Clinical Nutrition 38 (2019) 2659–2667

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Body fat percentage assessment by ultrasound subcutaneous fat thickness measurements in middle-aged and older adults

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ARTICLE INFO

Article history:
Received 5 January 2018
Accepted 25 November 2018

Keywords:
Ultrasonography
Obesity
Aging
Geriatrics
Body composition

SUMMARY

Background & aims: B-mode ultrasound accurately measures both muscle mass, body density and percent body fat (% BF) in younger adults, but how well it can estimate % BF in middle-aged and older adults using DXA-derived %BF as the criterion is unclear. We sought to develop % BF prediction equations for middle-aged and older adults using ultrasound subcutaneous fat thickness (SFT).

Methods: A cross-sectional study of Japanese adults (n = 414, 50–79 years) where 276 subjects were randomly assigned to a model development group and the other 138 subjects were assigned to a cross-validation group. B-mode ultrasound measured SFT at nine sites. Dual energy X-ray absorptiometry (DXA) measured % BF, arm fat mass (FM) and leg FM. Stepwise multiple linear regression developed prediction equations from anthropometric data (body mass, height, waist and hip circumference) and ultrasound SFT sites. Bland–Altman plots assessed validity of the prediction equations to measure % BF in the cross-validation group.

Results: The best prediction equation for % BF was the following: [% BF = 15.709 + (1.753*anterior trunk SFT) + (5.626*Sex) + (3.635*posterior upper arm SFT) - (4.428*anterior lower leg SFT) − (0.170*height) + (0.264*waist) + (anterior thigh SFT*2.241); r^2 = 0.809, standard error of the estimate (SEE) = 3.3 kg]. Arm FM and leg FM prediction equations had r^2 values ranging from 0.690 to 0.812 and SEEs of 0.29 and 0.75 kg. A small mean bias was noted for estimating % BF (−0.14%), but large limits of agreement were found (−8.0–7.7%) and systematic error was noted in all of the equations (r = 0.275 to 0.515, $p < 0.05$).

Conclusions: Despite high r^2 values and a small mean bias found between predicted and DXA % BF, wide limits of agreement were found with some systematic error present. Therefore, these prediction equations for middle-aged and older adults may not be sufficiently accurate to use in a clinical setting.

1. Introduction

With increasing age, there is a clear alteration in body composition that can adversely affect the health and function of an individual. One adverse outcome is the loss of skeletal muscle mass and function known as sarcopenia [1]. This loss may begin in the 4th or 5th decade of life [2], with the loss in skeletal muscle mass varying from ~0.6 to 1% per year [3]. Negative outcomes such as increased risk of falls [4], decreased insulin sensitivity [5] and increased mortality [6] are associated with this loss in skeletal muscle mass and function. Another adverse change in body composition with aging is the increase in fat tissue, particularly in visceral fat or intra-abdominal fat [7]. Excessive fat mass (FM) or obesity is strongly associated with several adverse outcomes, including metabolic syndrome [8], type-2 diabetes mellitus [9], cardiovascular disease [10] and cancer [11]. When both obesity and sarcopenia are present, a greater risk for negative health outcomes may be found [12].

Abbreviations: SFT, Subcutaneous fat thickness; % BF, Percent body fat; DXA, Dual energy X-ray absorptiometry; BIA, bioelectrical impedance; $ICC_{3,1}$, Intraclass correlation coefficient; SEM, Standard error of measurement; MD, Minimal difference; VIF, Variance inflation factor; SEE, Standard error of the estimate; TE, Total error; BMI, Body mass index; FM, Fat mass; BD, Body density.

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https://doi.org/10.1016/j.clnu.2018.11.017

In order to assess the magnitude of body composition changes with aging, valid methods are needed. Currently, dual-energy X-ray absorptiometry (DXA) and a densitometrical method (i.e. underwater weighing or air displacement plethysmograph) are common methods for quantifying whole-body adiposity [13]. However, these technologically complex methods are costly, and the devices have limited availability. A possible alternative method for estimating body composition may be bioelectrical impedance (BIA). However, this methodology is unable to image actual muscle and adipose tissue, and a number of BIA equations developed using a sample of young subjects resulted in a large bias for older individuals [14].

One promising method that can measure both a muscle and fat component is B-mode ultrasound. Some body compositions methods such as DXA and MRI can also estimate both muscle and fat but ultrasound may be more advantageous due to its portability, lower cost and no radiation or magnetic field exposure. Previous studies have validated the use of B-mode ultrasound to estimate appendicular lean tissue mass in older adults [15–17]. In addition, B-mode ultrasound may be an alternative method for measuring subcutaneous fat thickness (SFT) and then estimating body composition from the measured SFT. In the 1980s, Kuczmarski et al. [18] reported a significant relationship between body density (BD) by underwater weighing and SFT obtained by ultrasound. They also found that ultrasound was superior to the caliper method in measuring fat thickness in obese persons. Over the past 30 years, several studies have developed prediction equations for estimating BD and % body fat (% BF) from ultrasound measured SFT for healthy young and middle-aged adults [19–21] and young sports athletes [22,23]. These prediction equations have used various sites to estimate BD or % BF. For example, Abe et al. [19] used abdominal, subscapular, quadriceps and forearm sites. Kuczmarski et al. [18] used only biceps and thigh while Fanelli and Kuczmarski [20] included the waist, triceps, biceps and thigh. In athletic populations, the chest, triceps, subscapular, midaxillary, abdomen, suprailium, thigh, and tibialis were used [22,23]. Further, Saito et al. [23] also included the hip circumference in one of their equations.

