Eur J Nutr 42: 207–216 (2003)
DOI 10.1007/s00394-003-0416-4

ORIGINAL CONTRIBUTION

A. Bosy-Westphal
M. Mast
C. Eichhorn
C. Becker
D. Kutzner
M. Heller
M. J. Müller

Validation of air-displacement plethysmography for estimation of body fat mass in healthy elderly subjects

Summary *Background* Air-displacement plethysmography (ADP) is a non-invasive method for body composition analysis that divides the body into fat-free mass (FFM) and fat mass (FM) (= 2 compartment model, 2C). It places low demands on subject performance and is therefore most convenient in the elderly. *Objective* To validate ADP against dual energy X-ray absorptiometry (DEXA) and to compare it to a four-compartment model of body composition (4C; fat mass, total body water, bone mineral content and residual mass) in the elderly. *Methods* Body composition was assessed by ADP, DEXA and bioelectrical impedance analysis (BIA) in 26 healthy elderly subjects (15 women, 11 men) aged 60–82 years. *Results* Despite a high correlation of %FM assessed by ADP and DEXA we observed significant differences between the results of these methods for both sexes (2.5 ± 3.4 %; bias ± SD). Deviations of $\%FM_{ADP}$ from $\%FM_{DEXA}$ were dependent on bone mineral content (BMC_{DEXA}) fraction of FFM. A low BMC_{DEXA} was related to an overestimation of DEXA-derived %FM by ADP. There was a systematic bias between results from ADP and the 4C model. 76 % of its variance was explained by the assumption of a fixed density of FFM. 96 % of the variance in the density of FFM was explained by water content and only 4 % by BMC_{DEXA} of FFM. When compared to a 4C model, overestimation of $\%FM_{ADP}$ increases with increasing water fraction of FFM. *Conclusion* Although there is a tendency for overestimation of $\%FM_{ADP}$, ADP is a valid method for body composition measurement in the elderly. The bias in $\%FM_{ADP}$ is mainly related to water content of FFM and indicates that a correction factor for TBW may improve the accuracy of the ADP measurements in the elderly.

Key words ageing – body composition – air-displacement plethysmography – dual energy X-ray absorptiometry – bioelectrical impedance analysis

Received: 25 November 2002
Accepted: 20 February 2003

Dr. A. Bosy-Westphal · Dr. M. Mast ·
C. Eichhorn · Dr. C. Becker ·
Univ. Prof. Dr. med. Manfred J. Müller (✉)
Institut für Humanernährung
& Lebensmittelkunde
Christian-Albrechts-Universität zu Kiel
Düsternbroker Weg 17
24105 Kiel, Germany
Tel.: +49-431/8805670
Fax: +49-431/8805679
E-Mail: mmueller@nutrfoodsc.uni-kiel.de

Dr. med. D. Kutzner ·
Univ. Prof. Dr. med. M. Heller
Klinik für Radiologische Diagnostik
Christian-Albrechts Universität zu Kiel
Kiel, Germany

Abbreviations

ADP	air-displacement plethysmography
DEXA	dual energy X-ray absorptiometry
BIA	bioelectrical impedance analysis
FM	fat mass
FFM	fat-free mass
TBW	total body water
ICW	intracellular water
ECW	extracellular water
BMC	bone mineral content
LTM	lean tissue mass
MM	skeletal muscle mass
D	density
BMI	body mass index
R	resistance
Xc	reactance
HD	hydrodensitometry

EJN 416

European Journal of Nutrition, Vol. 42, Number 4 (2003)

Introduction

Age-dependent changes in body composition comprise progressive losses in bone and muscle mass as well as an increasing proportion of fat mass (FM). These alterations impair physical functional status, quality of life and increase the risk of comorbidities [1]. Body composition research may add to a better understanding of these age-related changes.

Densitometry is a well-established standard in the assessment of body composition. It is used as a reference for field methods like anthropometry and bioelectrical impedance analysis (BIA). When compared to hydrostatic weighing, the new air-displacement plethysmography (ADP) device provides a comfortable and simple measurement procedure that is more convenient for elderly or disabled subjects. The system consists of a plethysmograph, electronic weighing scale, calibration weights and cylinder, computer and software. The plethysmograph is divided into two chambers: a test chamber (for the subject) and a reference chamber. Briefly, the operating principle is based on an oscillating diaphragm between the chambers producing volume perturbations and pressure changes. The ratio of the pressures is a measure of the test chamber volume that is altered by the subject.

A recent review on validity and reliability of ADP summarized that ADP agreed well with hydrodensitometry (HD) and dual energy X-ray absorptiometry (DEXA) (within 1 %FM for adults), as well as with multicompartment models (underestimation of %FM by 2–3 % in adults [2]). However, studies using HD in the elderly have found a substantial overestimation in %FM when compared with four compartment (4C; fat mass, total body water, bone mineral content and residual mass) models. These differences were related to the underlying assumptions of the densitometric 2C model (i. e., a constant composition and density of FFM) and may limit the value of densitometric assessments of body composition in the elderly [3–5].

