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 with bioimpedance analysis

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- 32 **Running title:** Body shape and bioimpedance analysis
- 33

34 **ABSTRACT**

35 **Objective**: To test the hypothesis that variations in trunk circumferences 36 influence the accuracy of bioimpedance analysis (BIA) for assessment of 37 percent fat mass (%FM).

Subjects and methods: %FM was predicted with BIA and compared with airdisplacement plethysmography (ADP) in a small sample of 35 overweight (OW), 21 normal weight (NW), and 8 underweight (UW) volunteers. Waist and hip circumferences were assessed, and 15 of the OW subjects were measured before and after weight reduction.

43 **Results:** BIA and ADP provided similar cross-sectional estimates of group 44 mean %FM (28.9 \pm 10.0 and 31.3 \pm 13.0%, respectively). However within 45 individuals, there were large between-method differences (Diff_{BIA-ADP}) ranging 46 from -13 to +13 %FM. Furthermore, we found a systematic bias of BIA related 47 to the degree of adiposity. Consequently, %FM and fat mass loss during weight 48 reduction in OW were underestimated with BIA when compared with ADP. 49 Waist and hip circumference were inversely associated with resistance and 50 reactance (p < 0.01), and with Diff_{BIA-ADP} (p < 0.001). In women, the variability in 51 hip circumference explained 76%, and in men variability in waist circumference 52 explained 59% of Diff_{BIA-ADP}.

53 **Conclusion**: Resistance changes associated with variations in trunk 54 circumferences decrease resistance and therefore impair the accuracy of BIA to 55 assess %FM.

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57 Key words: body composition – fat mass - bioimpedance analysis – body
58 shape

60 Introduction

Bioimpedance analysis (BIA) is widely applied to assess body 61 62 composition in clinical practice and research settings. However the European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines guestion the 63 64 accuracy of BIA at extreme BMI ranges and suggest that longitudinal follow-up 65 data on body composition by BIA should be interpreted with caution (Kyle et al., 66 2004b). Correspondingly BIA considerably underestimated total and truncal fat 67 mass (Neovius et al., 2006) as well as fat mass loss in obese persons during 68 weight reduction (Fogelholm et al., 1997). The reasons for the discrepant 69 results are poorly understood. Therefore, the ESPEN guidelines suggest that 70 further validation studies of BIA should be performed to clarify the issue. BIA is 71 based on several assumptions that could lead to inaccurate results. First, the 72 hydration of human soft tissue is not constant. The hydration state is 73 significantly altered in overweight subjects (Haroun et al., 2005;Waki et al., 74 1991). Second, the measured body is not of cylindrical geometry. Large 75 variations exist in cross-sectional areas of human bodies that are likely to be 76 responsible for the lack of portability of BIA equations from one population to 77 another (Kyle et al., 2004b). In an increasingly overweight population, variations 78 in body shape might significantly influence BIA accuracy. However the effect of 79 variations in truncal circumferences on whole body impedance and thus the 80 accuracy of BIA to determine percent fat mass (%FM) in a healthy population 81 with varying nutritional status has never been quantified systematically. We 82 reasoned that BIA underestimates %FM and underestimates fat mass loss 83 achieved during weight reduction, in obese persons compared with ADP. In 84 addition, we hypothesized that trunk circumference variation affects impedance and could partly explain the different results in %FM obtained with BIA and

