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## Articles

# Fluid Imbalance Clarifies Differences in Fat Estimated with Bioelectrical Impedance Analysis Compared to Dual-Energy X-ray Absorptiometry and Single Lateral Standing Digital Image Analysis

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**Abstract:** Limitations of body mass index (BMI) as a measure of body fat and the need for practical methods to estimate body fat reinforce interest in smartphone two-dimensional digital imaging and bioelectrical impedance analysis (BIA). Compared to dual x-ray absorptiometry (DXA), we determined differences in body fat mass (FM) estimated with smartphone single lateral standing digital image (SLSDI) and bioimpedance analysis (BIA) in 188 healthy adults (69 females and 119 males). SLSDI FM estimates were similar to DXA values but BIA underestimated ( $p < 0.0001$ ) FM. We tested the hypothesis that fluid imbalance, expansion of the extracellular water (ECW), designated as ECW to intracellular water ratio (ECW/ICW), affects the BIA-dependent differences. With BMI  $> 25 \text{ kg/m}^2$ , 54 male rugby players, compared to 40 male non-rugby players, had greater ( $p < 0.001$ ) BMI and fat-free mass but less ( $p < 0.001$ ) FM and ECW/ICW. SLSDI and DXA FM estimates were not different in both groups; BIA underestimated ( $p < 0.001$ ) FM in the non-rugby men. This finding is consistent with expansion of ECW in individuals with excess body fat due to increased adipose tissue mass and its water content. Unlike SLSDI, BIA predictions of FM are affected by altered fluid distribution associated with increased adipose tissue. These findings establish the validity, practicality, and convenience of smartphone SLSDI to estimate FM for healthcare providers in clinical and field settings.

**Keywords:** smartphone; fat; extracellular water; body mass index; digital imaging; bioelectrical impedance

## Introduction

Obesity is a multifactorial chronic disease that increases the risk of long-term morbidity, reduces life span, and increases health care costs. More than 650 million people worldwide were identified as obese in 2016 representing a three-fold increase since 1975 [1]. Four million deaths in 2015 were attributed to obesity, and two-thirds ascribed to cardiovascular disease [2]. The global economic burden of obesity is expected to be 1.2 trillion USD in 2025 [3]. Thus, the availability of reliable and practical methods to identify individuals with excess body fat is a public health need.

Clinicians and health professionals use ranges of body weight relative to height, body mass index (BMI;  $\text{Wt}/\text{Ht}^2$ ), to stratify adiposity-related health risks in a population. The convenience of BMI in clinical and research settings is offset by its unreliability to estimate body fat and predict the risk of obesity-related diseases for an individual [3–10]. Because BMI is an insensitive indicator of body composition, it poorly predicts the adiposity of individuals with increased fat-free mass (FFM) [11,12]. Other methods to estimate body composition improve the specificity and precision of estimation of fat and lean body components but are limited by technical complexity, exposure to ionizing radiation, invasiveness, lack of mobility, and cost that prevent their widespread use for

routine assessment of body composition [13,14]. Recent reports describe the limitations of BMI and emphasize the need for practical and valid methods to identify overweight and obesity and thus improve the care of patients with excess body fat [15–17].

Two methods are amenable to meet this clinical need. Bioelectrical impedance analysis (BIA) relies on the conduction of a safe, administered alternating current to estimate total body water (TBW) [18]. Most BIA methods use 50 kHz phase-sensitive devices and rely on the assumption of constant hydration to estimate FFM and calculate body fat. Alternatively, smartphone two-dimensional standing digital image analysis (2DI) coupled with computational machine learning has recently been used to estimate total body adiposity [19–22]. Studies reported differences in body fat estimates using smartphone 2DI and BIA compared to DXA but have not provided explanations for the observations [20,21]. Whereas BIA is a valid method to assess TBW [18] and excess body fat increases ECW [23], reliance on the assumption of constant hydration of the FFM may overestimate FFM and, thus, underestimate body fat in adults who are overweight or obese [24,25]. We hypothesize that fluid imbalance is a factor explaining differences in fat estimation with BIA compared to 2DI.

The aim of the present study was to compare body fat mass (FM) estimates derived using BIA and smartphone single lateral standing digital image (SLSDI) relative to dual x-ray absorptiometry (DXA) in healthy adults and to determine whether fluid imbalance contributes to any observed differences in FM estimates.

## 2. Materials and Methods

### 2.1. Participants

We recruited healthy adult Caucasian women and men aged 19 to 64 y using advertisements and word of mouth to participate in this study, which was conducted at the Eubion Medical Center and Tor Vergata University, in Rome, Italy. Prospective participants underwent a clinical examination and completed a health questionnaire to establish the absence of an unhealthy condition prior to participation. Exclusion criteria include metal implants, current treatment for metastatic disease, diabetes, diuretic therapy, or limb loss. This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of the University of Tor Vergata. Each participant provided written informed consent prior to participation in any testing.

### 2.2. Body Composition Assessment

Volunteers, wearing form-fitting clothing without jewelry, came to the laboratory after consuming a light meal and emptying their bladders. Standing height and body weight were determined using standard medical equipment (SECA Stadiometer and SECA 762 scale; Hamburg, Germany).

Body composition estimation included BIA, SLSDI, and DXA administered in random order. Volunteers underwent whole-body BIA testing using a 50 kHz phase-sensitive impedance analyzer that introduced a sinusoidal, constant current (250  $\mu$ A RMS) (BIVA, EFG 3 Monitor; Akern, Florence, Italy) using a tetrapolar surface electrode placement with paired current-injecting and voltage drop-measuring electrodes (BIAtrodes; Akern, Florence, Italy) separated a minimum of 5 cm and placed on the right wrist and ankle. Volunteers rested supine on a bed without contact with metal for 10 min. This BIA instrument provided direct measurements of resistance (R), reactance (Xc), and phase angle (PhA). The technical accuracy and precision of the BIA instrument were determined with a calibrated precision parallel circuit formed by a 384  $\Omega$  ( $\pm 1\%$ ) resistor in parallel with a 780 pF ( $\pm 2\%$ ) capacitor yielding 47  $\Omega$  of Xc at 50 kHz.