To our knowledge there are only a few studies specifically investigating the validity of ADP in the elderly [6, 7]. In addition there are further studies addressing subjects of a wide age range [8, 9]. In contrast to earlier results from HD, Yee et al. [6] reported no significant difference between $\%FM_{ADP}$ and the $\%FM_{4C}$ in the elderly. Koda et al. [7] also reported a good agreement between $\%FM_{ADP}$ and $\%FM_{DEXA}$ in elderly women but not in men. There are some methodological differences between these studies, i. e., use of DEXA or 4C as a reference method, measurement of TBW by deuterium dilution or BIA as well as statistical analysis of the differences in %FM. Because of ethnical differences in body composition [10] the validation study of Koda et al. [7] in Asian subjects may not be transferable to European subjects. The observed discrepancies in the results and methodology of previous studies as well as population-based peculiarities warrant an additional validation study of ADP in the elderly.

In the present study ADP was applied for measurement of %FM in healthy elderly volunteers. It was validated against results of DEXA and compared to a 4C model based on density of the whole body measured by ADP ($D_{\text{whole body ADP}}$), total body water ($TBW_{BIA}$) as well as bone mineral content assessed by DEXA (BMC_{DEXA}). The 4C model differentiates fat mass, total body water, bone mineral content and residual mass. As deviations from the assumed constant composition of FFM are the main drawback for the validity of HD in the elderly [4], the influence of the variance of the density of FFM (D_{FFM}) on deviations in $\%FM_{ADP}$ from $\%FM_{4C}$ was quantified. We hypothesized that there is a bias in %FM estimation by ADP in the elderly that is mainly due to the limitations of the underlying 2C model. Therefore the age-related decrease in muscle mass will contribute to an increase in D_{FFM} and a concomitant underestimation of %FM by ADP. By contrast, the age-related loss in BMC leads to a lower D_{FFM} and a consecutive overestimation of $\%FM_{ADP}$. An attempt was made to provide some practical advice for an a priori identification of subjects with a substantial error of %FM estimation by ADP.

Subjects and methods

The study group consisted of 26 elderly Caucasian subjects (15 females and 11 males) aged between 60 and 82 years (mean age 67.7 ± 6.6 years) who were recruited by noticebord postings in local supermarkets. All participants were apparently healthy, weight stable and underwent basal physical examination (heart rate, blood pressure, blood glucose and lipid profile). Ten participants (38.5 %) had an elevated blood pressure defined as > 140/90 mmHg (mean values for the hypertensive subjects 161 ± 12 mmHg systolic blood pressure, 90 ± 6 mmHg diastolic blood pressure). One subject received antihypertensives. The prevalence of hypertriacylglycerolemia (> 2.29 mM) and hypercholesterolemia (> 5.2 mM) was 11.5 % and 38.5 %, respectively. One subject took a fibrate to control plasma lipoprotein concentrations. Dieting or physical exhaustion were avoided 7 days prior to the study. The study protocol was approved by the local ethical committee of the Christian-Albrechts University Kiel. Each subject provided informed written consent before participation.

Body composition analysis. Examinations took place between 7:00 and 9:00 o'clock in the morning after an overnight fast at the metabolic ward of the Institute for Human Nutrition. Height was measured to the nearest 0.5 cm with a stadiometer. Weight was assessed by an electronic scale coupled to the BOD POD® Body Composition System (Life Measurement Instruments, Concord, CA, USA).

2C model. Air-displacement plethysmography was performed by the BOD POD device as follows. A two-step calibration was carried out before each measurement. In the first step, the volume of the empty chamber, in the second step the volume of a 50-L-calibration cylinder was measured. When entering the BOD POD device all participants wore tight-fitting underwear (i. e., brassiere and pants) and a swim cap. They were instructed to sit motionless during the 50-s body volume measurement. Two repeated volume measurements were performed and averaged for further data analysis. Subsequently thoracic lung volume was also measured by the BOD POD device and subtracted from the body volume. For this part of the test, subjects were prompted to puff gently against an occluded airway while changes in body volume were recorded. In 5 subjects (2 females and 3 males) a valid measure could not be obtained after three trials and the volume of thoracic gas was predicted by the BOD POD software (version 1.69), which also calculated body density as body weight divided by body volume at the end of each test. Measured and predicted thoracic gas volume were compared in the remaining 21 subjects. Mean difference in measured and predicted lung volume was not significant (–0.3 ± 1.2 l; $p = 0.24$). Percentage of FM_{ADP} was calculated using Siri's equation (Eq. 1) [11]:

$$FM_{ADP}\,(\%) = (495/D_{ADP}) - 450 \qquad (1)$$

The CV for 3 repeated measurements of body and lung volume in six men were 0.14 ± 0.11 l and 6.49 ± 8.02 ml resulting in a CV of 2.8 ± 2.9 % of %FM. Differences between measured and predicted lung volume resulted in a non-significant deviation in %FM (–0.4 ± 3.1 %; $p = 0.57$).