86 ADP.

88 Subjects and methods

89 The ethical committee of the Charité approved the study and written, 90 informed consent was obtained from all participants. We enrolled 35 overweight 91 (OW; BMI \ge 25 kg/m²), 21 normal weight (NW; BMI between 19.0 and 25.0 kg/m^2) and 8 underweight (UW; BMI < 18.5 kg/m^2 , without clinical signs of 92 93 edema) volunteers into this study. We recruited NW volunteers among 94 university staff, and UW subjects from the Department of Psychosomatic 95 Medicine during their inpatient treatment of eating disorders. In addition we 96 recruited OW subjects at the start of a 6-months weight reduction program, and 97 fifteen of these OW volunteers were retested after weight reduction. We carried 98 out all tests in our Clinical Research Center in the morning after a 12 hours (OW 99 and NW) or 2 hours (UW) of fasting. Body height was assessed with a laser 100 stadiometer (Soehnle Leifheit AG, Germany), and body circumferences with a 101 non-stretchable measuring tape at standardized reference points (waist: half 102 way between lower rib and iliac crest; hip: at the level of trochanter major). After 103 resting for 10 minutes and voiding, BIA measurements (Helios, Forana, 104 Forschung und Analyse GmbH, Frankfurt am Main, Germany) were carried out 105 in the supine position. The subjects were carefully placed into a position 106 suitable for BIA measurements, assuring separate placement of legs in an 107 angle of about 30 degrees in order to avoid overestimation of the trunk length, 108 specifically in the OW. After cleaning the skin with disinfectant, we placed 109 single-use electrodes (BIA Classic Tabs, MediCal Healthcare GmbH, Karlsruhe, 110 Germany) on the dorsal surface of hand and foot of the dominant side, 111 according to manufacturer instructions. Whole body impedance was measured 112 at 50 kHz analysis and %FM was calculated according to an equation 113 previously published by Sun et al. (%FM_{BIA SUN}) which was developed using a 114 multi-compartment model (isotope dilution, densitometry and Dual-Energy X-115 Ray absorptiometry) as the reference method (Sun et al., 2003). In this study, 116 1095 healthy male and female subjects aged 12 – 94 with a broad range of BMI 117 $(23.6 \pm 5.6 \text{ to } 30.0 \pm 7.2)$ had been included. The standard error of estimate 118 when predicting fat mass with BIA has been shown to be between 1.9 and 3.6 119 kg (Kyle et al., 2004a). ADP was carried out immediately after BIA testing by 120 using the BodPod[®] (Life Measurement Inc. Concord, CA, U.S.A). After a 30 min 121 run-in phase, the BodPod was calibrated using a 50 I cylinder. Body weight was 122 measured with the scale attached to the BodPod which was calibrated daily. 123 Then, body volume was measured after adjustments for predicted thoracic lung 124 volume and estimated surface area artifact. Participants were dressed in tight 125 underwear and wore a swim cap during the measurement. Fat mass was 126 calculated according to the equation by Siri using the software provided by the 127 manufacturer. Body volume was measured in duplicate or triplicate when the 128 initial two measures differed by >150 ml

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130 Statistical analysis

131 We applied SPSS (version 18.0; SPSS Inc. Cary., NC) for statistical 132 analysis. Data are given as mean ± SD. Between-group comparison was 133 carried out with one-way ANOVA followed by 2-tailed post-hoc Dunnett t-tests. 134 We applied the paired t-test for intra-individual comparisons with p<0.05 135 considered as statistically significant. Bland & Altman analysis of agreement 136 was carried out for method comparison (Bland, 1986). To identify parameters 137 that explain the measurement difference between BIA and ADP, we carried out 138 a stepwise regression analysis with the measurement difference (Diff_{BIA-ADP}) as dependent, and with gender, body weight, age, BMI, waist and hipcircumference as independent variables.