Body composition was estimated by using the following BI prediction models. Kyle et al. [26] (BIA1):

$$\text{FFM} = 4.104 + 0.518 \text{ Ht}^2/\text{R} + 0.231 \text{ Wt} + 0.130 \text{ Xc} + 4.229 \text{ Sex}^* \text{ [1 for men and 0 for women]}$$

Sun et al. [27] (BIA2):

$$\text{Males: FFM} = -9.88 + 0.65 \text{ Ht}^2/\text{R} + 0.26 \text{ Wt} + 0.02 \text{ R}$$

$$\text{Females: FFM} = -11.03 + 0.70 \text{ Ht}^2/\text{R} + 0.07 \text{ Wt} + 0.02 \text{ R}$$

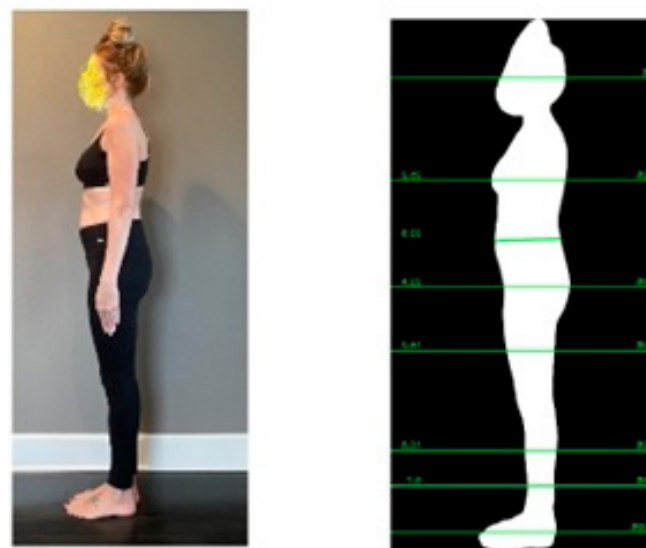
Units are FFM (kg), height (Ht)<sup>2</sup>/R (cm<sup>2</sup>/Ω), weight (Wt, kg), and Xc (Ω).

We also estimated body composition using the proprietary equations of the BIA instrument manufacturer (Bodygram PLUS; Akern, Florence, Italy) (BIA3). This approach estimates the actual hydration (water content) of the FFM of an individual and uses the measured hydration in lieu of the

customary 0.732 steady-state hydration ratio to estimate FFM (Akern Bodygram PRO; Florence, Italy). Fat mass is calculated as the difference between body weight and FFM.

A single SLSDI of each volunteer was obtained using the Fit.Your.Outfit (Pixelcando SL-Spain) smartphone APP implemented with a Cloud-based artificial intelligence (AI). An SLSDI of each volunteer was obtained without regard to background or illumination and transformed into an anonymous silhouette [22]. The individual stood upright with the head positioned in the horizontal plane, arms fully extended alongside the body with feet and legs touching and aligned sagittal to the camera to provide a lateral profile of the body (Figure 1). The smartphone cameras, either iOS- iPhone SE or iPhone 11 with CCD resolutions greater than 50 megapixels, were either pre-positioned on a stable tripod or held by a second individual who directed the handheld smartphone camera with the lens pointed at the middle of the standing height of each study participant. The AI system software automatically scaled all the digital pictures to a single homogeneous resolution of 5 megapixels and removed any background from the subject. The distance from the camera to the individual was 1.8 to 2.1 m with the feedback system of the smartphone APP. The operator downloaded and installed the Fit.Your.Outfit APP, available in iOS and Android from the APP stores, ensures the high technical quality of digital images for analysis to estimate body composition using proprietary software. The adequacy of the technical quality of the photograph is controlled and artifacts are prevented by using built-in sensors of modern smart devices and native libraries to detect specific anatomic nodal points (Figure 1) to identify parallaxes, improper distance from an individual to a camera, and to recognize incorrect arm extension and position, improper alignment of legs and feet, and non-horizontal head position. The quality control protocol provides visual warning instructions to the operator to ensure acquisition of high-quality digital images for analysis.

Precision, determined as coefficients of determination and concordance coefficients for repeated profiles of SLSDI images and FM estimates were 0.996 and 0.997 ( $p < 0.0001$ ), respectively, with no difference ( $<0.1$  kg) between repeated images.



**Figure 1.** Example of SLDDI photo and silhouette.

Reference whole-body composition was determined using DXA, a GE Lunar iDXAncore sn 200278 using software version 14.10.022 (Madison, WI). All participants were positioned supine and scanned within the dimensions of the DXA table. Precision estimates were 1.3% for FM and 0.5% for FFM.

Fluid distribution was evaluated using BIA measurements and bioelectrical impedance vector analysis (BIVA) [28]. This method uses tetrapolar BIA and a 50 kHz phase-sensitive impedance device and provides whole-body, direct serial measurements of resistance (R), reactance (Xc), and phase angle (PhA). One measure was the ratio of ECW to total body water (TBW), ECW%, which was estimated with a proprietary model (Akern BodyGram PLUS; Florence, Italy). We also assessed fluid distribution (ECW/ICW) using PhA, which is inversely related to ECW/ICW [29].

### 2.3. Statistical Methods

Statistical analyses were performed using SYSTAT version 13 (Systat Corporation; San Jose, CA, USA) and version 19.0.3 (MedCalc Software Bv, Ostend, Belgium). Descriptive data are expressed as mean  $\pm$  SD. Statistical significance was set at  $p < 0.05$ .