Dual energy X-ray absorptiometry (DEXA) was performed using a Hologic whole body absorptiometer (Hologic QDR 4500A, Hologic, Waltham, MA, USA). Subjects lay supine with arms and legs at their sides during the 10-minute scan. All scans were performed by a licensed radiological technician. Manufacturers software version V8.26a:3 was used for analysis of bone mineral content (BMC_{DEXA}), lean tissue mass (LTM_{DEXA}) and FM_{DEXA}, respectively. Fat-free mass (FFM_{DEXA}) resembles LTM_{DEXA} plus BMC_{DEXA} and was calculated as body weight minus FM_{DEXA}. Skeletal muscle mass (MM_{DEXA}) was calculated by the sum of appendicular LTM_{DEXA} (e. g. LTMarms + legs).

4C model. Bioelectrical impedance analysis (BIA) was performed as described previously [12]. We used a body bioelectrical impedance analyser (BIA 2000-S, Data Input, Frankfurt, Germany) and the formula of Visser et al. for data analysis [13]. This algorithm has been developed in European subjects aged 63–87 years based on isotope dilution. Intracellular and extracellular water (ICW_{BIA}, ECW_{BIA}) were estimated from phase angle derived by multifrequency data analyzed by manufactures software (Nutri 1.9 Europa Version; Data Input, Frankfurt, Germany).

Estimation of %FM by a 4C model was done applying Selinger's equation (Eq. 2) [14]:

$$FM_{4C}\,(\%) = [(2.747/D_{ADP}) - (0.714 \times TBW_{BIA}) + (1.146 \times BMC_{DEXA}) - 2.0503] \times 100 \qquad (2)$$

TBW_{BIA} and BMC_{DEXA} are given as a fraction of body weight.

Density of FFM was calculated using the following equation:

$$D_{FFM} = 1/(TBW_{BIA}/D_{water}) + (Mineral_{DEXA}/D_{minerals}) + (Protein_C/D_{protein}) \qquad (3)$$

TBW_{BIA}, $Mineral_{DEXA}$ and $Protein_C$ are given as a fraction of FFM_{DEXA}. $Mineral_{DEXA}$ is calculated according to Prior et al. [15] as BMC_{DEXA} times 1.2741 supposing that BMC_{DEXA} approximates bone ash [16]. This is the sum of total bone mineral without the components lost in ashing. The constant 1.2741 presumes that 4 % of bone mineral is lost during the ashing process [17] and that nonosseous mineral mass is 23 % of bone mineral ash [18]. Non-lipidic organic components are termed $Protein_C$ as protein is the main component. $Protein_C$ is calculated as $FFM_{DEXA} - TBW_{BIA} - (BMC_{DEXA}$ x 1.2741). Densities of water, minerals and protein are 0.9937, 3.038 and 1.34 g/cm^3, respectively.

Data analyses. All data are given as mean ± SD. Statistical analyses were performed using SPSS for Windows 8.0. The Mann-Whitney U-test was used for comparisons between sexes. Pearson's correlation coefficients were calculated for relationships between variables. Results for %FM by the 2C, DEXA and 4C model were analyzed for significant differences by the nonparametric Wilcoxon signed ranks test for related samples. A Bland-Altman plot was also used for comparison of different compartment methods for estimation of %FM [19]. A stepwise multiple regression analysis was performed to explain the variance in calculated D_{FFM}. All tests were two-tailed and a p-value of 0.05 was accepted as the limit of significance.

Results

Characteristics of the study population are given in Table 1. Significant differences for sex were observed for all measured parameters except age, body weight, body mass index (BMI), reactance and phase angle of bioimpedance analysis.

Table 1 Characteristics of the study population

	26 elderly subjects			
	All	female (15)	male (11)	p-value*
Age, years	68.2 ± 5.3	68.9 ± 5.2	67.3 ± 5.1	n. s.
Weight, kg	74.0 ± 13.1	70.0 ± 8.5	79.3 ± 15.6	n. s.
Height, m	1.67 ± 0.09	1.62 ± 0.06	1.74 ± 0.09	< 0.01
BMI, kg/m^2	26.4 ± 3.2	26.5 ± 2.6	26.1 ± 3.9	n. s.
R, Ω	570.9 ± 79.6	601.9 ± 59.1	528.6 ± 80.8	< 0.05
Xc, Ω	54.8 ± 7.9	56.9 ± 7.1	52.0 ± 7.7	n. s.
Phase angle, degrees	5.8 ± 1.5	5.4 ± 0.5	6.5 ± 2.0	n. s.
ICW/ECW	1.4 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	< 0.01
FFM_{DEXA}, kg	49.7 ± 12.2	42.4 ± 5.1	59.7 ± 11.4	< 0.01
MM_{DEXA}, kg	20.0 ± 5.4	16.5 ± 2.1	24.8 ± 4.6	< 0.001
BMC_{DEXA}, kg	2.3 ± 0.5	2.0 ± 0.3	2.8 ± 0.5	< 0.001
$D_{whole\ body\ ADP}$, g/cm^3	1.020 ± 0.02	1.038 ± 0.01	1.007 ± 0.01	< 0.001
FM_{ADP}, %	35.6 ± 9.3	41.7 ± 5.7	27.2 ± 5.6	< 0.001
FM_{DEXA}, %	33.1 ± 8.6	39.2 ± 4.9	24.6 ± 3.1	< 0.001
FM 4C, %	33.8 ± 7.4	38.8 ± 4.6	26.9 ± 4.0	< 0.001
ΔFM_{ADP}-FM_{DEXA}, %	2.5 ± 3.4	2.5 ± 3.2	2.6 ± 3.5	n. s.
ΔFM_{ADP}-FM 4C, %	1.6 ± 3.1	2.9 ± 2.4	–0.1 ± 3.0	< 0.05

