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The Comparison of Segmental Multifrequency Bioelectrical Impedance Analysis and Dual-Energy X-ray Absorptiometry for Estimating Fat Free Mass and Percentage Body Fat in an Ambulatory Population

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Abstract

Background: Despite malnutrition being associated with increased mortality and morbidity, there continues to be great difficulty in defining criteria and implementing widespread screening. Tools used to diagnose decreased fat-free mass (FFM [sarcopenia]) should be easy to use, relatively inexpensive, and safe. Bioelectrical impedance analysis (BIA) has the potential to meet these criteria, but reliability across body mass index (BMI) classes is a concern. *Methods:* A total of 176 healthy ambulatory participants (aged 18–65 years) were recruited equally (n = 44) in 4 BMI categories: (1) 18.5–24.9, (2) 25.0–29.9, (3) 30–34.9, and (4) ≥35.0. Participants were fasting overnight and had S-MFBIA (InBody 770) measurements the next morning, with DXA being performed subsequently within 30 minutes. *Results:* The measurement (mean ± SD) for FFM with DXA was 52.8 ± 11.0, and BIA was 53.6 ± 11.0. Delta (S-MFBIA vs DXA) was 0.8 ± 2.2 (5% limits of agreement −3.5 to +5.2), and concordance correlation coefficient (CCC) was 0.98 (95% CI, 0.97–0.98). The measurements (mean ± SD) for PBF with DXA was 37.5 ± 10.6% and S-MFBIA was 36.6 ± 11.3%. Delta (S-MFBIA vs DXA) was −0.9 ± 2.6 (5% limits of agreement 6.0 to +4.2), and CCC was 0.97 (95% CI, 0.96–0.98). The CCC according to the 4 BMI groups for FFM and PBF was between 0.96–0.98 and 0.90–0.94, respectively. *Conclusions:* FFM and PBF measured by S-MFBIA had good agreement with DXA across all BMI categories measured in the current study of ambulatory participants. (*JPEN J Parenter Enteral Nutr.* 2020;00:1–8)

Keywords

body composition; bioelectrical impedance analysis; dual-energy x-ray absorptiometry; enteral nutrition; Fat-free mass; percentage body fat

Clinical Relevancy Statement

In view of the rapid increase in prevalence of home enteral nutrition and its healthcare infrastructure, technologies used to measure body composition have become an integral part of home enteral nutrition programs and can guide caregivers and patients to achieve optimal nutrition in terms of fat-free mass across a wide spectrum of body mass indexes. Many such technologies are currently available, but there is paucity in data regarding their reliability. Current

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study evaluates a newer bioelectrical impedance analysis technology, and we found that it is a safe, economical, and reliable tool when compared to the gold-standard dual-energy x-ray absorptiometry and that it can be an ideal alternative in an ambulatory setting.

Introduction

The loss of fat-free mass (FFM), combined with low muscle strength and/or muscle performance, is the basis for the definition of age-related sarcopenia.[1,2] The prevalence of age-related sarcopenia ranges from 1% to 33% of older adults (aged >65 years) and depends on the setting (ie, acute hospital, long-term care, and community-dwelling facilities). When considering people aged ≥80 years, the prevalence of sarcopenia can be up to 50%.[3,4] Despite the high prevalence of sarcopenia in both older and obese individuals, the associated comorbidities, including mortality, as well as the availability of effective treatments of protein and exercise, it is a condition rarely diagnosed in ambulatory practice.[3] Medical definitions, such as age-related and obesity-associated sarcopenia, are only clinically useful if there are (1) clinical tools that can accurately diagnose the condition and (2) treatments that are readily available to improve the condition. In addition, the tools used to diagnose medical conditions should be easy to use, relatively inexpensive, and safe.