Estimates of FM from the smartphone SLSDI and each BIA prediction model were compared with the reference DXA values. Measurement agreement was evaluated with concordance correlation coefficient (CCC) [29], standard error of the estimate (SEE) between DXA and each method. Reference and estimates of body fat values in each sex group were compared separately with a paired t-test. Bland–Altman plot [30,31] was used to determine bias and limits of agreement (LOA) for the SLSDI and BIA methods compared to DXA determinations.

Effects of fluid distribution were evaluated in a sub-group of males with BMI greater than 25 kg/m<sup>2</sup>, the population indicator for overweight and obesity to contrast the effects of differences in adipose tissue with an unpaired t-test. Differences between group vector distributions were determined using Hotelling's T2 test.

### 3. Results

Table 1 describes the physical characteristics of the participants and shows the wide ranges of BMI and FM in the groups.

**Table 1.** Physical characteristics of the study participants. Values are mean  $\pm$  SD (min – max).

	Females	Males	Males BMI <sup>A</sup> >25 kg/m <sup>2</sup>	
	Total 69	Total 119	Rugby 54	Non-rugby 40
N				
Height, cm	162.7 $\pm$ 6.3 (150.0-178.0)	182.5 $\pm$ 8.7 (160.0-202.0)	186.2 $\pm$ 7.2 (173.0-202.0)	179.7 $\pm$ 9.2 (163.0-198.0)
Weight, kg	67.8 $\pm$ 14.5 (41.8-103.6)	93.2 $\pm$ 13.3 (61.1-125.6)	104.8 $\pm$ 11.4 (79.4-123.0)	91.4 $\pm$ 12.2 (69.5-124.6)
BMI, kg/m <sup>2</sup>	25.6 $\pm$ 5.4 (16.1-37.2)	27.6 $\pm$ 4.0 (19.5-37.0)	30.4 $\pm$ 3.3 (25.2-36.6)	28.7 $\pm$ 3.0 (25.1-37.1)
Fat mass <sup>B</sup> , kg	24.3 $\pm$ 11.6 (6.4-52.3)	18.8 $\pm$ 7.67 (5.6-35.2)	19.3 $\pm$ 6.5 (9.0-35.2)	23.3 $\pm$ 6.9 (10.3-35.1)
Fat <sup>B</sup> , %	34.2 $\pm$ 10.2 (12.1-50.5)	19.8 $\pm$ 6.7 (8.3-36.8)	18.2 $\pm$ 4.7 (9.8-28.9)	25.4 $\pm$ 6.2 (11.4-36.8)
Fat-free mass <sup>B</sup> , kg	43.5 $\pm$ 5.6 (31.0-55.8)	74.4 $\pm$ 12.7 (47.4-103.2)	84.7 $\pm$ 6.6 (68.9-103.2)	68.1 $\pm$ 10.1 (50.6-97.2)

<sup>A</sup> BMI: Body Mass Index; <sup>B</sup> Dual x-ray absorptiometry.

Levels of agreement differed between the reference and candidate methods (Table 2). FM values were similar for SLSDI-estimated and DXA-determined FM for females (24.1  $\pm$  11.3 and 24.3  $\pm$  11.6 kg) and males (18.8  $\pm$  7.2 and 18.8  $\pm$  7.6 kg) with no significant difference between methods (Table 2). All BIA equations underestimated ( $p < 0.0001$ ) FM (1.1  $\pm$  2.4, 3.2  $\pm$  2.4, and 3.6  $\pm$  2.3 kg) in females. One BIA model underestimated FM (2.4  $\pm$  3.7 kg) and two BIA equations overestimated ( $p < 0.0001$ ) FM (1.44  $\pm$  3.9 and 2.9  $\pm$  3.7 kg) in males.

**Table 2.** Levels of agreement (F and M) for body fat estimates using smartphone single lateral standing digital image analysis and 50 kHz bioelectrical impedance analysis compared to dual x-ray absorptiometry.