* sex differences, Mann-Whitney U test; Data are given as means ± SD
BMI Body Mass Index; *BIA* Bioelectical Impedance Analysis; *R* resistance; *Xc* reactance; *ICW* intracellular water; *ECW* extracellular water; *FFM* fat-free mass; *FM* fat mass; *DEXA* Dual-energy X-ray absorptiometry; MM_{DEXA} skeletal muscle mass; BMC_{DEXA} bone mineral content; *ADP* Air-Displacement Plethysmography; $D_{whole\ body}$ body density measured by ADP; FM_{ADP} fat mass calculated according to Siri (Eq. (1) [11]); *FM 4C* fat mass calculated according to Lohman (Eq. (2) [14])

Estimates of $\%FM_{ADP}$ were 1.6 ± 3.1 % and 1.0 ± 2.8 % higher than $\%FM_{DEXA}$ or the $\%FM_{4C}$. The bias between $\%FM_{ADP}$ and $\%FM_{DEXA}$ was significant for both sexes ($p < 0.01$). The bias between $\%FM_{ADP}$ and $\%FM_{4C}$ was significant only in females ($p < 0.01$). In Fig. 1 the regression plots for $\%FM_{ADP}$ vs. $\%FM_{DEXA}$ and $\%FM_{ADP}$ vs. $\%FM_{4C}$ are given. Despite the consistency of the results from different methods Bland-Altman plot (Fig. 2) shows (i) a mean overestimation of $\%FM_{ADP}$ compared to the 4C model as well as (ii) a systematic error between %FM estimated by the 2C and 4C model. Thus, the 2C model overestimates the 4C-derived data with increasing %FM. In Bland-Altman analysis the 95 % level of agreement (± 2SD) between ADP and DEXA or 4C model was 6.8 % and 6.2 %, respectively.

The composition and density of FFM (D_{FFM}) are shown in Table 2. Significant sex differences were observed in water and protein fraction but not for mineral content of FFM_{DEXA}. Mean water content of FFM_{DEXA} was closer to the assumed value of 73.2 % [37] in females (74.9 %) than in males (68.2 %). Since mineral content of FFM_{DEXA} was lower than the standard 6.8 % [18] the fraction of protein exceeded the assumed value of 19.4 % [18] in men. In 9 subjects (7 women and 2 men) bone mineral density was reduced when compared with age- and sex-specific reference ranges [20]. Calculated D_{FFM} was higher in men than in women due to a lower water and higher protein fraction of FFM_{DEXA}. The deviation between the assumed D_{FFM} (1.1 g/cm^3; [18]) and the calculated D_{FFM} was higher in men than in women (Table 2).

Water content of FFM_{DEXA} as well as the ratio ICW_{BIA}/ECW_{BIA} were inversely correlated with the calculated D_{FFM} (Table 3) and also with density of the whole body measured by ADP (Fig. 3). Water content of FFM_{DEXA} was associated with the ratio of ICW_{BIA}/ECW_{BIA} ($r = –0.40$; $p < 0.05$). The ratio of ICW_{BIA}/ECW_{BIA} and skeletal $muscle_{DEXA}$ content of FFM_{DEXA} were positively associated with the density of the whole body (Table 3). In a multiple stepwise regression analysis 96 % of the variance in calculated D_{FFM} was explained by water content of FFM_{DEXA} and the additional 4 % was explained by the mineral content of FFM_{DEXA}.

The difference in %FM estimated by the 2C and 4C model was dependent on water fraction of FFM: it was positively correlated with TBW_{BIA}/FFM_{DEXA} (Fig. 4, Table 3) and negatively with ICW_{BIA}/ECW_{BIA} (Table 3) and phase $angle_{BIA}$ ($r = –0.61$; $p < 0.01$), respectively. By contrast, the difference between $\%FM_{ADP}$ and $\%FM_{DEXA}$ showed a negative association with the BMC_{DEXA} fraction of FFM (Table 3). Variability in BMC_{DEXA} explained 27 % of the difference between $\%FM_{ADP}$ 2C and $\%FM_{DEXA}$.