Most of those studies used the underwater weighing method to determine BD as a reference value while one study used DXA-derived % BF as a reference value and that was in young adults [21]. Currently, only two studies have developed ultrasound SFT prediction equations to estimate BD in adults older than 50 but younger than 70 years old [18,19]. However, no studies have used DXA-derived % BF as the criterion measure for middle-aged and older adults. It is well known that body fat distributions, i.e. subcutaneous and visceral fat distribution, are strongly influenced by aging. Waist circumference is highly correlated with intrabdominal fat [24] and height has been found to be a significant predictor of % BF [25] so including different anthropometric measures may also help improve the prediction of % BF. However, it is unclear which ultrasound sites and anthropometric measures can best predict % BF in middle-aged and older adults when using DXA as the criterion method. DXA also allows for the measurement of leg and arm fat mass (FM) which can be used to estimate fat-free adipose tissue of the extremities [26,27]. Adjusting for fat-free adipose tissue in appendicular lean mass may be important in the diagnosis of sarcopenia [28]. However, equations using ultrasound to estimate leg and arm FM have not been developed. Thus, the purpose of this study was to test the validity of previously developed % BF prediction equations and develop new prediction equations to estimate % BF and arm and leg FM in middle-aged and older men and women using ultrasound SFT and anthropometric measures.

2. Materials and methods

2.1. Subjects

Subjects (n = 414) ranged in age from 50 to 79 years, were Japanese and were recruited starting in July 2015 from the university campus of the National institute of Fitness and Sports in Kanoya, Kagoshima, Japan (NIFS-K) and local area and data was collected during the period of October 2015 to November 2016. Subjects were randomly separated into a model development group (n = 276) and cross-validation group (n = 138) so that the model developmental group had two times the number of subjects than the cross-validation group [29]. % BF, arm FM and leg FM were estimated from anthropometric measures, sex, age and subcutaneous fat thicknesses of subjects. Subjects were considered healthy and free from chronic conditions such as cardiovascular disease, cancer, myositis and neuromuscular disorders from self-report questionnaires. Approximately 70% of subjects reported that they participated in some type of recreational sports activity, such as ground golf, at least twice a week. The study was conducted according to the Declaration of Helsinki and approved by the institutional review board of the NIFS-K. A written description of the purpose and safety of the study was given to subjects, and they signed a written informed consent prior to beginning the study.

2.2. Anthropometric data

Height was measured to the nearest 0.1 cm on a height scale (YL-65, Yagami Inc., Nagoya, Japan) and weight was measured to the nearest 0.1 kg on an electronic weight scale (Tanita WB-260A, Tokyo, Japan). Waist and hip circumference were measured to the nearest 0.1 cm using a measuring tape. Body mass index (BMI) and waist to hip ratio (WHR) were calculated from these measurements.

2.3. Ultrasound measurements

All ultrasound measurements were made using B-mode ultrasound (Aloka SSD-500, Tokyo, Japan) with a 5-MHz linear transducer head. Subcutaneous fat thicknesses from ultrasound measurements were made at the following sites which have previously been used to estimate body fat percentage in younger adults [19]: 30% proximal between the radial head and ulnar styloid process on the anterior forearm, 60% distal between the acromion process and lateral epicondyle of the humerus on the anterior and posterior upper arm, halfway between the greater trochanter and lateral condyle of the femur on the anterior and posterior thigh, approximately 3 cm lateral to the umbilicus (anterior trunk) and approximately 5 cm below the inferior angle of the scapula (posterior trunk), and 30% proximal to fibula lateral malleolus and tibia lateral condyle of the anterior and posterior lower leg as described in a previous study [19]. Subjects stood quietly during the measurements for approximately 5 min. Water-soluble transmission gel covered the transducer head and was placed perpendicularly at each site with as little pressure as possible to ensure that the skin surface was not depressed. Two images were taken and printed (SONY UP-897MD, Tokyo, Japan). Subcutaneous fat thickness was defined as the distance from the subcutaneous fat tissue-muscle interface to the subcutaneous fat tissue-skin interface as shown on the ultrasound image (Fig. 1). The SFT from the two images were measured by a ruler, averaged and then used for analysis. Test-retest reliability measures of intraclass correlation coefficient ($ICC_{3,1}$), standard error of measurement (SEM), and minimal difference (MD) to be considered real were determined for SFT and are presented in Table 1.

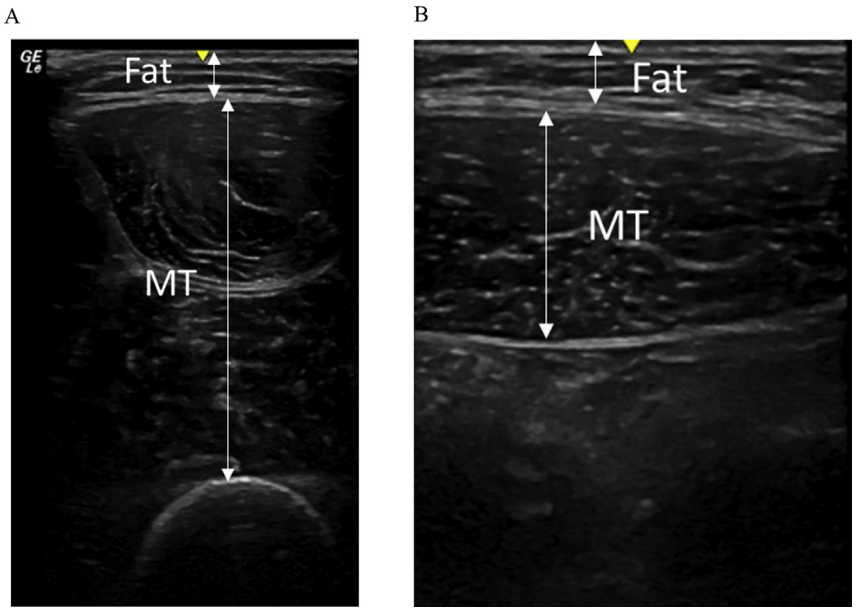

Fig. 1. Ultrasound measured subcutaneous fat thickness (FAT) and muscle thickness (MT) of the anterior thigh (A) and anterior trunk (B).