Currently, in most ambulatory settings, body mass index (BMI) is the most common data point used to distinguish between malnourished and overweight or obese individuals. However, BMI fails to account for many variables that are essential for risk stratification, including location of adipose tissue stores (upper body vs lower body or subcutaneous vs visceral), FFM vs fat mass, and overall fluid status. Because of this, a number of additional methods for directly estimating body composition have been used. Dual-energy x-ray absorptiometry (DXA) is one of the most well-validated techniques that allows for determination of bone mineral and soft-tissue densities, which can be further delineated into whole-body fat mass and FFM, as well as segmental analysis.[5] Unfortunately, it is not routinely available in the ambulatory practice outside of research settings because of a number of factors, including space requirement, cost, and specialty training. A computed tomography (CT) scan improves upon the details of body composition provided by DXA by allowing for further details, such as delineation of visceral, intramuscular, and subcutaneous adipose tissue, but similar to DXA, its limitations also include cost, space, and specialty training.[6] FFM can also be further subdivided into skeletal muscle groups or visceral organs. A CT scan does have risk of radiation exposure and thus is more often available for body composition assessment for inpatients, as they may have undergone imaging as part of their clinical assessment. Skeletal muscle ultrasonography is also emerging as a body composition measurement technique that can capture loss of muscle mass over time without radiation exposure.[5] However, currently, there is lack of protocol consensus, and further validation is necessary prior to more widespread use in the ambulatory setting.

Another body composition measurement technique that is becoming more readily available in the ambulatory setting is bioelectrical impedance analysis (BIA). BIA is a generic term to describe this technology, including bioimpedance spectroscopy, and uses a low-amplitude alternating electrical current at either single-frequency (SF) or multifrequency (MF) to characterize the conductive and nonconductive tissue components of the body. These measurements can be either whole-body (wrist to ankle on 1 side, (Figure 1, left side) or segmental (Figure 1, right side).[7,8] BIA has some significant advantages over the other techniques, including relatively low cost, avoidance of radiation exposure, ease of use, and the ability to be performed by both patients and allied health staff. Although SF-BIA is limited in the body composition variables that it can provide, newer segmental multifrequency BIA (S-MFBIA) machines have touted the ability to improve the accuracy of FFM and percentage body fat (PBF) estimates while providing additional body composition data, including extracellular and intracellular water, visceral fat, and regional FFM, FM, and bodywater measurements.

Despite these advantages, a number of issues have been raised with the use of BIA, including accuracy and reliability in determining body composition measurements across the spectrum of body weights (and thus BMIs) encountered in ambulatory practices when compared to DXA.[9] The primary aim of the current study was to evaluate FFM and PBF measurements by S-MFBIA and DXA in participants recruited equally in 4 BMI (calculated as weight in kilograms divided by height in meters squared) categories: (1) 18.5–24.9, (2) 25.0–29.9, (3) 30–34.9, and (4) ≥35.0. Our hypothesis was that the S-MFBIA would have a concordance correlation coefficient (CCC) for both FFM and PBF of ≥0.90 across all 4 BMI groups compared to DXA and, with the ease of use, could be a comparable alternative to DXA.

Methods

The present study was conducted at the Mayo Clinic Department of Medicine Clinical Trials Unit (DOM CTU) and, in accordance with the Declaration of Helsinki, was approved by the institutional review board (IRB) (ID 14–004659). We registered the trial with clinicaltrials.gov (NCT02635958) prior to the start of the study. We recruited potential participants using IRB-approved recruitment through newspaper classifieds and radio advertisements. Mayo Clinic IRB–approved written informed consent was obtained for all study participants prior