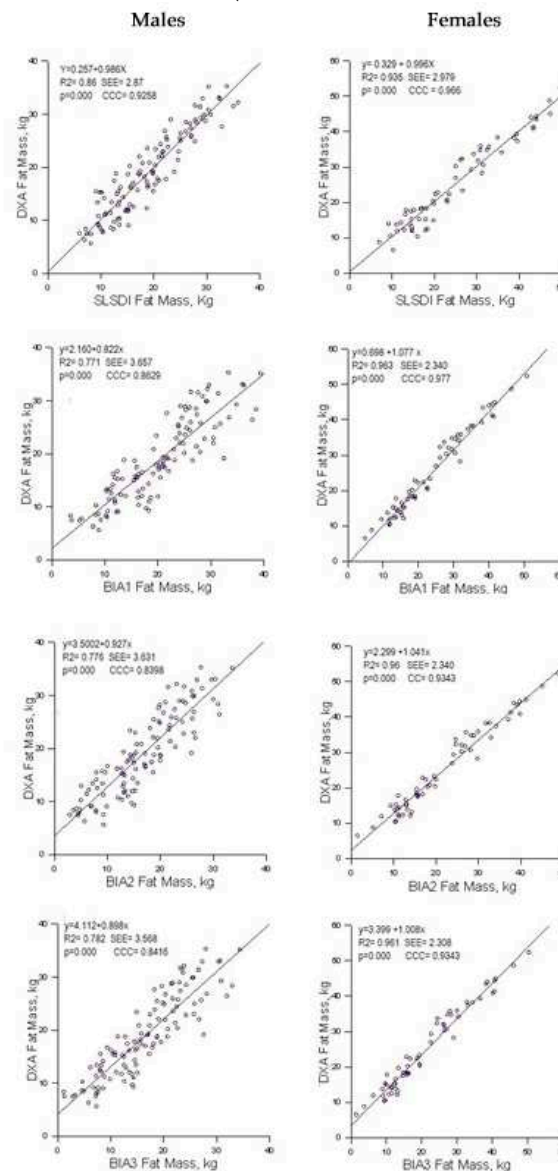
Group	Method	Fat mass, kg	Bias, kg	p	SEE, kg	CCC	MAE, kg	MAPE, %	LOA, kg
Females	DXA	24.3 $\pm$ 11.6							
	SLSDI	24.1 $\pm$ 11.3	0.2 $\pm$ 3.3	0.29	3.0	0.96	2.5	8.6	6.0, -5.5
	BIA1	23.2 $\pm$ 10.6	1.1 $\pm$ 2.4	0.0001	2.3	0.98	2.2	8.9	5.8, -3.6
	BIA2	21.1 $\pm$ 10.9	3.2 $\pm$ 2.4	0.0001	2.3	0.94	3.4	13.6	7.8, -1.5
Males	BIA3	20.7 $\pm$ 11.3	3.6 $\pm$ 2.3	0.0001	2.3	0.93	3.6	15.1	8.1, -0.9
	DXA	18.8 $\pm$ 7.6							
	SLSDI	18.8 $\pm$ 7.2	0 $\pm$ 2.9	0.97	2.9	0.93	2.3	11.5	5.6, -5.6
	BIA1	20.3 $\pm$ 8.2	-1.4 $\pm$ 3.9	0.0001	3.7	0.86	3.3	16.9	6.2, -9.1
	BIA2	21.1 $\pm$ 7.3	-2.9 $\pm$ 3.7	0.0001	3.6	0.84	3.6	19.0	9.5, -4.9
	BIA3	16.4 $\pm$ 7.5	2.4 $\pm$ 3.6	0.0001	3.6	0.84	3.6	18.1	9.6, -4.7

RMSE = root mean square; Bias = DXA - method; p=probability value; CCC = concordance correlation coefficient; MAE = mean absolute error; MAPE = mean absolute percent error; LOA = limits of

agreement; image analysis [22]; BIA1 = Kyle et al. [26]; BIA2 = Sun et al. [27]; BIA3 = Akern (proprietary model) DXA = dual x-ray absorptiometry; SLSDI = single lateral standing digital image.

Figure 2 reveals differences between SLSDI and BIA FM estimates compared to DXA. According to McBride [29], SLSDI shows substantial concordance (0.96) in females and moderate concordance in males (0.93) relative to DXA fat mass. Among females, BIA1 showed substantial concordance (0.98) while BIA2 and BIA3 showed moderate concordance (0.94 to 0.93) with DXA. In contrast, all male samples showed poor CCC (< 0.90) between the BIA prediction models and DXA.

For females, BIA, compared to SLSDI, had similar mean absolute error (MAE; 2.2 to 3.6 vs 2.5 kg) and greater MAE in males (3.3 to 3.6 vs 2.3 kg). Mean absolute percent error (MAPE) was less with SLSDI than BIA for females and males (8.6 vs 8.9 to 15% and 11.5 vs 16.9 to 19%, respectively).

