
BMI (kg/m^2)	25.8 (3.7) [18.5–33.0]	28.9 (4.4) [19.0–39.5]	0.0029
FM_{DXA} (kg)	24.3 (7.6) [12.4–41.4]	23.7 (7.2) [9.5–36.5]	0.7658
FM_{DXA} (%)	36.9 (6.8) [23.5–53.6]	26.8 (4.8) [15.1–34.9]	<0.0001
FFM_{DXA} (kg)	39.2 (4.2) [32.5–48.4]	62.6 (6.8) [52.2–79.3]	<0.0001
FFM_{ADP} (kg)	37.9 (4.7) [29.6–49.1]	58.3 (6.2) [49.5–75.4]	<0.0001
Z_5 (Ω)	605 (60) [464–718]	569 (49) [488–685]	0.0110
Z_{50} (Ω)	542 (62) [406–654]	508 (46) [452–612]	0.0186
Z_{100} (Ω)	514 (51) [403–614]	475 (41) [417–591]	0.0019

Abbreviations: ADP, air-displacement plethysmography; BMI, body mass index; DXA, dual-energy X-ray absorptiometry; FM, fat mass; FFM, fat-free mass; Z_x, body impedance at a frequency of *x* (in kHz).
Values are means, s.d. (parentheses) and ranges (brackets).
Between-group comparisons were carried out using Student's *t*-test.

−0.8 (0.2) kg ($P<0.0001$). This difference is low and unlikely to be of practical relevance (Bedogni *et al.*, 2001).

To identify predictors for BIA algorithms, we carried out a bootstrapped stepwise linear regression using FFM_{DXA} as the outcome variable and gender, age, weight, ZI_5, ZI_{50} and ZI_{100} as predictors. Gender and weight were predictors in 1000 out of 1000 bootstrap samples, ZI_{100} in 442, ZI_5 in 427, age in 418 and ZI_{50} in 181. Thus, we choose gender, weight and ZI_{100} as predictors for BIA algorithms and developed three prediction models for FFM_{DXA}: (1) based on ZI_{100} and gender (not shown), (2) based on weight and gender (not shown) and (3) based on ZI_{100}, weight and gender (Table 2). The 95% confidence intervals of the regression coefficients of model (3) were calculated using bootstrap analysis. On the basis of the standardized regression coefficients, gender was the strongest predictor of FFM, followed by weight and ZI_{100}.

Table 3 gives the mean, s.d. and limits of agreement of the bias and the Pitman test for ADP, ZI_{100}, weight, ZI_{100} and weight vs DXA and Figure 1 gives the corresponding Bland–Altman plots.

The 95% limits of agreement of ADP vs DXA were wider in males (−11.0 to 2.4 kg) than in females (−4.8 to 2.2 kg). There was no evidence of proportional bias for any gender.

In males, the estimate of FFM obtained from ZI_{100} (−12.2 to 12.2 kg) was more biased than that obtained from weight (−6.0 to 6.0 kg), and the combination of ZI_{100} and weight (−6.0 to 6.0 kg) did not reduce the bias as compared to weight alone. In females, the prediction obtained from ZI_{100}

Table 2 Prediction equation of FFM from gender, weight and the impedance index at 100 kHz

	β	*95% CI* β *(bootstrap)*	P-*value*	*Standardized* β *(bootstrap)*
Male gender	13.163	10.916–15.409	0.0001	6.289
Weight (kg)	0.363	0.302–0.425	0.0001	5.463
ZI_{100} (cm^2/Ω)	0.141	0.034–0.248	0.0170	1.205
Intercept	8.770	3.062–14.478	0.0030	—

Abbreviations: *β*, regression coefficient; CI, confidence interval; ZI_{100}, impedance index at 100 kHz.

(−6.8 to 6.8 kg) was slightly more biased than that obtained from weight (−6.0 to 6.0 kg), and the combination of ZI_{100} and weight (−5.0 to 5.0 kg) improved the prediction only slightly. In most cases, the prediction of FFM from weight, ZI_{100} or their combination was associated to a negative proportional bias.

Discussion

We evaluated the agreement between ADP and BIA vs DXA for the assessment of FFM in a convenience sample of healthy elderly subjects. The main rationale for doing this is that, contrary to DXA, ADP does not expose to ionizing radiations and BIA is more portable than both DXA and ADP. A good agreement of ADP and BIA with DXA would imply the possibility of using them as surrogates of DXA in epidemiological studies of the elderly. Our study suggests however that neither ADP nor BIA is interchangeable with DXA, especially in males.