The difference in %FM estimated by the 2C and 4C model is closely related to the difference between assumed and calculated D_{FFM} (Fig. 5); 76 % of the variance of the difference between the two methods is explained by the bias resulting from the assumption of a fixed D_{FFM}. By contrast, the difference between $\%FM_{ADP}$ and $\%FM_{DEXA}$ was not related to the difference in assumed and calculated D_{FFM}.

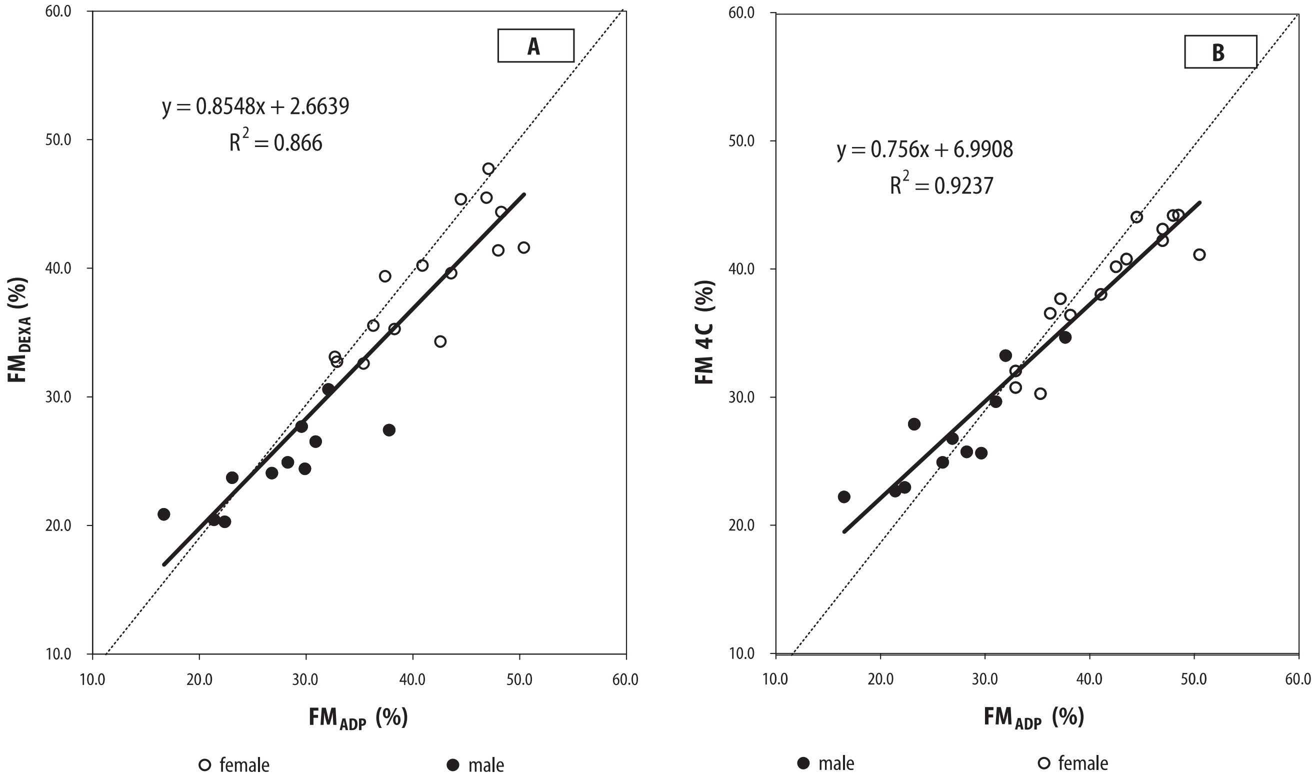

Fig. 1 Relationship between $\%FM_{DEXA}$ and $\%FM_{ADP}$ (**A**). Relationship between $\%FM_{4C}$ and $\%FM_{ADP}$ (**B**) in 26 elderly subjects. The *dashed line* indicates the line of identity

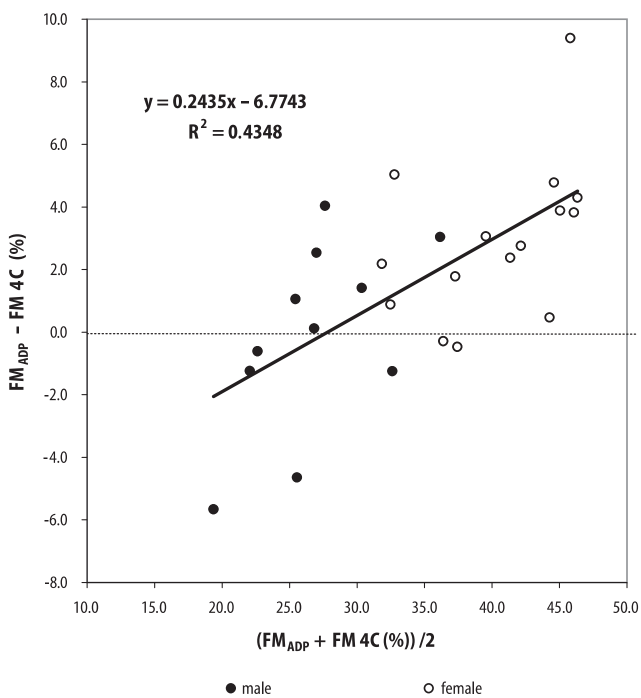

Fig. 2 Bland-Altman plot for the relationship between $\%FM_{ADP}$ and $\%FM_{4C}$ in 26 elderly subjects