Table 1
Reliability measures of ultrasound sites. Intraclass correlation coefficient ($ICC_{3,1}$), standard error of measurement (SEM), and minimal difference (MD).

Ultrasound Site	$ICC_{3,1}$	SEM (cm)	MD (cm)
Anterior Forearm	0.990	0.02	0.06
Anterior Upper arm	0.972	0.05	0.10
Posterior Upper arm	0.959	0.06	0.17
Anterior Trunk	0.993	0.08	0.22
Anterior Thigh	0.984	0.06	0.15
Posterior Thigh	0.994	0.04	0.08
Anterior Lower Leg	0.987	0.02	0.06
Posterior Lower Leg	0.977	0.03	0.09

2.4. Dual energy X-ray absorptiometry (DXA) measurements

A whole-body scan using DXA (Discovery A, Hologic Inc., Bedford, MA, USA) was used to estimate % BF of subjects. On the day of data collection and prior to the whole-body scans, quality assurance testing and calibration were performed. Subjects were asked not to eat at least 3 h prior to testing and not to perform vigorous exercise at least 24 h prior to testing. In addition, they were provided water ad libitum. Lab test-retest reliability data (ICC, SEM, and MD) for percent body fat previously done in 6 young adults were 0.987, 0.63%, and 1.74%, respectively.

2.5. Statistical analysis

IBM SPSS statistics 24 was used to analyze the data. Bland–Altman plots were used to test the validity of previously developed % BF equations [19] using all subjects. The difference between the measured and predicted % BF were plotted against the average of the measured and predicted % BF with 95% confidence intervals included. Pearson correlations were also used to determine if there was any systematic error found in the prediction equations by correlating the difference between DXA and predicted % BF and the average of DXA and predicted % BF.

After testing old prediction equations, new prediction equations were developed. Independent t-test analyses (or approaches) were used to evaluate the differences in subject characteristics between the model developmental and cross-validation groups. Percent BF, arm FM and leg FM prediction equations were the criterion variables used in the stepwise multiple linear regression analyses. The predictor variables were anthropometric data, sex, age and subcutaneous fat thicknesses. To determine which subcutaneous fat thicknesses and anthropometric data best predicted % BF of middle-aged and older adults, we used stepwise multiple linear regression analysis. The Shapiro–Wilk test was used to determine normality of the criterion variable. Variance inflation factor (VIF) and Pearson correlations were used to test if multicollinearity was present among any of the predictor variables. If VIF was greater than 10 or Pearson correlation between two predictors was greater than 0.85, than the predictor with the highest correlation to the criterion was kept and the other predictor was excluded. Also, if predictors were not significantly correlated with the criterion, they were excluded from the model development analysis. Prediction equation total error (the average amount of deviation away from the line of identity) was also calculated.

Bland–Altman plots were used to investigate the accuracy of the prediction model from the cross-validation sample. The difference between the measured and predicted % BF, arm FM or leg FM were plotted against the average of the measured and predicted % BF, arm FM or leg FM with 95% confidence intervals (limits of agreement) included. Pearson correlations were calculated between DXA % BF, arm FM or leg FM and predicted % BF, arm FM or leg FM. Correlations were also used to determine if there was any systematic error found in the prediction equations by correlating the difference between DXA and predicted % BF, arm FM or leg FM and the average of DXA and predicted % BF, arm FM or leg FM. The data are represented as mean (standard deviation) throughout the manuscript. An alpha level of $p < 0.05$ was used to determine statistical significance.

3. Results

3.1. Validation of body fat equations

The only study that had subcutaneous fat measurement sites at the same measurement site as the current study was Abe et al. [19]. We tested the accuracy of the equations by Abe et al. [19] using the

participant data from the current study. We developed Bland–Altman plots and calculated correlation coefficients between predicted and DXA measured % BF equations. For the equation predicting male % BF, there was a mean bias of 6.07% and the correlation between the mean and difference of DXA and predicted % BF was r = 0.391, p < 0.001 (Fig. 2). For the female prediction equation, there was a mean bias of 8.59% and the correlation between the mean and difference of DXA and predicted % BF was r = 0.174, p = 0.007 (Fig. 2). A significant correlation was found between predicted and measured % BF for females (r = 0.72, p < 0.001) and males (r = 0.53, p < 0.001). Based on the large mean differences and some systematic error found in the equations, we developed a new % BF equation for middle-aged and older Japanese adults.