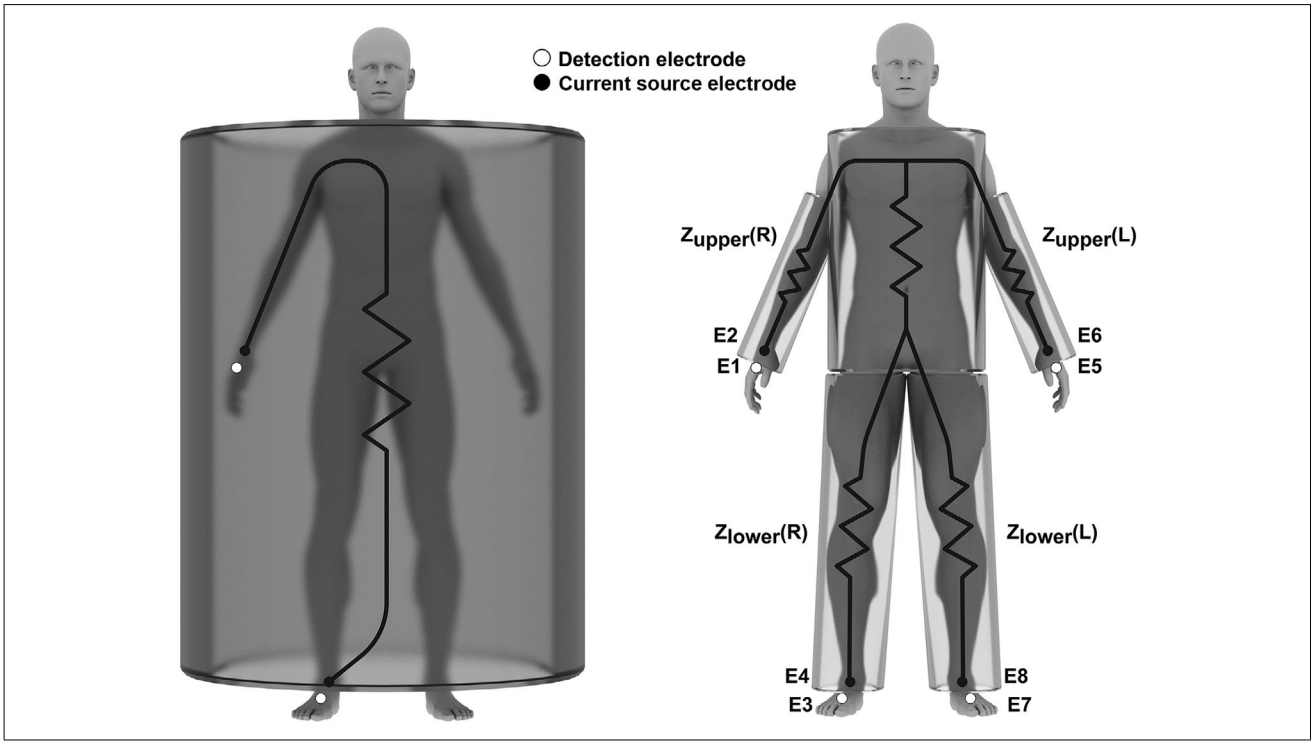

Figure 1. Schematic diagram of Bioelectrical Impedance Analysis (BIA) showing whole-body (left) and segmental (right) analysis.

to study participation. Interested individuals contacted a member of the study team who then performed a prescreening, evaluating eligibility by phone using an IRB-approved telephone script. Inclusion criteria included the following: adults aged 18–65, a BMI $\geq$ 18.5, and weight $<$ 450 lb (204 kg) (the upper weight limit of the iDXA machine [GE Healthcare; Wauwatosa, WI, USA]). Exclusion criteria included height $>$ 198.1 cm, a pacemaker, a defibrillator, an artificial lung, an artificial heart, previous history of a nuclear scan 5 days prior to testing, metal implants outside of dental work, and those currently pregnant. Individuals who passed the prescreen phone interview were scheduled for an in-person, private appointment to review the informed consent/assent form. After signing the informed consent and undergoing the study-screening process, including a formal recheck of the study-entry criteria, those who met the inclusion and exclusion criteria were stratified into 1 of the 4 BMI classes ([1] 18.5–24.9, [2] 25.0–29.9, [3] 30–34.9, and [4] $\geq$35.0) based on self-reported weight and height initially (later confirmed by actual measurements). The goal was to recruit a total of 176 participants, with 44 in each BMI category (Figure 2). Females of childbearing potential, who provided consent, were administered a pregnancy test, which was required to be negative to proceed with the trial.

Participants were instructed to present to DOM CTU the morning after undergoing an overnight fasting period after midnight. Prior to height and weight measurements, participants were instructed to void urine in the DOM CTU restroom. Height was measured with stadiometer to the nearest cm by having participants place both bare feet flat on the ground. Following height measurements and standing for a period of $\geq$10 minutes, participants were then asked to step on the S-MFBIA scale (InBody 770, Inbody Co. Ltd. , Seoul, South Korea). The participants followed the S-MFBIA commands, and a study assistant was present to ensure compliance. The process of measuring body composition using the S-MFBIA scale used in the present study (InBody 700 series) has been described elsewhere.[10,11] Briefly, the participants grasped the handles, with the palms and thumbs making contact with the electrodes. The S-MFBIA uses 8 polar tactile electrodes, with 2 that are in contact with the palms, 2 with each thumb, 2 with the anterior, and 2 with the posterior aspect of each foot (Figure 1, right). The patient was instructed to remain motionless, and the study assistant was present to ensure this command was followed. The S-MFBIA estimated the body composition across 5 segments (right leg, left leg, right arm, left arm, and trunk), using the frequencies 1, 5, 50, 250, 500, and 1000 kHz. Body composition results are calculated with use of proprietary prediction algorithms that are built into the firmware of the device and apply only to the device being studied. The total testing time, including removal of socks and shoes and entering participant information, was