142 **Results**

143 Hypothesis 1: the difference between BIA and ADP

144 Demographic subject characteristics and body composition of the study participants are shown in Table 1. The mean %FM was 28.9 ± 10.0 based on 145 146 BIA_{SUN}, which was slightly but not significantly different from %FM as assessed 147 with ADP (31.3 ± 13.0). However Bland & Altman Analysis of agreement 148 showed wide limits of agreement, and maximum individual differences between 149 BIA_{SUN} and ADP ranged from -13 to +13 %FM (Fig.1). The difference between 150 %FM determined by ADP and BIA_{SUN} was inversely associated with mean %FM 151 (r = -0.57, p < 0.001). This finding indicated a systematic bias in the estimation 152 of body fat according to BIA_{SUN} which was related to the nutritional status of the subject, and lead to an overestimation of %FM in lean, as well as an 153 154 underestimation of %FM in overweight subjects. When estimates of percent 155 %FM were compared within the three study groups separately, BIA_{SUN} 156 significantly underestimated %FM in the OW group (p < 0.05) while in the NW 157 and UW group %FM determined by BIA_{SUN} and ADP gave similar results (Table 158 1). After the weight loss program, the OW participants had lost an average of 159 6.6 ± 2.7 kg (range: 0.9 – 10.4 kg). ADP indicated a reduction in fat mass of 6.4 160 \pm 2.6 kg in the OW group while BIA_{SUN} indicated a loss of only 3.7 \pm 2.1 kg. This 161 result was significantly less in comparison with ADP (p < 0.01). When compared 162 with ADP, individual difference with BIA_{SUN} ranged from underestimating fat 163 mass loss by 7.4 kg to overestimating fat mass loss by 1.9 kg.

165 Hypothesis 2: Variation in trunk circumference is responsible for the systematic
166 difference between BIA and ADP

167 In the entire 64 participants study group, waist and hip circumference were consistently and inversely correlated with resistance (R_{50} ; waist r = -0.54, 168 169 p < 0.01; hip r = -0.47; p < 0.01), reactance (Xc₅₀; waist r = -0.47, p < 0.01; hip r 170 = -0.50; p < 0.01) as well as with the measurement difference between BIA and 171 ADP (Diff_{BIA-ADP}; Figure 2). To assess whether or not individual variability in 172 truncal circumferences independently affected Diff_{BIA-ADP}, we performed a 173 stepwise regression analysis with Diff_{BIA-ADP} as dependent, and with gender, 174 age, body weight, BMI and truncal circumferences as independent variables. 175 Gender (p = 0.007), BMI (p = 0.039) and hip circumference (p = 0.062) entered the model as independent predictors and together explained 70% of the 176 177 measurement difference. Although based on a relatively small sample size, we aimed at further exploring the role of gender differences in the variance of 178 179 measurement differences, and repeated the stepwise regression analysis for 180 women (n = 48) and men (n = 16) separately. In women, hip circumference was 181 the single significant predictor explaining 76% of the variance of the 182 measurement difference. In contrast in males, the single significant predictor 183 was waist circumference, explaining 57% of the observed variance. The results 184 of the regression analysis are shown in table 2.

186 **Discussion**

187 The equation of Sun et al. to predict fat mass performed relatively well to 188 estimate group average fat mass in the normal- and underweight subjects, but 189 led to an underestimation in the overweight group (table 1). Similar findings 190 have been documented earlier (Deurenberg, 1996;Bosy-Westphal et al., 2008) 191 and led to the development of obese-specific equations. BIA underestimates fat 192 mass loss in obese women during weight reduction by -2.8 to +1.6 when 193 compared with dual-energy X-Ray absorptiometry (DEXA) (Webber et al., 1994) 194 or with a criterion 4C model (Fogelholm et al., 1997). While showing the same 195 trend, our results comparing BIA with ADP suggested a more pronounced 196 absolute bias (table 1, figure 1).

197 We extrapolated the extremes of the bias derived from the cross-198 sectional part of our study in the OW group to a longitudinal "worst case" 199 scenario, and found that FM could be under- and overestimated by 13% both 200 before and at the end of a weight reduction program by BIA_{SUN}. In a given 201 subject with a loss of 24% FM according to ADP (i.e. from 49 to 25%), BIA_{SUN} 202 could thus estimate a gain of 2% FM (36 to 48%). On average, the difference 203 between ADP and BIA_{FOR} can be expected to be much less extreme. In our 204 study, ADP estimated a fat mass loss from 45.4 to 41.4 % (- 4%), and BIA_{SUN} 205 from 39.8 to 38.5 % (= - 1.3%). Still this difference should not be neglected. We 206 do not know if more pronounced weight loss would lead to an even greater 207 discrepancy.