3.2. % Body fat prediction equations

No statistically significant differences were found between the model developmental group and the cross-validation group for age, height, weight, body mass index (BMI), arm FM, leg FM, % BF and SFTs (Table 2). Also, multicollinearity was not present in the final prediction equations (VIF < 10, r < 0.85). The best fit model for estimating % BF for males and females combined included anterior trunk SFT, sex, posterior upper arm SFT, anterior lower leg SFT, height, waist, and anterior thigh SFT (adjusted $r^2 = 0.804$, standard error of the estimate (SEE) = 3.3%, total error (TE) = 4.0%, Table 3). A cross-validation sample revealed that DXA % BF was significantly correlated with predicted % BF from this model (r = 0.833, p < 0.001, Fig. 3A). We also found a small mean bias between DXA and the predicted % BF (−0.14%). However, some systematic error was noted in the prediction equation such that our equation was associated with overestimating % BF at lower % BF values and underestimating % BF at higher % BF values (r = 0.275, p = 0.001, Fig. 3B). Individual prediction equations were also developed for male and female % BF (Table 3). The predicted % BF values were significantly correlated with those of DXA % BF (Males: r = 0.600, p < 0.001; Females: r = 0.765, p < 0.001); however, some systematic bias was found in the Bland–Altman analysis (Males: r = 0.515, p < 0.001; Females: r = 0.468, p < 0.001).

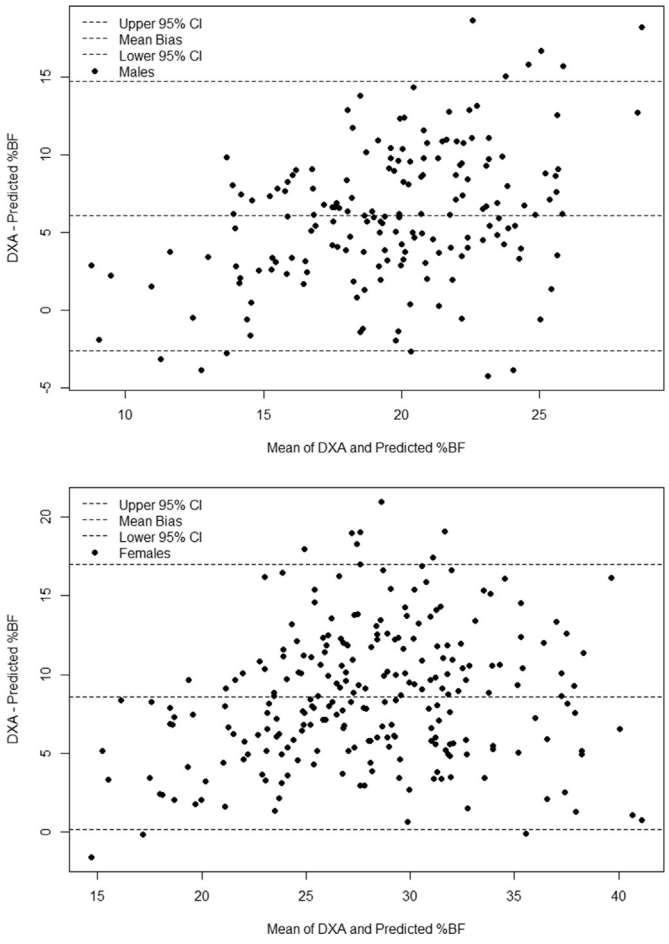

Fig. 2. Bland–Altman plots between dual-energy X-ray absorptiometry (DXA) % body fat (BF) and predicted % BF from previously developed equations by Abe et al. [19].

Table 2
Group Characteristics. Model development group had 120 males and 156 females while the cross-validation group had 55 males and 83 females. Mean (standard deviation). SFT = subcutaneous fat tissue.

	Model Development	Cross-validation	P-value
Age (years)	69.5 (6.6)	69.6 (5.9)	0.888
Height (cm)	156.0 (8.5)	155.8 (7.7)	0.821
Weight (kg)	57.6 (9.3)	57.1 (10.0)	0.676
BMI (kg/m^2)	23.6 (3.0)	23.4 (2.9)	0.547
Waist (cm)	82.6 (8.9)	82.8 (8.9)	0.857
Hip (cm)	92.4 (5.2)	92.4 (5.5)	0.964
Waist to Hip Ratio	0.89 (0.07)	0.90 (0.07)	0.810
Anterior Forearm SFT (cm)	0.42 (0.21)	0.43 (0.21)	0.695
Anterior upper arm SFT (cm)	0.44 (0.24)	0.44 (0.23)	0.842
Posterior upper arm SFT (cm)	0.95 (0.41)	0.93 (0.39)	0.729
Anterior Trunk SFT (cm)	2.57 (1.13)	2.59 (1.01)	0.885
Posterior Trunk SFT (cm)	1.09 (0.49)	1.01 (0.42)	0.115
Anterior thigh SFT (cm)	0.92 (0.46)	0.89 (0.43)	0.506
Posterior thigh SFT (cm)	0.69 (0.33)	0.68 (0.31)	0.782
Anterior lower leg SFT (cm)	0.37 (0.18)	0.36 (0.16)	0.637
Posterior lower leg SFT (cm)	0.52 (0.21)	0.49 (0.18)	0.136
Arm Fat Mass (kg)	1.83 (0.66)	1.81 (0.70)	0.789
Leg Fat Mass (kg)	4.63 (1.68)	4.57 (1.72)	0.759
% Body Fat	28.2 (7.5)	28.2 (7.2)	0.994

Statistical significance p ≤ 0.05.