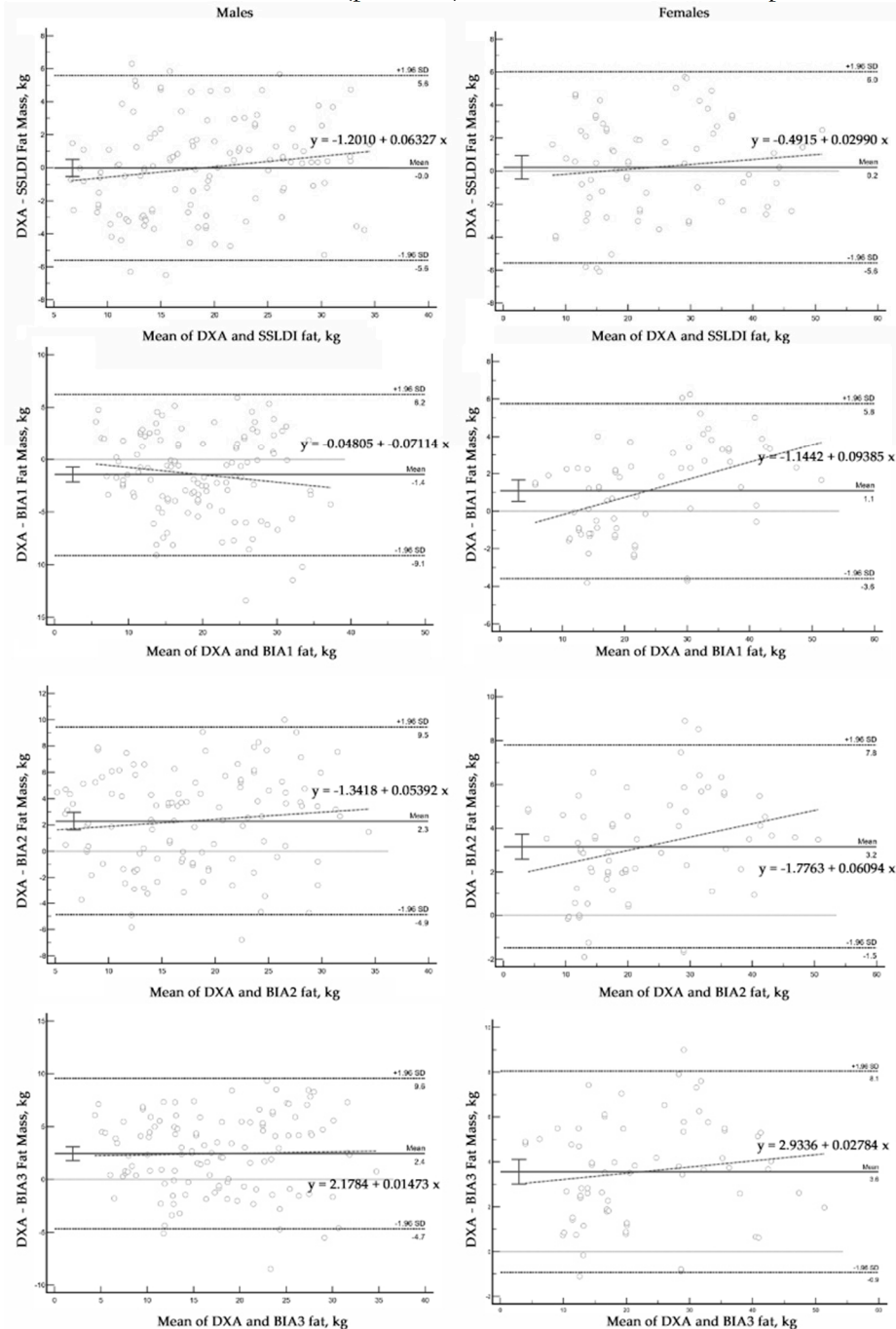


**Figure 2.** Linear regression plots of fat mass (FM) estimated with smartphone single lateral standing digital image (SLSDI) and bioelectrical impedance equations (BIA) compared to reference dual x-ray absorptiometry (DXA) in males and females.

The SLSDI predictions of FM for females and males were distributed equivalent with the line of identity with the slopes (0.995 and 0.986) the same as 1, and the intercepts (0.37 and 0.26 kg) not different than 0. The SEE values were 3.0 and 2.9 kg for females and males, respectively. In contrast, the intercepts for the BIA2 and BIA3 (2.3 and 3.4 kg;  $p < 0.0001$ ) prediction models in females and BIA1, BIA2, and BIA3 (2.16, 3.50 and 4.11 kg;  $p < 0.0001$ ) in males were different from 0. The slopes for these

lines were variable from 1.0 to 1.08 in females and from 0.82 to 0.99 for males, but not different than 1. The SEE in females (2.3 kg) was less than SEE in males (3.7 kg) (Figure 2).

Figure 3 shows the Bland-Altman plots of SLSDI and BIA compared to DXA. On average, smartphone SLSDI had similar FM values as DXA FM in females and males (0.2 and 0 kg). Compared to DXA, the BIA predictions of FM had significant bias in females (1.1 to 3.6 kg;  $p < 0.0001$ ) and males (2.3 and 2.4 kg;  $p < 0.0001$ ) with one BIA equation overestimating FM (-1.4 kg;  $p < 0.0001$ ). The limits of agreement (LOA) for SLSDI were smaller compared to the BIA FM estimates. The slopes of the lines relating the differences between DXA and individual methods were not different from 0 for SLSDI in the females and males but were different ( $p < 0.0001$ ) than 0 for two of the BIA equations in the females.



**Figure 3.** Bland-Altman plots of fat mass estimated with smartphone single lateral standing digital image (SLSDI) and bioelectrical impedance equations (BIA) compared to reference dual x-ray absorptiometry (DXA) in males and females.

We tested the hypothesis that excess fat affected fluid balance in men with BMI  $> 25 \text{ kg/m}^2$  by comparing male elite male rugby players with men who were non-rugby players (Table 2). Compared to non-rugby men, male rugby players had greater BMI ( $30.04 \pm 3.3$  vs  $28.7 \pm 3.0 \text{ kg/m}^2$ ;  $p < 0.05$ ) and

FFM ( $84.7 \pm 6.6$  vs  $68.1 \pm 10.1$  kg;  $p < 0.0001$ ) with less FM ( $19.3 \pm 6.6$  vs  $23.3 \pm 6.8$  kg;  $p < 0.01$ ), %fat ( $18.2 \pm 4.7$  vs  $25.4 \pm 6.2\%$ ;  $p < 0.0001$ ) and ECW% ( $36.2 \pm 2.4$  vs  $39.54 \pm 3.5\%$ ;  $p < 0.001$ ).

Estimates of FM using SLSDI were not different than DXA-determined FM in the rugby and non-rugby men (Table 3). Compared to DXA, one BIA equation overestimated FM ( $4.1 \pm 3.3$  kg;  $p < 0.0001$ ) in rugby players and two BIA models underestimated FM ( $3.7 \pm 3.5$  and  $4.2 \pm 3.6$  kg;  $p < 0.0001$ ) in non-rugby males. Concordance was moderate between SLSDI and DXA FM and poor for all BIA equations. For both groups, BIA, compared to SLSDI, had a greater MAE (2.6 to 4.8 vs 2.6 kg and 2.7 to 34.3 vs 2.1 kg, respectively) and MAPE (11.3 to 21.4 vs 7.9% and 8.3 to 20.9 vs 10.8%, respectively).

**Table 3.** Levels of agreement R and Non-rugby for body fat estimates using smartphone single lateral standing digital image analysis and 50 kHz bioelectrical impedance analysis compared to dual x-ray absorptiometry of men with body mass index greater than 25 kg/m<sup>2</sup>.