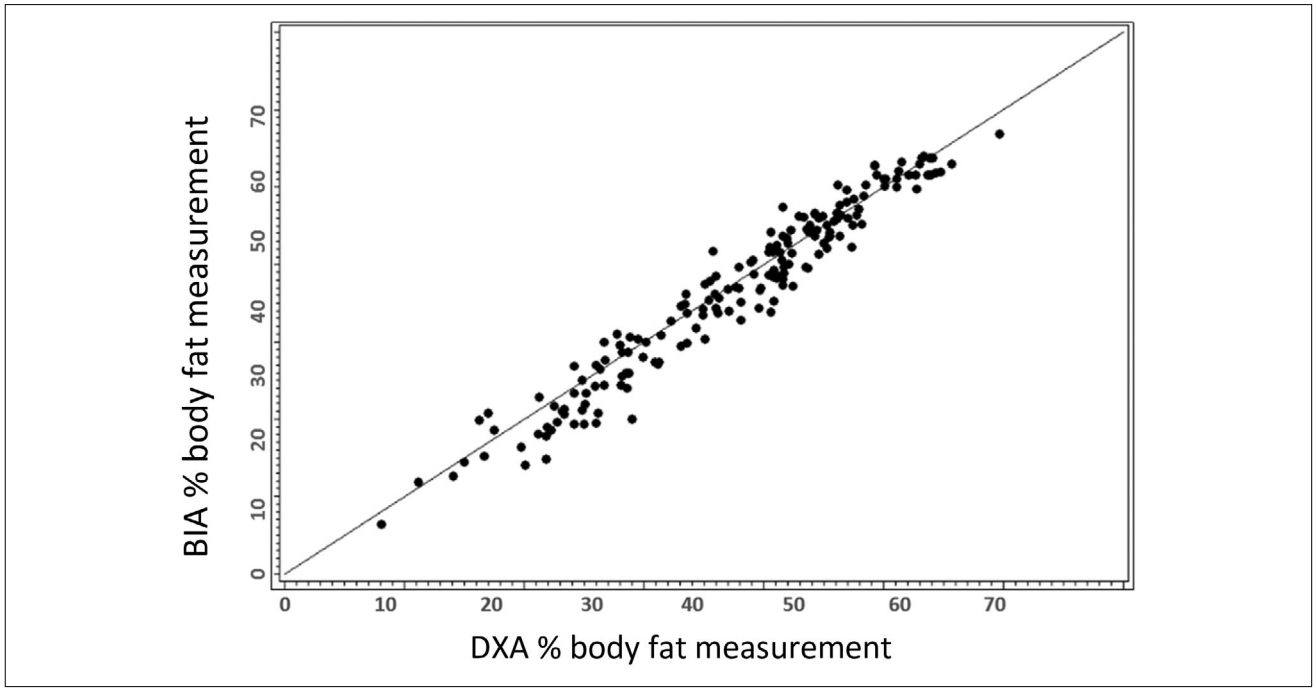

Figure 2. Percentage body fat measurement: S-MFBIA vs DXA. S-MFBIA segmental multifrequency bioelectrical impedance analysis; DXA, dual-energy x-ray absorptiometry.

~2 minutes. All measurements were then automatically sent from the S-MFBIA to a printer. Immediately after the S-MFBIA measurement, the participants were transported via wheelchair to the iDXA location in the adjacent building in the Mayo Clinic DOM CTU, and the iDXA scan was performed. The DOM CTU technician had the participants lie flat on the DXA table. During the scan, the participants were instructed to remain motionless, and the technician was there to ensure compliance as with the S-MFBIA measurement. The DXA measurements for FFM and PBF were completed within 30 minutes of the S-MFBIA. Participants remained in a fasting state throughout the study protocol.