3.3. Arm FM prediction equations

The best fit model for estimating arm FM when using both males and female data included BMI, posterior upper arm SFT, sex, WHR, height and weight. The adjusted r^2 was 0.808, SEE was 0.29 kg and TE was 0.32 kg (Table 4). Using a cross-validation group, predicted arm FM was significantly correlated with DXA arm FM (r = 0.895, p < 0.001, Fig. 4A). In addition, we found a mean bias of 0.02 kg, but arm FM tended to be overestimated at lower values and underestimated at higher values (r = 0.438, p < 0.001, Fig. 4B). Individual prediction equations were also developed for male and female arm FM (Table 4). The predicted arm FM values were significantly correlated with those of DXA arm FM (Male: r = 0.758, p < 0.001; Female: r = 0.908, p < 0.001); however, some systematic bias was found in the Bland–Altman analysis (Males: r = 0.421, p < 0.001; Females: r = 0.375, p < 0.001).

3.4. Leg FM prediction equations

The best fit model for estimating leg FM in males and females included anterior thigh SFT, hip, sex, posterior lower leg SFT, weight, posterior thigh SFT, and anterior thigh SFT (Table 5). There was a significant correlation between DXA leg FM and predicted leg FM (r = 0.888, p < 0.001, Fig. 5A) in the cross-validation group. Further, the mean bias was −0.01 kg, but the equation was associated with overestimating lower values of leg FM and underestimating higher leg FM values (r = 0.400 p < 0.001, Fig. 5B). Individual prediction equations were also developed for male and

Table 3
% BF prediction equations.

Models	U–β	S–β	P-value	R^2	Adjusted R^2	SEE	TE
Body fat % (Males and Females)				0.809	0.804	3.3	4.0
Constant	15.709		0.016				
Anterior Trunk SFT	1.753	0.264	<0.001				
Sex (males = 1; females = 2)	5.626	0.372	<0.001				
Posterior Upper Arm SFT	3.635	0.199	<0.001				
Anterior Lower Leg SFT	−4.428	−0.108	0.003				
Height (cm)	−0.170	−0.191	<0.001				
Waist (cm)	0.264	0.315	<0.001				
Anterior Thigh SFT (cm)	2.241	0.136	0.003				
Body fat % (Males Only)				0.623	0.606	3.2	4.0
Constant	−22.914		<0.001				
BMI (kg/m^2)	0.442	0.248	0.007				
WHR	32.753	0.377	<0.001				
Anterior Trunk SFT (cm)	1.550	0.234	0.002				
Posterior Upper Arm SFT (cm)	6.928	0.312	<0.001				
Posterior Thigh SFT (cm)	−6.327	−0.234	0.005				
Body fat % (Females Only)				0.655	0.641	3.4	4.2
Constant	25.581		0.002				
Anterior Trunk SFT (cm)	1.905	0.370	<0.001				
BMI (kg/m^2)	0.614	0.329	<0.001				
Posterior Upper Arm SFT (cm)	3.271	0.191	0.007				
Anterior Lower Leg SFT (cm)	−4.677	−0.160	0.007				
Height (cm)	−0.112	−0.114	0.025				
Anterior Thigh SFT (cm)	1.725	0.135	0.040				

Unstandardized B = U–β, Standardized B = S–β, SEE = standard error of the estimate, TE = total error, SFT = subcutaneous fat tissue; Trunk SFT = Sum of anterior and posterior trunk SFT; Arm fat = sum of anterior forearm fat, posterior and anterior upper arm fat; Sum of all 9 SFT = Sum of anterior forearm, anterior and posterior upper arm, anterior and posterior trunk, anterior and posterior lower leg SFT; WHR = waist-to-hip ratio; BMI = body mass index.

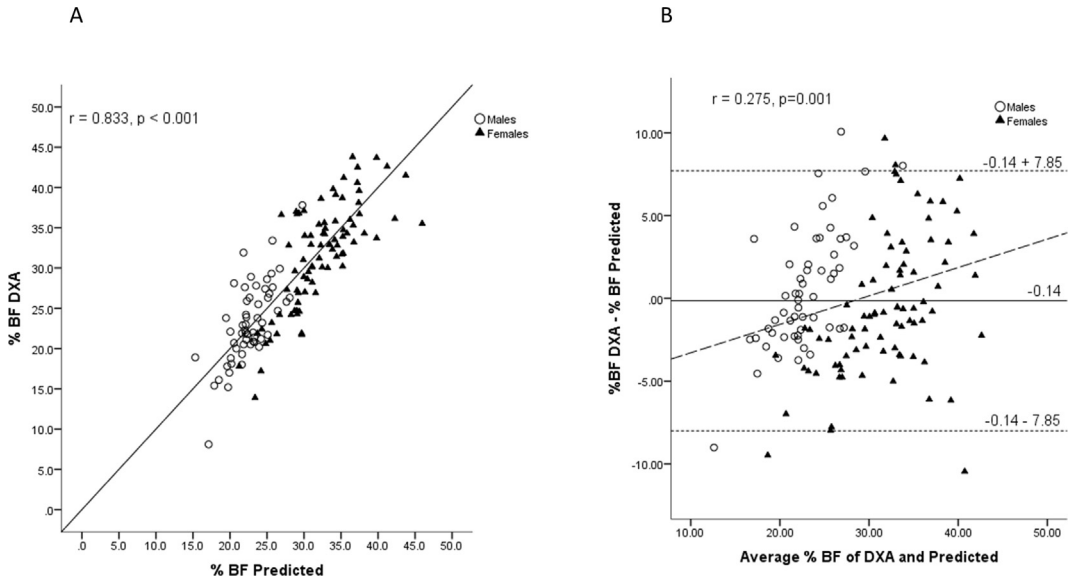

Fig. 3. A: Association between dual-energy X-ray absorptiometry (DXA) % body fat (BF) and predicted % BF. – represents line of identity. B: Bland–Altman plots for % BF with 95% limits of agreement. – represents mean bias. - - - represents 95% limits of agreement. — — represents line of best fit.

female leg FM (Table 5). The predicted leg FM values were significantly correlated with those of DXA leg FM (Males: r = 0.775, p < 0.001; Females: r = 0.870, p < 0.001); however, some systematic bias was found in the Bland–Altman analysis (Males: r = 0.437, p = 0.001; Females: r = 0.361, p = 0.001).