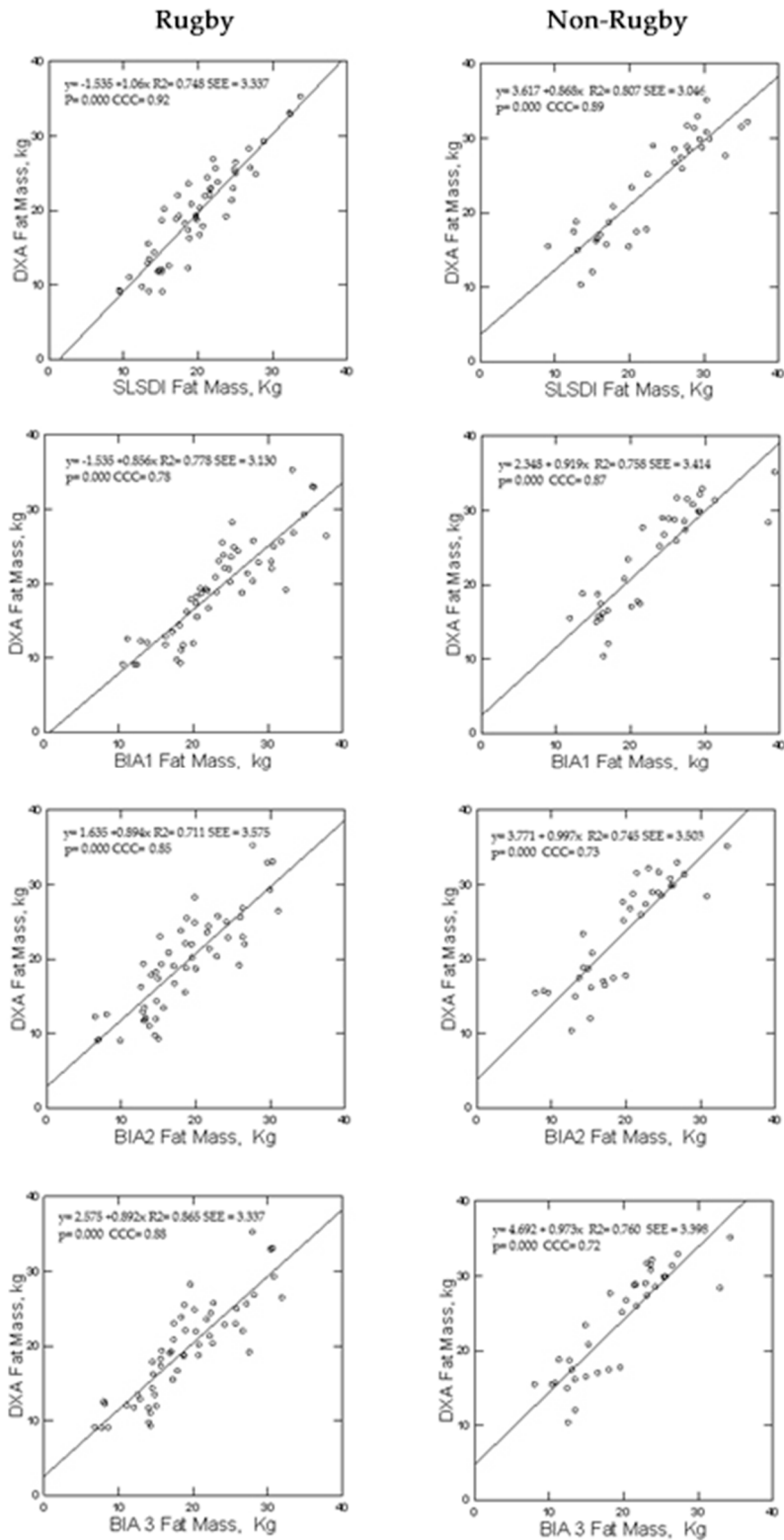
Group	Method	Fat mass, kg	Bias, kg	p	SEE, kg	CCC	MAE, kg	MAPE, %
Rugby	DXA	19.3±6.6						
	SLSDI	19.7±5.7	0.4±2.6	0.33	2.6	0.92	2.1	7.9
	BIA1	23.4±6.8	4.0±3.3	0.0001	3.1	0.78	4.3	21.4
	BIA2	18.5±6.2	-0.9±3.6	0.08	3.5	0.85	3.0	14.3
	BIA3	18.8±6.4	-0.5±3.4	0.24	3.3	0.88	2.7	11.3
Non-rugby	DXA	23.3±6.8						
	SLSDI	22.7±7.1	-0.6±3.1	0.22	3.1	0.89	2.6	10.8
	BIA1	22.8±6.5	-0.5±3.4	0.36	3.4	0.87	2.6	8.3
	BIA2	19.7±5.9	-3.7±3.5	0.0001	3.5	0.73	4.4	19.1
	BIA3	19.1±6.1	-4.2±3.6	0.0001	3.4	0.72	4.8	20.9

RMSE = root mean square; Bias = DXA - method; p=probability value; CCC = concordance correlation coefficient; MAE = mean absolute error; MAPE = mean absolute percent error; LOA = limits of agreement; image analysis [22]; BIA1 = Kyle et al. [26]; BIA2 = Sun et al. [27]; BIA3 = Akern (proprietary model) DXA = dual x-ray absorptiometry; SLSDI = single lateral standing digital.