The mean (s.d.) bias of ADP vs DXA was −4.3 (3.4) kg in men and −1.3 (1.7) kg in women. Even if the mean bias was significantly different from 0 in both cases, the limits of agreement were much better in females (−4.8 to 2.2 kg) than in males (−11.0 to 2.4 kg). Our outcome variable (FFM) differs from that commonly employed in validation studies of ADP (percent FM), but a comparison with the available literature is nonetheless important. In a study of 26 elderly Caucasians, Bosy-Westphal *et al.* (2003) obtained a mean (s.d.) bias of 2.5 (3.2)% in females and one of 2.6 (3.5)% in males for the assessment of percent FM by ADP vs DXA. Alemán-Mateo *et al.* (2007) validated ADP against a four-compartment model for the assessment of percent FM in 202 elderly Mexicans and found higher limits of agreement in males (−6.3 to 8.3%) than in females (−3.1 to 5.1%). In the study of Bosy-Westphal *et al.* (2003), however, the validation of ADP vs a four-compartment model gave a greater bias in females than in males (2.9 (2.4) vs −0.1 (3.0)%, mean (s.d.)). Taken together, these data show that gender is likely to influence the interchangeability of ADP with other body composition methods in the elderly.

In our study, gender influenced also the agreement between FFM estimated by BIA and FFM measured by DXA.

Table 3 Agreement between ADP and DXA and BIA and DXA for the assessment of FFM

	Mean bias (kg)	*s.d. bias (kg)*	P-*bias*	*ULA (kg)*	*LLA (kg)*	r-*Pitman*	P-*Pitman*
ADP vs DXA–males	−4.3	3.4	<0.0001	−11.0	2.4	−0.18	0.390
ADP vs DXA–females	−1.3	1.7	<0.0001	−4.8	2.2	0.26	0.096
ZI_{100} vs DXA–males	0.0	6.6	1.0000	−12.2	12.2	−0.85	0.004
ZI_{100} vs DXA–females	0.0	3.4	1.0000	−6.8	6.8	−0.65	0.001
Wt vs DXA–males	0.0	3.0	1.0000	−6.0	6.0	−0.55	0.026
Wt vs DXA–females	0.0	2.8	1.0000	−5.6	5.6	−0.07	0.638
ZI_{100} and Wt vs DXA–males	0.0	3.0	1.0000	−6.0	6.0	−0.57	0.022
ZI_{100} and Wt vs DXA–females	0.0	2.5	1.0000	−5.0	5.0	0.02	0.923

Abbreviations: ADP, air-displacement plethysmography; DXA, dual-energy X-ray absorptiometry; LLA, lower limit of agreement; ULA, upper limit of agreement; Wt, weight; ZI_{100}, impedance index at 100 kHz.