Assuming the PBF and FFM means and SDs, as reported in Faria et al,[12] we estimated that with 44 participants in each of the 4 BMI classes ([1] 18.5–24.9, [2] 25.0–29.9, [3] 30–34.9, and [4] ≥35.0), we had a 90% power to detect a 0.5 SD difference between DXA and S-MFBIA, assuming a 2-sided significance level of .05. Data are presented as mean, SD, and range. A 2-sided *P*-value > .05 signified that there was no statistical difference between the groups. Agreement between the S-MFBIA and DXA was assessed overall and according to BMI category. The mean difference between methods (S-MFBIA vs DXA) was compared to 0 using the 1-sample *t*-test, and 95% limits of agreement were calculated as the mean difference ± 1.96 SDs.[13] In addition, Lin's CCC was computed with a 95% CI, calculated using Fisher *z*-transformation.[14]

Results

The baseline demographics of all participants were similar in regard to race, sex, alcohol and tobacco use, and activity (Table 1). There were no adverse events reported by any participant in the current study. The overall (n = 176) measurements (mean ± SD) for PBF with DXA was 37.5 ± 10.6% and S-MFBIA was 36.6 ± 11.3% (Figure 2). The overall delta (S-MFBIA vs DXA) was −0.9 ± 2.6% (95% limits of agreement −6.0 to +4.2) and CCC was 0.97 (95% CI, 0.96–0.98) (Table 2). The overall (n = 176) measurements (mean ± SD) for FFM with DXA was 52.8 ± 11.0 kg and S-MFBIA was 53.6 ± 11.0 kg (Table 2). The overall delta (S-MFBIA vs DXA) was 0.8 ± 2.2 kg (95% limits of agreement −3.5 to + 5.2) and CCC was 0.98 (95% CI, 0.97–0.98) (Figure 3). The CCC according to the 4 BMI groups for PBF ranged from 0.90 to 0.94. The lowest BMI category (18.5–24.9 [n = 44]) had the lowest CCC of 0.90 (Table 2). The CCC according to the 4 BMI groups for FFM ranged from 0.96 to 0.98 (Table 2). The highest BMI category (≥35) had the lowest CCC of 0.96 (Table 2).

Discussion

Having the ability to accurately estimate FFM and PBF are important parts of our evolving understanding of the pathophysiology of malnutrition and sarcopenia. Although there are a number of available techniques for measuring

Table 1. Participant Characteristics.

Characteristic	Overall (N = 176)	BMI category 18.5–24.9 (N = 44)	25.0–29.9 (N = 44)	30.0–34.9 (N = 44)	≥ 35.0 (N = 44)
Age, y					
Mean ± SD	38.1 ± 12.4	30.8 ± 10.9	39.3 ± 11.9	43.7 ± 11.5	38.7 ± 11.8
Median (min, max)	35.5 (19, 65)	27 (19, 60)	39 (19, 61)	43 (25, 63)	36 (19, 65)
Sex, n (%)					
Male	43 (24)	9 (20)	16 (36)	11 (25)	7 (16)
Female	133 (76)	35 (80)	28 (64)	33 (75)	37 (84)
Race					
White	162 (92)	37 (84)	42 (95)	42 (95)	41 (93)
Asian	5 (3)	2 (5)	2 (5)	1 (2)	0 (0)
Black	2 (1)	1 (2)	0 (0)	1 (2)	0 (0)
>1 race	7 (4)	4 (9)	0 (0)	0 (0)	3 (7)
Current tobacco user					
No	164 (93)	41 (93)	42 (95)	41 (93)	40 (91)
Yes	12 (7)	3 (7)	2 (5)	3 (7)	4 (9)
Alcohol use					
Never	33 (19)	5 (11)	7 (16)	12 (27)	9 (20)
Monthly or less	47 (27)	11 (25)	9 (20)	11 (25)	16 (36)
2–4 times per month	57 (32)	17 (39)	14 (32)	11 (25)	15 (34)
2–3 times per week	36 (20)	11 (25)	13 (30)	10 (23)	2 (5)
≥4 times per week	3 (2)	0 (0)	1 (2)	0 (0)	2 (5)
Current level of activity					
Extremely inactive/sedentary	35 (20)	5 (11)	6 (14)	9 (20)	15 (34)
Moderately active	104 (59)	28 (64)	23 (52)	26 (59)	27 (61)
Vigorously active	27 (15)	6 (14)	11 (25)	8 (18)	2 (5)
Extremely active	10 (6)	5 (11)	4 (9)	1 (2)	0 (0)
Current stress (1–10)					
Mean ± SD	4.2 ± 2.1	4.3 ± 1.9	4.1 ± 2.1	4.3 ± 2.2	4.1 ± 2.0
Median (min, max)	4 (1, 9)	4 (1, 8)	4 (1, 8)	4 (1, 8)	4 (1, 9)