4. Discussion

Overall, several key findings resulted from this study. First, previously developed % BF equations developed in younger individuals resulted in a large amount of error when used with older adults. Second, a large amount of the variability found in % BF, arm FM and leg FM in middle-aged and older Japanese adults was accounted for by ultrasound SFT and anthropometric measures. In addition, a small amount of bias was found between predicted and DXA measured % BF, arm FM and leg FM. However, large limits of agreement existed for each of the prediction equations and some systematic error was present.

Prior to developing new % BF equations for older Japanese individuals, we investigated how well previous equations developed by Abe et al. [19] estimated % BF in our population. The equations by Abe et al. [19] estimated body density and then the Brozek equation was used to determine % BF. We found that equations underestimated % BF by roughly 6% for males and 8% for females. In

Table 4
Arm fat mass prediction equations.

Models	U–β	S–β	P-value	R^2	Adjusted R^2	SEE	TE
Arm Fat Mass (Males and Females)				0.812	0.808	0.29	0.32
Constant	−11.697		<0.001				
BMI (kg/m2)	0.255	1.149	<0.001				
Posterior Upper Arm SFT (cm)	0.554	0.346	<0.001				
Sex	0.541	0.407	<0.001				
WHR	1.598	0.172	<0.001				
Height (cm)	0.050	0.643	0.003				
Weight (cm)	−0.054	−0.762	0.016				
Arm Fat Mass (Males)				0.718	0.708	0.27	0.32
Constant	−4.330		<0.001				
BMI (kg/m^2)	0.096	0.551	<0.001				
Posterior Upper Arm SFT (cm)	0.548	0.252	<0.001				
WHR	1.765	0.207	0.002				
Height (cm)	0.010	0.114	0.024				
Arm Fat Mass (Females)				0.803	0.798	0.30	0.32
Constant	−3.301		<0.001				
BMI (kg/m^2)	0.105	0.483	<0.001				
Posterior Upper Arm SFT (cm)	0.528	0.266	<0.001				
Weight (kg)	0.019	0.203	0.002				
WHR	1.505	0.138	0.002				

Unstandardized B = U–β, Standardized B = S–β, SEE = standard error of the estimate, TE = total error, SFT = subcutaneous fat tissue; Trunk SFT = Sum of anterior and posterior trunk SFT; Arm fat = sum of anterior forearm fat, posterior and anterior upper arm fat; Sum of all 9 SFT = Sum of anterior forearm, anterior and posterior upper arm, anterior and posterior trunk, anterior and posterior lower leg SFT; WHR = waist-to-hip ratio; BMI = body mass index.

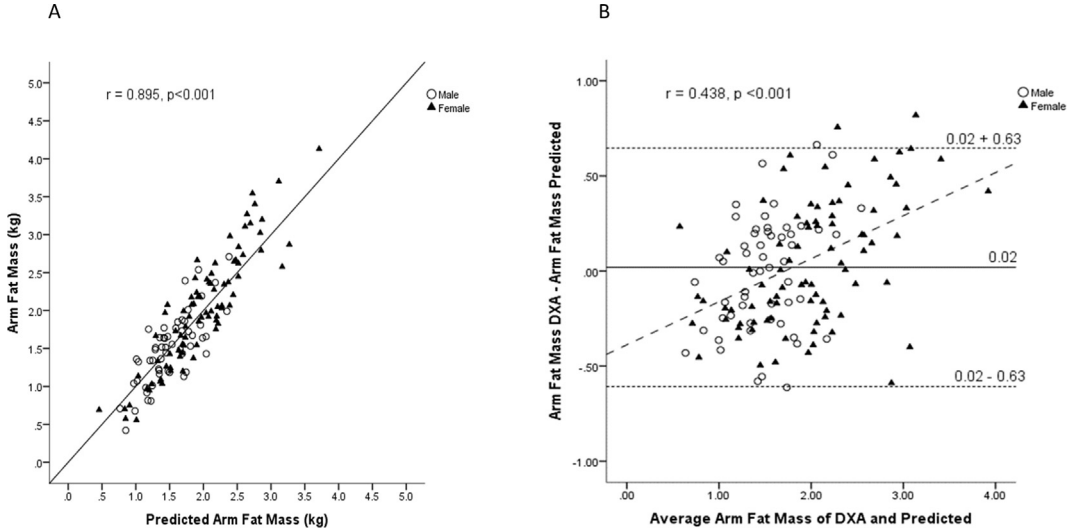

Fig. 4. A: Association between dual-energy X-ray absorptiometry (DXA) arm fat mass and predicted arm fat mass. – represents line of identity. B: Bland–Altman plots for arm fat mass with 95% limits of agreement. – represents mean bias. - - - represents 95% limits of agreement. — — represents line of best fit.

addition, some systematic error was also noted. This is contrary to the findings originally reported which showed that ultrasound SFT accurately predicted body density when hydrodensitometry was used as the criterion. Although the previously developed equations were appropriate for Japanese adults between 18 and 51 years, they do not appear appropriate to use in an older Japanese population. Based on this information we developed new equations from ultrasound SFT taken in older Japanese adults.