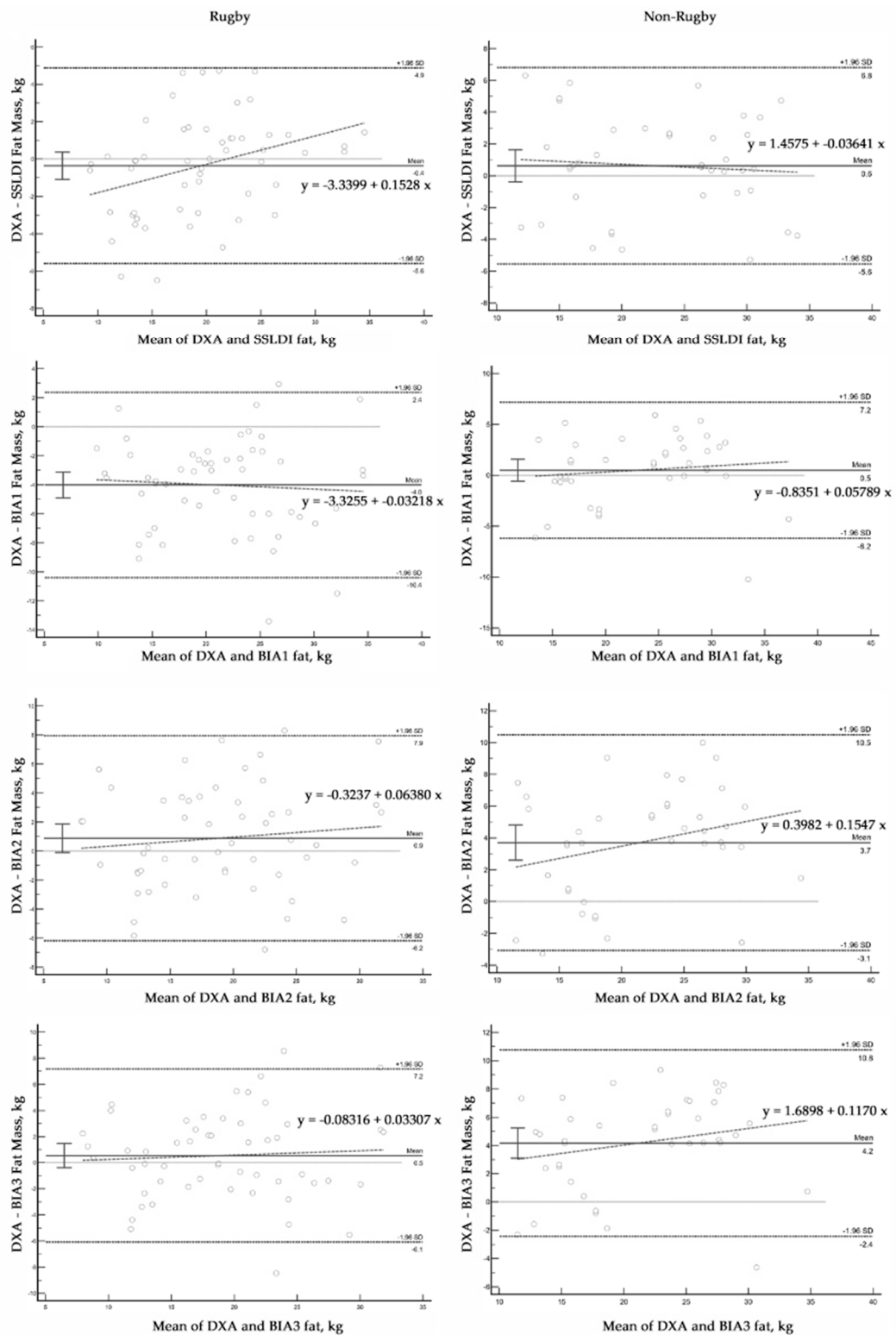
Compared to DXA FM values, the SLSDI FM estimates were distributed with slopes not different than 1 and intercepts not different than 0 whereas BIA predictions differed significantly from the line of identity (Figure 4). CCC values also were greater for SLSDI compared to BIA.

Bland-Altman analysis revealed significant bias and greater variability of data with BIA equations compared to SLSDI (Figure 5 and Table 3). No bias was detected with SLSDI in either group of men. Fat mass was significantly overestimated ( $4.0 \pm 3.3$  kg) with one BIA equation in rugby players. One BIA equation underestimated FM ( $-0.9 \pm 3.6$  kg) in the rugby males and all BIA equations significantly underestimated FM in the non-rugby players ( $-3.7 \pm 3.5$  and  $-4.2 \pm 3.6$  kg). The slopes of the lines relating the differences between DXA and individual methods were not different from 0 for SLSDI in both groups but were different ( $p < 0.0001$ ) than 0 for the BIA equations in all male groups. The LOA were greater for all BIA equations compared to SLSDI.

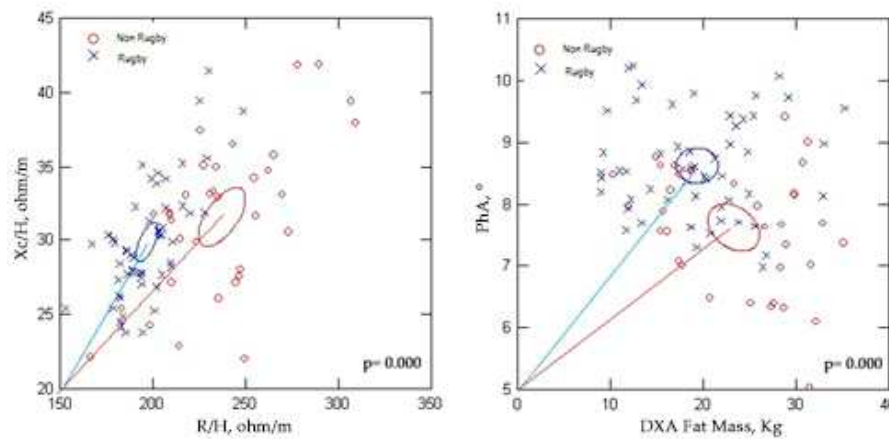
Figure 6 shows the significant group differences ( $p < 0.0001$ ) between the 95% confidence ellipses of men with BMI > 25 kg/m<sup>2</sup>. The rugby players had a shorter impedance vector displaced to the left indicating greater conductive mass and structure than the non-rugby males. The larger PhA of the rugby players designates less relative ECW or ECW/ICW compared to the non-athletic men associated with the lesser FM.



**Figure 4.** Linear regression plots of fat mass (FM) estimated with smartphone single lateral standing digital image (SLSDI) and bioelectrical impedance equations (BIA) compared to reference dual x-ray absorptiometry (DXA) in rugby and non-rugby men.