BMI calculated as weight in kilograms divided by height in meters squared.
BMI, body mass index.

body composition, many are restricted to the research setting, have associated radiation exposure, or are too expensive and specialized in use to be utilized in the ambulatory setting. DXA is one such modality and typically considered the gold standard because of its accuracy in measuring certain body composition metrics, such as FFM and fat mass. Unfortunately, the cost of both the machine and individual measurements, technician requirements for use, and size of the DXA equipment make routine ambulatory clinic use impractical. Because of this, BIA is emerging as a viable approach in this patient population. In the current study, an S-MFBIA demonstrated a high CCC with DXA in both PBF and FFM measurements. The main and novel finding of the present study was the high CCC (>0.90) remained preserved across the 4 BMI categories evaluated for both FFM and PBF. This finding is strengthened by the fact that participants were equally (n = 44) recruited across the BMI categories, as compared with other studies that recruit across a range. This is important clinically because obesity is defined according to BMI class and cutoffs (eg, BMI > 30.0).[15]

There are a number of studies that have compared the S-MFBIA with DXA in varying patient populations.[10,12-18] A previous study used an earlier version of the S-MFBIA tested in the present study (InBody 720 vs 770) in patients with obesity (>35).[12] One of the main objectives of this study was to conduct a validation test for S-MFBIA in patients with a BMI > 35, given the question of decreased accuracy of BIA as BMI increases.[12] The average BMI in the 73 participants was 40.17 ± 4.08, and most of the participants were women (89%). There was a high correlation between fat (intraclass correlation coefficient (ICC) = 0.832), as measured by the DXA and S-MFBIA. Like the present study, there was a high correlation between DXA and S-MFBIA with FFM (ICC = 0.899). Another trial compared S-MFBIA (InBody 720) with DXA in

Table 2. Agreement Between DXA and S-MFBIA.[a].

Time (comparison)	N	Measurement		Delta (S-MFBIA vs DXA)		Concordance correlation
		DXA (mean ± SD)	S-MFBIA (mean ± SD)	Mean ± SD	95% Limits of agreement	Coefficient (95% CI)
Fat Free mass, kg						
Overall	176	52.8 ± 11.0	53.6 ± 11.4	0.8 ± 2.2[b]	−3.5 to +5.2	0.98 (0.97, 0.98)
According to BMI						
18.5–24.9	44	46.7 ± 9.0	47.9 ± 9.3	1.2 ± 1.9[b]	−2.6 to +5.0	0.97 (0.94, 0.98)
25.0–29.9	44	52.1 ± 11.6	53.3 ± 12.6	1.2 ± 2.2[b]	−3.2 to +5.6	0.98 (0.96, 0.99)
30.0–34.9	44	53.6 ± 11.2	53.9 ± 11.4	0.3 ± 2.0	−3.7 to +4.3	0.98 (0.97, 0.99)
≥35.0	44	58.7 ± 8.7	59.2 ± 9.5	0.6 ± 2.5	−4.4 to +5.5	0.96 (0.93, 0.98)
Percentage body fat, %						
Overall	176	37.5 ± 10.6	36.6 ± 11.3	−0.9 ± 2.6[b]	−6.0 to +4.2	0.97 (0.96, 0.98)
According to BMI						
18.5–24.9	44	27.4 ± 7.7	25.4 ± 7.7	−2.0 ± 2.8[b]	−7.5 to +3.5	0.90 (0.83, 0.94)
25.0–29.9	44	33.5 ± 8.4	32.3 ± 9.1	−1.3 ± 2.9[b]	−7.0 to +3.4	0.94 (0.89, 0.96)
30.0–34.9	44	40.5 ± 6.7	40.3 ± 6.9	−0.2 ± 2.3	−4.7 to +4.3	0.94 (0.90, 0.97)
≥35.0	44	48.6 ± 5.3	48.4 ± 5.2	−0.1 ± 2.0	−4.1 to +3.9	0.92 (0.87, 0.96)

BMI calculated as weight in kilograms divided by height in meters squared.
BMI, body mass index; DXA, dual-energy x-ray absorptiometry; S-MFBIA, segmental multifrequency bioelectrical impedance analysis.
[a] Agreement was assessed overall and according to BMI category. The mean difference between methods (InBody [DXA]) was compared to 0 using the 1-sample *t*-test, and 95% limits of agreement were calculated as the mean difference ± 1.96 SDs.[22] In addition, Lin's concordance correlation coefficient was computed with a 95% CI, calculated using Fisher *z*-transformation.[23]
[b] One-sample *t*-test $P < .05$ comparing the mean difference (InBody [DXA]) to 0.