In the current study, we found that the variance in % BF that could be explained by our prediction equations varied from an r^2 of 0.62–0.81. Several studies using B-mode ultrasound found similar or higher r^2 values than the current study. One study that used B-mode ultrasound on male collegiate football players (n = 58) found an r^2 of 0.83 and a SEE of 2.64% [22]. The criterion used in that study was a 3-compartment water (3C-W) model (body density from BodPod with thoracic volume estimated, total body water from BIA and body mass) and ultrasound SFT sites included the chest, triceps, subscapular, midaxillary, abdomen, suprailium and thigh. In the current study, nine subcutaneous fat sites were measured (anterior forearm, anterior and posterior upper arm, anterior and posterior trunk, anterior and posterior thigh, and anterior and posterior lower leg) and DXA was used as the criterion. However, unlike the previous study by Hyde et al. [22] that only had the sum of ultrasound subcutaneous fat in their prediction equation, our prediction equations also included anthropometric data.

A second study also found that B-mode ultrasound SFT explained a high degree of variance in % BF (~0.83–0.90%) [21]. Interestingly, their prediction equation only included the abdomen and front thigh SFT for men, and abdomen and medial calf for women with a SEE ranging from 1.9 to 3.0%. In our study, the prediction equation that explained the greatest proportion of % BF included the anterior trunk (abdomen) SFT, sex, posterior upper arm SFT (triceps), anterior lower leg (Tibialas anterior), height, waist circumference and anterior thigh SFT. The top predictors

Table 5
Leg fat mass prediction equations.

Models	U–β	S–β	P-value	R^2	Adjusted R^2	SEE	TE
Leg Fat Mass (Males and Females)				0.804	0.799	0.75	0.80
Constant	−9.855		<0.001				
Anterior Thigh SFT (cm)	1.222	0.331	<0.001				
Hip (cm)	0.089	0.276	<0.001				
Sex	1.188	0.351	<0.001				
Posterior Lower leg SFT (cm)	1.399	0.177	0.001				
Weight (kg)	0.043	0.237	<0.001				
Posterior Thigh SFT (cm)	0.696	0.136	0.003				
Anterior Lower Leg SFT (cm)	−1.078	−0.118	0.006				
Leg Fat Mass (Males)				0.690	0.682	0.69	0.74
Constant	−12.085		<0.001				
Anterior Thigh SFT (cm)	1.879	0.360	<0.001				
Hip	0.117	0.485	<0.001				
WHR	4.019	0.195	0.001				
Leg Fat Mass (Females)				0.754	0.747	0.80	0.85
Constant	−11.215		<0.001				
Hip	0.154	0.509	<0.001				
Anterior Thigh SFT (cm)	1.034	0.288	<0.001				
Posterior Thigh SFT (cm)	0.713	0.152	0.007				
Posterior Lower Leg SFT (cm)	1.070	0.141	0.016				

Unstandardized B = U–β, Standardized B = S–β, SEE = standard error of the estimate, TE = total error, SFT = subcutaneous fat tissue; Trunk SFT = Sum of anterior and posterior trunk SFT; Arm fat = sum of anterior forearm fat, posterior and anterior upper arm fat; Sum of all 9 SFT = Sum of anterior forearm, anterior and posterior upper arm, anterior and posterior trunk, anterior and posterior lower leg SFT; WHR = waist-to-hip ratio; BMI = body mass index.

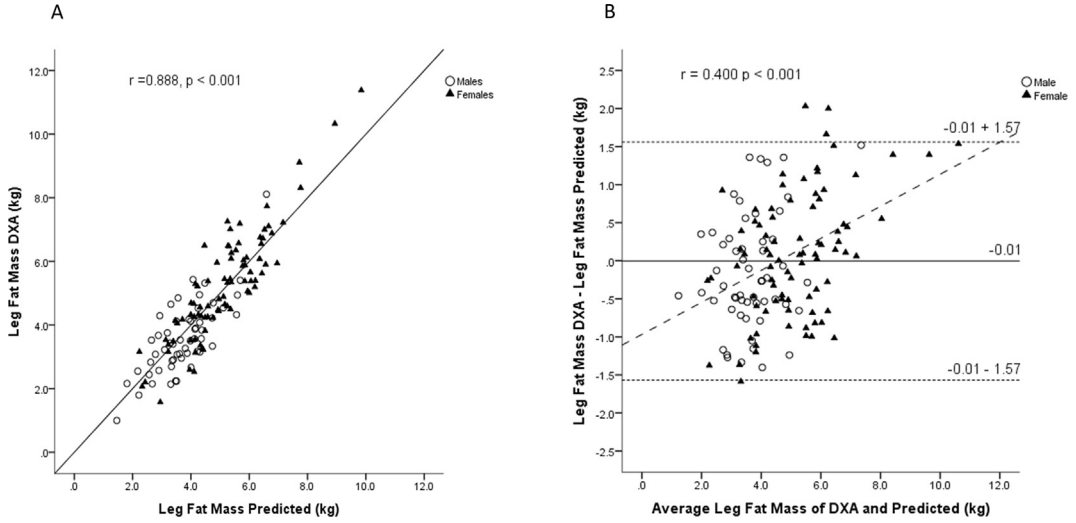

Fig. 5. A: Association between dual-energy X-ray absorptiometry (DXA) leg fat mass and predicted leg fat mass. – represents line of identity. B: Bland–Altman plots for leg fat mass with 95% limits of agreement. – represents mean bias. - - - represents 95% limits of agreement. — — represents line of best fit.