Discussion

Validation of ADP against DEXA

We found a high correlation of $\%FM_{ADP}$ and $\%FM_{DEXA}$ in the elderly (Fig. 1). This finding confirms previous results from other authors [7]. The amount of shared variance between $\%FM_{ADP}$ and $\%FM_{DEXA}$ was 87%. However, the slope of the regression line and also the intercept significantly differed from 1 and 0, respectively. The deviation of %FM between the two methods was significant in both sexes ($p < 0.01$) and ranged from −4% to +10% (Fig. 2). It increased with increasing proportion of BMC_{DEXA} of FFM_{DEXA} (Table 3). This finding was also reported by Koda et al. [7] and by others based on differences in %FM as measured by DEXA and HD, respectively [21–23]. Because (i) the fraction of BMC_{DEXA} was not related to calculated D_{FFM} (Table 3) and (ii) the fraction of BMC_{DEXA} explained only a minor part in variance of D_{FFM} in a multivariate regression analysis, the deviation in %FM between DEXA and the 2C model is unlikely to be caused by the error of assumption of a fixed D_{FFM} alone. It might be due to methodological issues of BMC measurements by DEXA. DEXA is proposed to underestimate %FM in people with larger sagittal abdominal diameters or waists be-

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Table 2 Composition and density of fat-free mass

	26 elderly subjects			
	All	female (15)	male (11)	p-value*
TBW_{BIA}, l	35.3 ± 6.3	31.6 ± 2.3	40.3 ± 6.2	< 0.001
$Protein_C$, kg	11.5 ± 5.5	8.3 ± 3.0	15.8 ± 4.9	< 0.001
$Mineral_{DEXA}$, kg	3.0 ± 0.7	2.6 ± 0.3	3.5 ± 0.6	< 0.001
TBW_{BIA}/FFM_{DEXA}, %	72.1 ± 5.7	74.9 ± 4.8	68.2 ± 3.9	< 0.01
$Protein_C/FFM_{DEXA}$, %	21.9 ± 5.9	19.0 ± 5.1	25.9 ± 4.0	< 0.01
$Mineral_{DEXA}/FFM_{DEXA}$, %	6.0 ± 0.7	6.1 ± 0.7	5.9 ± 0.6	n. s.
$D_{FFM\ calc}$, g/cm³	1.101 ± 0.017	1.092 ± 0.014	1.113 ± 0.012	< 0.05
$\Delta D_{FFM\ ADP}$-$D_{FFM\ calc}$, g/cm³	–0.001 ± 0.017	0.008 ± 0.014	–0.013 ± 0.012	< 0.05

* sex differences, Mann-Whitney U test; Data are given as means ± SD
TBW_{BIA} total body water = FFM_{BIA} x 0.73; *$Mineral_{DEXA}$*, BMC_{DEXA} x 1.2741; *$Protein_C$*, FFM – TBW – Mineral; *FFM* body weight – FM_{DEXA}; *ADP* air displacement plethysmography; *$D_{FFM\ calc}$* density of FFM calculated according to Eq. (3); *$D_{FFM\ ADP}$* assumed density of FFM = 1.1 g/cm³

Table 3 Correlation coefficients between parameters of FFM composition and results from different compartment models

	BMC_{DEXA}/FFM_{DEXA}	TBW_{BIA}/FFM_{DEXA}	ICW_{BIA}/ECW_{BIA}	MM_{DEXA}/FFM_{DEXA}
$D_{FFM\ calc}$, g/cm³	–	–0.98***	0.62**	–
$\Delta D_{FFM\ ADP}$-$D_{FFM\ calc}$, g/cm³	–	0.98***	–0.62**	–
$D_{whole\ body\ ADP}$, g/cm³	–	–0.65***	0.47*	0.61**
FM_{ADP}, %	–	0.66***	–0.47*	–0.60**
FM_{DEXA}, %	–	0.70***	–0.41*	–0.66***
FM 4C, %	–	0.49*	–	–0.68***
ΔFM_{ADP}-FM_{DEXA}, %	–0.52**	–	–	–
ΔFM_{ADP}-FM 4C, %	–	0.80***	–0.68*	–

Significant Pearson correlation coefficients, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$
$D_{FFM\ calc}$ density of FFM calculated according to Eq. (3); *ADP* air displacement plethysmography; *$D_{FFM\ ADP}$* assumed density of FFM = 1.1 g/cm³; *BMC_{DEXA}* bone mineral content; *ICW_{BIA}* intracellular water, *ECW_{BIA}* extracellular water; *MM_{DEXA}* skeletal muscle mass; *TBW_{BIA}* total body water = FFM_{BIA} x 0.73; *FFM* body weight – FM_{DEXA}