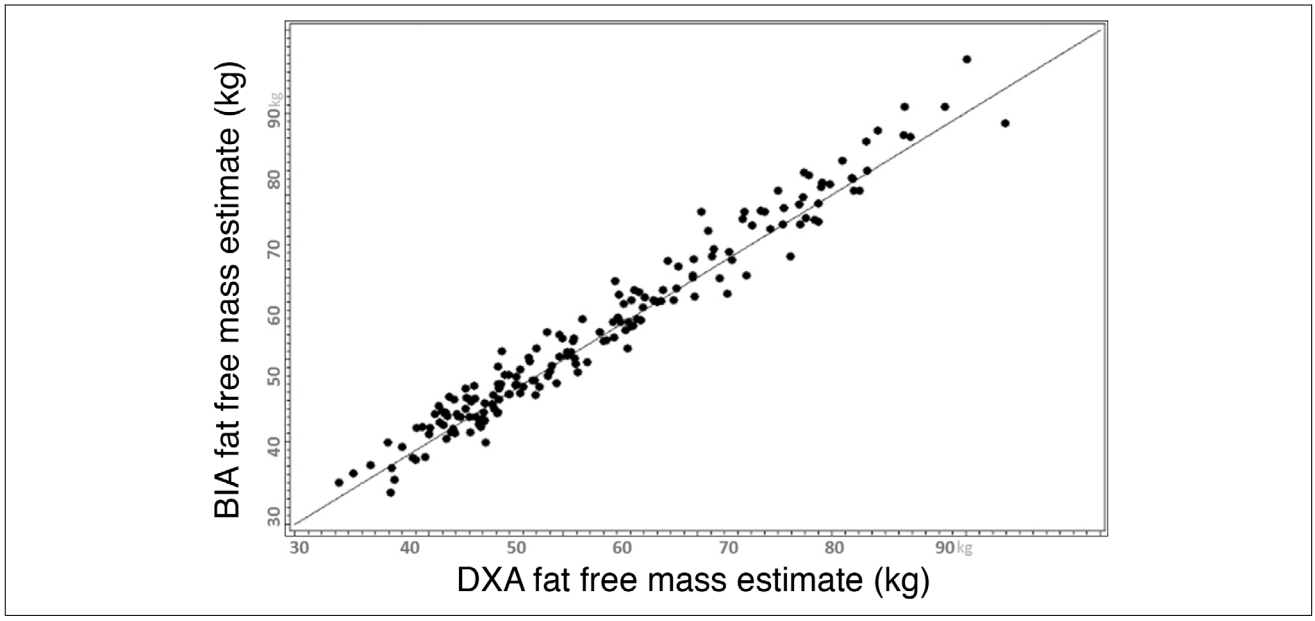

Figure 3. Fat-free mass estimate (kg): S-MFBIA vs DXA. BIA, S-MFBIA segmental multifrequency bioelectrical impedance analysis; DXA, dual-energy x-ray absorptiometry.

college women athletes (n = 45; average age, 21.2 ± 2 years; average weight, 62.6 ± 9.9 kg).[10] Compared with DXA, the S-MFBIA provided slightly lower PBF levels (−3.3%) and higher FFM (+2.1 kg). The authors concluded that the S-MFBIA and DXA had excellent agreement for measuring total and segmental FFM.[10] Ling et al evaluated S-MFBIA vs DXA in 484 patients, with mean age in the 60s and mean BMI in the overweight range.[17] Similar to other trials, they noted excellent agreements between both techniques in whole-body lean mass (ICC women = 0.95,