208 Our findings imply that fat mass of overweight subjects, and the success 209 of weight reduction programs in terms of fat mass reduction, will be 210 underestimated with BIA_{SUN}. The idea that BIA accuracy depends on the choice 211 of an adequate population-specific prediction equation (Kyle et al., 2004b) is well accepted obese people. However, how could BIA be applied to a group of people with heterogeneous nutritional status, or to subjects undergoing transition from an overweight to a normal weight state? We suggest that instead of accumulating an increasing amount of population-specific BIA equations, a better understanding of the strengths and weaknesses of the BIA technique could be more beneficial.

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219 The physical principle behind BIA is that resistance (R) of a homogeneous 220 conductive material of uniform cross-sectional area is proportional to its length 221 (L) and inversely proportional to its cross sectional area. Although the body is 222 not a uniform cylinder and its conductivity is not constant, an empirical relationship can be established between the impedance quotient (length²/R) 223 224 and the volume of water, which contains electrolytes that conduct the electrical 225 current through the body (Kyle et al., 2004a). According to previous studies 226 (Hoffer et al., 1969;Lukaski et al., 1985) correlations between impedance and 227 total body water are uniformly high suggesting that the theory behind this 228 measurement is valid. However, one of the assumptions behind impedance 229 measurements clearly relates to a uniform geometry of the measured body, 230 which apparently is not the case with a human body.

The extremities account for 91% of whole body resistance while the contribution of the trunk was only < 10% (Zhu et al., 1998). Despite the small contribution of the trunk to whole body impedance, we found that truncal circumferences were consistently and inversely associated with whole body impedance. In addition, we were able to demonstrate that individual variation in hip circumference in women, and waist circumference in men, explained a major part of the observed difference between ADP and BIA, independent of variations in weight or BMI. We are aware that our study is small and suggest that our findings should be interpreted with caution. Future studies with larger sample sizes of mixed gender are needed to characterize the effect of variations in body shape on body impedance in better detail. Nonetheless, we believe that our data provide clear indicators of what parameters should be studied, and what results could be expected.

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248 **Conflict of interest:** we are not aware of interest conflicts.

249 Study limitations

We used a comparison with ADP and not a 4C-model to assess body fat. In addition, we predicted lung volume in the overweight subjects rather than measuring this parameter directly. These issues could have introduced a systematic measurement bias in our study.

254

255 **Conclusions and outlook**

Our study provides specific insight into the limitations of BIA to accurately predict fat mass in a heterogeneous group and in overweight subjects during weight loss. In addition, our study also identified gender-specific variations in central body shape as one of the major underlying reasons causing the different results on %FM between BIA and ADP. In future studies, the accuracy of BIA could be improved by adjusting measured resistance for the subject's trunk circumference.

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- Table 1. General characteristics, bioimpedance, and body composition of overweight
- (OW), normal weight (NW) and underweight (UW) subjects, as well as of the subgroup
- of overweight subjects before and after weight reduction.