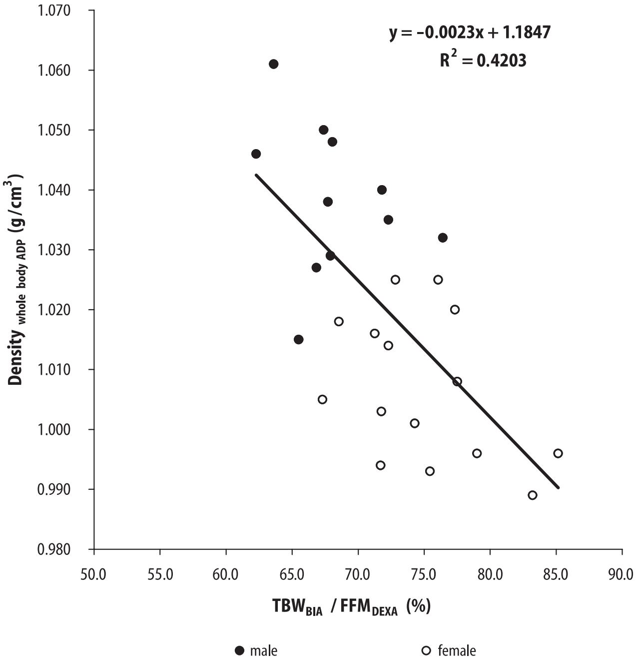
cause the BMC/FFM ratio and fat thickness may have an influence on estimates of %FM [3, 7]. However, other authors found that DEXA overestimates %FM at a higher tissue thickness [24].

In a previous study, comparison of %FM from DEXA and a 4C model in older adults revealed a substantial error of ± 5 %FM [25]. These authors therefore suggested that the isolated use of DEXA is unacceptable in a research setting in an older population. However, a large variation in water content of FFM might have limited the validity of the 4C model in this study. By contrast, a more recent report found an excellent agreement of %FM as assessed by DEXA and 4C model. These data suggested that DEXA is an accurate method for measurement of fat mass in the elderly [26]. This is also supported by the present study. The deviation between $\%FM_{DEXA}$ and the $\%FM_{4C}$ and the 95 % level of agreement between the two methods were –0.7 % and 5.7 %, respectively.

Comparison of ADP with a 4C model

We also tried to compare the results from the 2C model with a 4C model considering the density of whole $body_{ADP}$ as well as BMC_{DEXA} and TBW_{BIA}. The comparison was not used for validation purposes but intended to estimate the bias from the assumption of a fixed density of FFM. In this respect, water and mineral content of FFM that were measured by independent methods (BIA and DEXA) contribute to explain the limits of ADP as a 2C model. Although the agreement between the 2C and 4C model exceeded the agreement of the 2C model vs. DEXA (shared variance of 93 %) there was (i) a higher intercept of the respective regression line with a significant deviation from the line of identity (Fig. 1) as well as (ii) a systematic error between the estimated $\%FM_{ADP}$ and $\%FM_{4C}$ (Fig. 2).

The difference between $\%FM_{ADP}$ and $\%FM_{4C}$ was significant only in the subgroup of females ($p < 0.01$). This sex difference is in accordance with a previous validation study comparing the 2C model (as assessed with HD) against a 4C model [5]. Other authors also reported significant differences between %FM estimated by the 2C and 4C model in elderly women [4] and in elderly subjects of both sexes [3]. By contrast, a recent ADP validation study in the elderly did not find any significant differences between the results from different compartment models [6]. However, the magnitude of absolute differences in %FM from ADP and the 4C model resembled those observed in earlier reports [4] but exceeded



our own results (2.2 %FM by Yee et al. vs. 1.6 % in the present study, Table 1). The lack of significance in the study of Yee et al. [6] is likely to be due to the different method of statistical evaluation. Differences between the results of compartment models were evaluated by a three-way analysis of variance with Bonferroni's post hoc test for correction of multiple pairwise comparisons by Yee et al. [6]. By contrast, we used the Wilcoxon signed ranks test for analysis. Thus, using a three-way ANOVA in our study resulted in a lack of significance in females (results not shown). However, the difference for women reported by Yee et al. was more than 2.6 times higher than in males which also supports a sex difference [6].

Composition and density of FFM

FFM composition can be described by individual organ contribution [27]. Alternatively FFM is characterized chemically by the proportion of water, mineral and protein. The 2C model divides the body into FFM and FM. The analysis is based on the assumption of a constant density of the two compartments (0.9007 g/cm^3 for FM and 1.1 g/cm^3 for FFM). A fixed density of FFM (D_{FFM}) implies a constant composition of FFM. However, proportions of FFM as water (73 %, 0.9937 g/cm^3), mineral (6.8 %, 3.038 g/cm^3) and protein (19.4 %, 1.34 g/cm^3) tend to vary in certain conditions such as growth, intensive exercise, race/ethnicity, severe illness and aging [4, 15, 28–33]. In each of these circumstances possible shortcomings of ADP have to be carefully examined.