were sex, waist and anterior trunk SFT. In order to explain a similar amount of the variance in % BF, our prediction equations required more predictors than previous studies; however, the anterior trunk and anterior thigh were significant predictors used in all of the studies. The age of our subjects was significantly older than the studies by Hyde et al. [22] and Leahy et al. [21] (~70 years vs. ~ 20 years) which could explain why potentially more predictors were needed to account for a similar amount of variance in % BF. With aging there is a significant redistribution and increase in body fat [7] and a decline in muscle mass [3]. While subcutaneous fat is present in older adults, a great deal of fat is found in the abdominal area and around visceral organs [7]. Waist circumference is highly correlated with intra-abdominal fat [24] and by including this measurement it is likely that some of the intrabdominal fat was accounted for which helped to increase the predictive ability of our equations for % BF.

Despite the fact that the current ultrasound % BF prediction equations described a large degree of variance in predicted % BF scores ($r^2 = 0.809$) and a small mean bias (−0.14%), wide limits of agreement were found and also some systematic error or bias was present ($r = 0.250$, $p < 0.001$). Our findings are similar to Leahy et al. [21] who also used DXA as a criterion to develop ultrasound prediction equations. Little mean bias was present in the current study (−0.14%) and in the study by Leahy et al. [21] (0.09 and −0.42%) and both studies had large limits of agreement (- 6.2 to 5.4 and − 8.0 to 7.7%). However, Leahy et al. [21] failed to report if any systematic error was present in the Bland–Altman plots. These studies suggest that when DXA is used as the criterion method in both younger and older adults that a small mean bias is present but large limits of agreement in % BF estimations exist. Ideally, when estimating % BF, the limits of agreement would be smaller for the equation to be considered more accurate.

In contrast to our findings, other studies using B-mode ultrasound report no systematic bias or smaller limits of agreement for estimating % BF than the current study. Fanelli et al. [20] used ultrasound SFT from the waist and thigh to estimate body density

with hydrodensitometry as the criterion. They found that their prediction equation for estimating body density from ultrasound SFT had an r = 0.809 and that it resulted in % BF similar to hydrodensitometry, although the range of % BF, systematic error, and limits of agreement with hydrodensitometry were not reported. Abe et al. [19] also found that ultrasound SFT accurately predicted body density when hydrodensitometry was used as the criterion without having any systematic error or bias. Two other studies investigating B-mode ultrasound SFT to estimate % BF in athletes also found very good accuracy of their prediction equations [22,23]. Saito et al. [23] used ultrasound to estimate % BF in sumo wrestlers who were classified as obese. The prediction equation had an r^2 of 0.94 with the abdomen SFT, hip circumference and triceps SFT as the best predictors of body density. However, these authors also failed to use a cross-validation group with their prediction equations so it is unclear how accurately these prediction equations were at estimating % BF in other sumo wrestlers. The most recent study used a 3C-W model as the criterion for % BF to form a prediction equation from ultrasound SFT. The sum of the 7 ultrasound SFT sites was used and limits of agreement were roughly around −3 to 1% with no systematic error being reported. Overall, studies reporting accuracy in estimating body density or % BF from ultrasound used either hydrodensitometry or 3C-W model as the criterion measures and also examined younger and middle-aged adults.

Part of the reason for the larger variance found in our study could be a result of the different criterion measures used or the older age of our current subjects. DXA is used to estimate body composition [13], but some studies have shown that it underestimates % BF [29–32] or overestimates % BF [33,34] when compared to a 4–compartment (4-C) model. Van der Ploeg et al. [30] also noted a large amount of individual difference between DXA and 4-C model (−2.6–7.2%) and that lower % BF was underestimated and higher BF% was overestimated. The authors suggested that this bias may result from beam-hardening errors due to differences in anterior and posterior tissue thickness. Therefore, some of the inherent variation found in this study could partially be explained from the variation in the criterion method used. Future studies, should investigate how different criterion devices influence the predictive ability of ultrasound estimated % BF.

One limitation of this study was the use of stepwise multiple linear regression due to the possibility that the model can be unstable when used in other samples. However, it was unclear to the authors which variables would be best to use in older populations when anthropometric and ultrasound measures were used so stepwise linear regression was deemed appropriate for the purposes of this manuscript. In addition, we used a cross-validation group to examine how well the equations predicted % BF. Another limitation is that we did not include arm and thigh circumferences for the leg and arm FM prediction equations. These may be other variables that could improve the estimation of arm and leg FM and should be investigated in future studies. In addition, prediction equations were not developed for android and gynoid depots and future research may investigate the ability of ultrasound to predict these depots.

5. Conclusion

Ultrasound SFT can be used to estimate % BF in middle-aged and older adults; however, large individual differences were found between prediction equations and DXA % BF with some systematic error. Therefore, caution should be used when applying these equations in middle-aged and older adults to estimate % BF. This is the first study to estimate % BF using B-mode ultrasound and anthropometric data in middle-aged and older adults so more research is needed to verify these findings.

Funding

This study received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Statement of authorship

The authors' responsibilities were as follows RST, TAB, JPL, EF and TAK: designed research project; TAB, EF, and TAK: conducted research; RST, EF and TAB: analyzed data or performed statistical analysis; RST and TAB: wrote the manuscript and had primary responsibility for final content; and all authors: read and approved the final manuscript.

Conflicts of interest

No conflicts of interest, financial or otherwise, are disclosed by the authors.

Acknowledgements

The authors would like to thank the study subjects for participating in this study.

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