ICC men = 0.96), fat mass (ICC women = 0.97, ICC men = 0.93), and PBF (ICC women = 0.93, ICC men = 0.88) measurements. Additionally, with lean muscle mass, they also found excellent agreement in terms of segmental analysis, including the upper limbs (ICC women = 0.91, ICC men = 0.87) and lower limbs (ICC women = 0.83, ICC men = 0.85). Furstenberg et al compared S-MFBIA to DXA for assessing body composition in 104 stable peritoneal dialysis patients.[18] They also noted good correlation between the two methods in terms of both FFM ($r = 0.95$, $P < .0001$; bias, −0.88 kg; 95% CI, −1.53 to 0.23 kg), as well as fat mass ($r = 0.93$, $P < .0001$; bias, 0.69 kg; 95% CI, 0.03–1.36 kg). Segmental analysis of FFM also revealed strong correlations for trunk and left and right arms and legs ($r =$ 0.90, 0.84, 0.86, 0.89, and 0.90, respectively, $P < .0001$).[18]

As the results of these studies reveal, BIA technology has improved significantly over the past 30 years with the introduction of MF segmental devices. This advancement of the BIA technology demonstrates an understanding that the body is made up of 5 cylinders (2 legs, 2 arms, and the trunk) with distinct differences in composition (Figure 1).[11,16] In the current study and others, S-MFBIA has demonstrated a high correlation with FFM and fat mass to DXA.[10,12,16] S-MFBIA has a number of potential advantages over DXA, including lack of radiation exposure and decreased cost.[16] BIA can also be performed by a clinical assistant with minimal training, with the patient following simple commands to complete the ~2-minute test. In addition to the body composition data provided, raw parameters, such as capacitance and phase angle, have been linked to severity of illness and can be of further assistance in risk stratifying patients and predicting survival.[8,19,20]

Our study has several limitations. This was a single-center study using relatively healthy ambulatory participants who were normal weight, overweight, or obese class I, II, or III. We used a single type of S-MFBIA machine (InBody 770) that we routinely use at our institution, making it difficult to generalize the findings of this study to other BIA devices, given the proprietary algorithm used to obtain body composition data. We compared the FFM and PBF estimates to DXA, which, although considered the gold standard for body composition, is still an estimate rather than direct measurement. DXA, similar to other studies, can also be associated with technical errors of measurement despite best efforts to standardize the technique, including conducting both measurements within 30 minutes. Additionally, limits of agreement analysis between S-MFBIA and DXA was noted to be 8.1% for FFM and 13.6% for fat mass. However, given the inherent errors associated with DXA (gold standard), we felt that these differences were still minimal enough to allow usage in clinical setting. In fact, many researchers in this field have recognized the need to shift focus from statistical significance to clinically meaningful difference, which may be more meaningful in clinical practice.[21]

Conclusions

To have widespread ability to accurately estimate FFM and PBF, and thus diagnose sarcopenia and malnutrition, devices must possess a number of essential characteristics, including ease of use, low cost, and accurate measurements across the spectrum of patients being tested. We feel the main one of these criteria is the ease of use in ambulatory practices, as this is one of the only ways we will be able to diagnose and effectively treat those patients with malnutrition and sarcopenia. The S-MFBIA can be used in most ambulatory practices and requires very minimal training to use. In fact, BIA takes the same amount of space as a scale and allows our clinical assistants in the ambulatory nutrition clinic to obtain body composition measurements in a few minutes while obtaining other vitals (eg, weight, heart rate, and blood pressure). Given the high level of agreement to DXA across BMI categories in estimating FFM and fat mass, S-MFBIA allows providers to further risk stratify patients and develop treatment plans. Additionally, body composition can be reliably followed in the ambulatory setting with serial measurements to assess how various treatments (such as nutrition therapy) are affecting body composition and nutrition status. Future comparison studies of S-MFBIA and DXA in patients diagnosed with malnutrition, sarcopenia, or fluid overload will allow for further applicability of this technique.

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Statement of Authorship

R. T. Hurt and M. S. Mundi equally contributed to the conception and design of the research; J. O. Ebbert and I. Croghan contributed to the design of the research; S. Nanda and S. R. Velapati contributed to the acquisition and analysis of the data; D. R. Schroeder contributed to the analysis of the data; L. M. Teigen contributed to the acquisition, analysis, and interpretation of the data. All authors drafted the manuscript, critically revised the manuscript, agree to be fully accountable for ensuring the integrity and accuracy of the work, and read and approved the final manuscript.

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