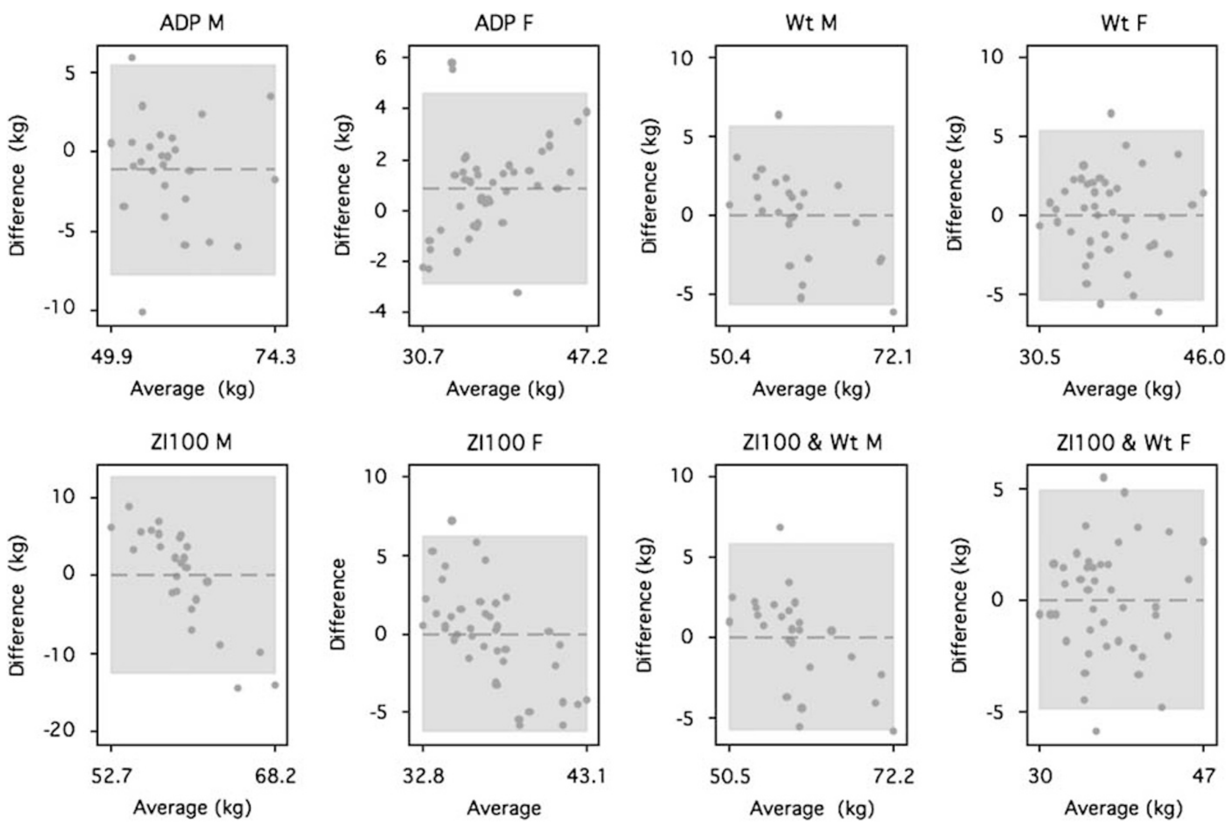

Figure 1 Bland–Altman plots of FFM measured by air-displacement plethysmography (ADP) and estimated by BIA, weight and their association vs FFM measured by DXA. Gray areas indicate 95% limits of agreement. Abbreviations: M, males; F, females; ZI_{100}, impedance index at 100 kHz; Wt, weight.

The limits of agreement of FFM estimates obtained from ZI_{100} were two times wider in males (−12.2 to 12.2 kg) than in females (−6.8 to 6.8 kg) and in both cases there was evidence of negative proportional bias. As we have observed in many of our studies of four-polar BIA (Bedogni *et al.*, 2003), weight was more accurate than ZI at predicting FFM. The only advantage of the combination of weight and ZI_{100} was a modest decrease of the bias in females (from −5.6 to 5.6 kg to −5.0 to 5.0 kg). Thus, even if ZI_{100} was a predictor of FFM independent of gender and weight (Table 2), its practical contribution to the estimate of FFM was quite low.

It is important to note that ADP outperformed ZI_{100} for the prediction of FFM in both males and females, even if its performance in males was not acceptable for the reasons stated above. Somewhat surprisingly, however, ADP was not superior to weight for assessing body composition in males, especially considering that it was superior also to the combination of ZI_{100} and weight in females. Even if the interindividual variability of FFM was similar in males and females, as detected by a CV of 11% for both genders, differences in FFM composition may be the main reason for the different performance of ADP and BIA in males and females (Bosy-Westphal *et al*., 2003).

This study has several limitations. First, it was carried out in a convenience sample of individuals who self-referred to our center so that its findings cannot be extended to the general population. Second, our subjects were in a good state of health so that our results cannot be generalized to ill subjects. Third, a four-compartment model of body composition is clearly preferable to DXA as a reference method to evaluate ADP (Pietrobelli *et al*., 2001). However, it is important to know the interchangeability of DXA and ADP because both are increasingly used in the epidemiological field. On the contrary, a point of strength of the study is the high variability of the subjects' body composition. Such variability provides in fact a hard test for the accuracy of body composition techniques (Brambilla *et al*., 2006).

In conclusion, in healthy elderly subjects (1) ADP and DXA are not interchangeable for the assessment of FFM, especially in males and (2) ZI_{100} is not superior to weight for the prediction of FFM and their combination is of little advantage and only in females.

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