**Figure 5.** Bland-Altman plots of fat mass estimated with smartphone single lateral standing digital image (SSLDI) and bioelectrical impedance equations (BIA) compared to reference dual x-ray absorptiometry (DXA) in rugby and non-rugby men.



**Figure 6.** Resistance-reactance (R-Xc) plots showing 95% confidence ellipses (left panel) and the effect of fat mass on phase angle (PhA) (right panel) for rugby and non-rugby men.

#### 4. Discussion

Awareness of the limitations of BMI coupled with interest by clinicians and public health researchers for point-of-contact methods to estimate body adiposity is increasing [15–17]. Characteristics such as validity, high reproducibility, mobility, cost-effectiveness, and simplicity for operators focused research on SLSDI and BIA to meet this need. The findings of the present study demonstrate a high level of agreement between SLSDI FM estimates compared to reference DXA FM measurements with significant underestimation of FM with BIA. These results are consistent with other comparative studies of 2DI and BIA relative to reference DXA determinations of body fat [20,21]. However, they provide the first evidence that fluid imbalance contributes to the errors of BIA in estimating body fat.

A few reports speculated that increased body fat could adversely impact the validity of BIA predictions of FFM [24,25,33,34]. The researchers proposed that an expansion of ECW would contribute to an overestimation of FFM but provided no evidence. We evaluated this hypothesis in a subgroup of men with BMI > 25 kg/m<sup>2</sup> with different body composition profiles. Male rugby players, compared to non-rugby playing men, had decreased specific resistivity (R/Ht; 196.98±17.21 vs 237.22±30.97 ohm/m;  $p < 0.0001$ ) and increased FFM (84.7 vs 68.1 kg;  $p < 0.001$ ) with decreased FM (19.3 vs 23.3 kg;  $p < 0.001$ ) and ECW% (36.23±2.4 vs 39.54±3.5%;  $p < 0.001$ ). The decreased specific resistivity, or increased conductivity, reflects greater TBW and FFM in the rugby players. The increased ECW% among the non-rugby men indicates altered fluid distribution in association with the increased body fat and reduced ICW due to less FFM. Increased body fat is associated with an expanded adipose tissue volume that is directly related to an expanded ECW, absolute and relative, previously reported among adults classified as obese using BMI criteria [23,34]. Chemical analyses demonstrated the appreciable average water content of adipose tissue as 15% [35], which contributes to its conductivity but to a lesser degree than muscle. Thus, inter-individual differences in body fat contribute to the variability of ECW in adults.

Raw BIA measurements provide additional support for the finding of increased ECW. The significantly decreased PhA values in the non-rugby men indicate an expansion of ECW based on tracer dilution determinations of fluid volumes and demonstrate an inverse relationship between PhA and ECW/ICW [32]. Also, a decreased PhA has been associated with an expansion of ECW in novice runners with increased risk of acute kidney injury after a marathon [36]. The findings of imbalanced fluid distribution, ECW/ICW, contribute to the concerns regarding the assumption of constant hydration inherent in the application of BIA to estimate FFM and predict body fat [37–39].

1. The independent variables in the BIA prediction models offer potential sources of error in the prediction of FFM. The common predictors of  $Ht^2/R$  and  $R$  are significantly related to TBW. Thus, an increase of ECW in individuals with excess body fat increases TBW and decreases  $R$ , which assuming constant hydration of FFM, can overestimate FFM and thus underestimate FM. The BIA models also include body weight, which is highly correlated with body fat, and can vary depending on environmental, dietary, and physical activity conditions and hence affect hydration. Additionally, the application of BIA prediction equations in groups in whom the original model was not developed can lead to errors. It is well established that body geometry

(e.g., limb length, cross-sectional area, and volume) and fluid content (total and distribution) directly affect resistivity and contribute to inter-individual differences in whole body and regional BIA measurements [37,40]. Additionally, regional BIA measurements using different electrode placements, such as foot-to-foot and hand-to-hand, yield discordant estimates of body composition compared to whole-body measurements [41]. These factors contribute to the errors in BIA predictions of body fat using various BIA methods and models and DXA in the present study and other reports [20,21].

Our finding of a high level of agreement between SLSDI and DXA fat measures is consistent with two previous studies that used smartphone digital imaging with machine learning and proprietary prediction models to determine body fat in adults [20,21]. Although these studies utilized standing body positions for 2DI, they differed in body positions in relation to the smartphone camera. Nana et al. [20] used front and side positions with 24 replicate digital images for analysis, and Majmudar et al. [21] employed front and back poses. In contrast, the present study utilized a single lateral pose to obtain body contour silhouettes for analysis. Use of multiple body positions and repetitive digital images resulted in greater variability in body fat estimates ( $\pm 1.5$  %fat) compared to 0 in the present study.

This study has some limitations. The inference of expansion of ECW relies on qualitative assessments using BIA. Future studies should incorporate tracer dilution determinations of ECW and TBW and obtain threshold values at which altered fluid distribution affect errors in estimating body fat using BIA. Additionally, investigators should determine the effect of graded body fat levels on errors relative to criteria reference methods particularly in adults with sarcopenic obesity.

## 5. Conclusions

Smartphone SLSDI provides highly reliable and accurate estimates of FM comparable to DXA of adults with a wide range of body fat. The accuracy and reproducibility, coupled with the convenience, practicality, and cost-efficiency facilitate an innovative method to assess body composition to enable routine assessment of body fat for health care personnel in many environments and for biomedical researchers outside the laboratory. It surmounts the limitations of BMI as an index of body fat [15–17] and provides a novel method to estimate abdominal FM [22]. Moreover, SLSDI image-based FM estimates provide better performances than impedance-based methods seemingly because they are not influenced by variable hydration states.

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