In the present study, 76 % of the difference between $\%FM_{ADP}$ and $\%FM_{4C}$ is explained by the difference in the assumed and calculated D_{FFM} (Fig. 5). When the calculated D_{FFM} exceeded the assumed D_{FFM} of 1.1 g/cm^3 %FM was underestimated by ADP. Conversely when calculated D_{FFM} was lower than the assumed constant %FM was overestimated by ADP. The inaccuracy of the $\%FM_{ADP}$ estimation resulting from deviations of calculated and assumed densities of FFM is shown in an example assuming a body composition of 75% FFM and 25% FM and the mean densities used in Siri's formula [11]. In this condition D_{ADP} is calculated as: $D_{ADP} = 1/[(0.75/1.1) + (0.25/0.9007)]$, yielding $D_{ADP} = 1.0423$. Accordingly %FM is calculated yielding %FM = 24.9 (see Eq. 1). Varying the density of FFM from an assumed density of 1.1 g/cm^3 applying minimal and maximal results from Eq. 3 (i.e., applying the minimal and maximal calculated D_{FFM} of 1.065 g/cm^3 or 1.133 g/cm^3) resulted in a substantial overestimation of %FM by 11% and an underestimation of 10%, respectively.

Variations in body water and mineral are among the most variable components of FFM [14, 34]. It is believed that the effect of bone proportion on D_{FFM} might compensate the effect of water content because of the higher density of mineral compared to water [35]. However, in the present study calculated D_{FFM} and density of whole body as well as the difference in $\%FM_{ADP}$ and $\%FM_{4C}$ were closely correlated with water content of FFM_{DEXA} (Table 3, Figs. 3 and 4). By contrast, there was no association with fraction of BMC_{DEXA}. Using a multivariate regression analysis (i) fraction of FFM_{DEXA} as water explained 96% and (ii) mineral $content_{DEXA}$ explained an additional 4% of the variance in D_{FFM} only. Thus, when compared with water content there is only a minor influence of age-dependent decrease in BMC on the overestimation of %FM by ADP. In addition, water occupies about an 11 times greater proportion of the FFM than mineral [15]. Thus, small changes in water will have a greater effect on D_{FFM} simply because of the composition of FFM.

There are some contradictory reports on water content of FFM in the elderly. Earlier studies report a tendency for increasing dehydration with advanced age [36] as well as an increasing proportion of TBW on FFM [4, 37]. However, this could not be confirmed by other studies showing a fairly constant TBW/FFM ratio with advanced age [6, 27, 38–40]. In our study, TBW was measured by bioelectrical impedance analysis. The mean TBW_{BIA}/FFM_{DEXA} ratio was 72.1% (Table 2) which was in good agreement with the 71.5% measured by deuterium dilution in a comparable group of elderly subjects [6]. However, in elderly men we observed a lower water content of FFM_{DEXA} (Table 2). This might have contributed to an underestimation of D_{FFM} and consequently in $\%FM_{ADP}$ in men. However, in males there was no significant difference between $\%FM_{ADP}$ and $\%FM_{4C}$.

The use of BIA to assess TBW may violate our approach of a 4C model. However, BIA predicted TBW showed a very close agreement with TBW measured by deuterium dilution [41]. This finding was also supported in a group of elderly diseased patients [42]. In addition, a delayed isotopic equilibration was observed in the elderly which may reduce the accuracy of the isotope dilution techniques in this population [43]. Taken together we feel that BIA can be used with certain confidence in elderly subjects.

Since ICW/ECW is related to water content of FFM (compare results [44]), the results from mutifrequency bioelectrical impedance analysis might be useful for a priori identification of subjects with a substantial error of %FM estimation by ADP. An ICW_{BIA}/ECW_{BIA} ratio < 1.3 and > 1.8 is associated with a substantial increase (i.e., by more than ± 1 SD of 3%) in the deviation of %FM between the 2C and 4C model, since this cut off for water content can be easily acquired by the bedside technique. Thus BIA might be a practical approach in assessing the applicability of ADP in certain clinical conditions with altered water content of FFM.

Given that the density of fat-free muscle is 1.066 g/cm^3, a decreased muscularity is expected to increase the D_{FFM} [15]. We therefore hypothesized that an age-related decrease in muscle mass would contribute to a higher D_{FFM} and would favor underestimation of %FM by ADP. However, there was no correlation between the proportion of skeletal muscle $mass_{DEXA}$ of FFM_{DEXA} and D_{FFM}. Although our results argue against an underestimation of $\%FM_{ADP}$ caused by the age-related loss of muscle mass such an effect might have been compensated by the concurrent loss in BMC.

Conclusion

We conclude that ADP is a valid method of body composition measurement in the elderly. The bias in %FM measurement by the BOD POD device is mainly related to water content of FFM and indicates that a correction factor for TBW may improve the accuracy of the ADP measurements in elderly females.

Acknowledgment This work was supported by a grant from the Deutsche Forschungsgemeinschaft (DFG Mü 714/8–1).

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