	OW	NW	UW	OW T0	OW T1
n (m/f)	(9/26)	(7/14)	(0/8)	(0/15)	(0/15)
· ·	41 ± 10*	34 ± 8	27 ± 11	40 ± 11	41 ± 11
age (y)	(25 – 61)	(23 – 54)	(19 – 47)	(28 – 61)	(28 – 61)
	1.69 ± 0.10*	1.76 ± 0.09	1.65 ± 0.05**	1.67 ± 0.07	1.67 ± 0.07
height (m)	(1.54 – 1.88)	(1.62 – 1.94)	(1.60 – 1.73)	(1.54 – 1.80)	(1.54 – 1.80)
	90.5 ± 15.3***	67.3 ± 10.6	45.3 ± 6.3	91.6 ± 11.5	85.1 ± 10.6***
weight (kg)	(61.2 – 135.2)	(55.3 – 98.4)	(38.4 – 55.0)	(75.2 – 110.5)	(71.1 – 101.3)
2	31.5 ± 4.1***	21.6 ± 1.7	16.6 ± 1.8	32.7 ± 3.0	30.4 ± 2.7***
BMI (kg/m²)	(25.1 – 41.3)	(19 – 25)	(14.0 – 18.6)	(28.2 – 61.0)	(26.1 – 34.5)
	98 ± 12***	76 ± 8	65 ± 5	99 ± 10	95 ± 9***
waist (cm)	(75 – 124)	(56 – 91)	(57 – 72)	(77 – 120)	(76 – 110)
	112 ± 9***	94 ± 5	77 ± 7	116 ± 7	114 ± 6
hip (cm)	(92 – 126)	(84 – 103)	(71 – 92)	(105 – 126)	(103 – 125)
	489 ± 64***	604 ± 75	732 ± 51	501 ± 52	539 ± 64**
R (ohm)	(331 – 606)	(479 – 717)	(686 – 851)	(405 – 603)	(419 – 649)
	50 ± 7***	58 ± 9	56 ± 8	48 ± 6	52 ± 4***
Xc (ohm)	(33 – 67)	(46 – 75)	(45 – 71)	(38 – 59)	(47 – 60)
	36.5 ± 11.1***	15.0 ± 3.2	7.0 ± 3.8	41.7 ± 7.1	35.3 ± 6.4***
FM _{ADP} (kg)	(8.6 – 56.3)	(9.5 – 19.4)	(0.8 – 13.6)	(30.4 – 53.1)	(25.3 – 43.9)
	40.1 ± 9.7***	22.9 ± 6.1	14.9 ± 6.6	45.4 ± 3.7	41.4 ± 3.9***
FM_{ADP} (%)	(10.7 – 54.3)	(10.9 – 31.7)	(2.1 – 25.7)	(40.5 – 54.3)	(34.5 – 49.4)
	31.9 ± 8.1***	15.9 ± 2.3	6.6 ± 4.2	36.5 ± 5.8	32.9 ± 5.4***
FM _{віа sun} (kg)	(16.8 – 45.6)	(10.5 – 19.1)	(0.4 – 12.1)	(25.6 – 45.6)	(22.3 – 42.7)
	38.6 ± 6.2***	23.9 ± 3.7	13.8 ± 7.6	39.8 ± 2.7	38.6 ± 3.3*
FM _{BIA} SUN (%)	(20.3 – 46.2)	(17.8 – 28.8)	(0.9 – 22.0)	(34.0 – 43.5)	(31.1 – 43.6)
BMI. Body Mass Index: R. Resistance: Xc. Reactance: FM. Fat Mass: FM ADP. FM measured with					

air-displacement plethysmography, FM BIA SUN, FM measured with bioelectrical impedance and predicted according to Sun et al. ***, p < 0.001; **, p < 0.01; *, p < 0.05 when comparing OW and UW with NW, and OW T0 with OW T1;

Table 2. Effect of variation in truncal circumferences (waist, hip) on the measured

- difference between bioelectrical impedance analysis (BIA) and air-displacement
- plethysmography (ADP): multiple stepwise regression analysis.

independent variable	ß		Р	adj. R ²
Model 1	all subjects (n=64)			0.702
Gender		0.194	0.007	
BMI		-0.444	0.039	
Hip		-0.400	0.062	
Model 2	Women (n = 48)			0.764
Нір	· · ·	-0.877	0.000	
Model 3	Men (n = 16)			0.594
Waist		-0.771	0.000	
Waist, waist circumference (cm); Hip, hip circumference (cm)				

Figure 1. Bland & Altman plot for analyzing the agreement between BIA_{SUN} and ADP for assessing percent body fat in overweight (OW), normal weight (NW) and underweight (UW) subjects.





