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Development of a bedside-applicable ultrasound protocol to estimate fat mass index derived from whole body dual-energy x-ray absorptiometry scans

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ABSTRACT

Objectives: Precise measures of adiposity are difficult to obtain in clinical settings due to a lack of access to accurate and reliable techniques. The aim of this study was to develop and internally validate a bedside-applicable ultrasound protocol to estimate fat mass index.
Methods: We conducted an observational cross-sectional study of 94 university and community dwelling adults who attended a single data-collection session. Adipose tissue thickness was quantified in a supine or prone position using the four-site protocol (images two anterior sites on each thigh) and the nine-site protocol (images nine anterior and posterior sites). Adipose tissue thicknesses from the four-site protocol were compared against the fat mass index that was derived from dual-energy x-ray absorptiometry scans. Subsequently, we optimized the accuracy of the four-site protocol with the addition of bedside-accessible adipose tissue thicknesses from the nine-site protocol and easily obtained covariates.
Results: The four-site protocol was strongly associated ($R^2 = 0.65$) with fat mass index but wide limits of agreement (-3.53 kg/m^2 and 3.50 kg/m^2) were observed using the Bland-Altman analysis. With the addition of the anterior upper arm and abdomen adipose tissue thicknesses as well as the covariates age, sex, and body mass index, the model accuracy improved ($R^2 = 0.93$) and the Bland-Altman analysis displayed narrower limits of agreement (-1.57 kg/m^2 and 1.60 kg/m^2).
Conclusions: This optimized protocol developed can be applied bedside and provide accurate assessments of fat mass index.

Introduction

The prevalence of obesity is increasing world-wide and requires urgent intervention to mitigate the substantial health risks that are associated with this condition [1,2]. Not only does obesity increase the risk of developing noncommunicable diseases such as hypertension, type 2 diabetes, coronary heart disease, stroke, and many cancers [1], obesity also negatively affects quality of life [3] and increases the risk of premature death [4].

Obtaining non-invasive, accurate, and reliable measures of adiposity in clinical or community facilities is challenging. Currently, body mass index (BMI) is the most common tool to indirectly measure adiposity due to its simplicity of assessment and interpretation. However, BMI cannot distinguish specific tissues, and changes in body composition can be highly variable among individuals and have a significant influence on a patient's response to treatment, quality of life, and health-oriented outcomes [5]. Applying accurate and precise body composition modalities such as computed tomography (CT), magnetic resonance imaging (MRI), and dual-energy x-ray absorptiometry (DXA) may be useful to quantify adiposity and track changes over time, but these approaches are impractical due to costs, radiation exposure (in the case of CT scans), and limited accessibility [6]. These challenges in obtaining accurate measures of adiposity have been recognized in the strategic plan for obesity research that was released by the National Institutes of Health, with an emphasis on developing clinically applicable approaches [7].

Ultrasonography, which is a non-invasive and readily available tool, has been utilized to quantify adipose tissue thickness and has demonstrated strong associations with whole body adiposity measured using DXA [8–11], bio-electrical impedance analysis [12],

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hydrostatic weighing [13,14], air displacement plethysmography [12,15,16], and multicompartment models [17,18]. However, the majority of these protocols are applied in a non-supine posture and include posterior adipose tissue thicknesses, which limits the clinical application in individuals with reduced mobility or patients who are confined to a hospital bed.

Here, we sought to develop and internally validate a bedside-viable ultrasound protocol to predict whole body adiposity. Specifically, we assessed the agreement between a four-site protocol (images in four locations on the anterior thigh compartment) and DXA-based fat mass index, and subsequently. optimized the accuracy of the four-site protocol by incorporating additional bedside-accessible adipose tissue thicknesses and easily obtained covariates.

Methods

Study design and participants

This observational study recruited 94 participants to attend a single data collection session at the University of Waterloo between August 2015 and May 2016. Participants underwent anthropometric measures, a whole body DXA scan, and ultrasound assessments in a supine or prone position using the previously established nine-site [19] and four-site [20] protocols. This study was reviewed and cleared by a University of Waterloo Clinical Research ethics committee. Written informed consent was obtained from all participants in accordance with established protocols for human research.

Participants (≥18 y of age) were recruited from the University of Waterloo student population, the University of Waterloo Research Aging Participant Pool, and the surrounding Kitchener-Waterloo community. Participants were screened using a health questionnaire and excluded if they had a previous history of neuromuscular disorders, were currently or suspected being pregnant, had undergone a barium swallow or nuclear medicine scan within the past 3 wk, had a stroke within the past 5 y, had a prosthetic joint replacement, or had an implantable electronic device. Participants were instructed to refrain from alcohol consumption for 24 h and moderate-to-vigorous physical activity for 48 h before the scheduled data collection session.

Dual-energy x-ray absorptiometry imaging procedures

Height and weight data were obtained for all participants in lightweight clothing or a cloth hospital gown using a balance beam scale or stadiometer. DXA scans were performed as previously described [21]. Briefly, participants were positioned supine on the scanning table and 1 to 2 whole body DXA scans were performed (Hologic Discovery QDR 4500, Hologic, Toronto, Ontario, Canada). Using Hologic software (version 13.2), whole body scans were segmented into the head, trunk, upper limbs, and lower limbs by a single trained investigator as previously described [22]. Fat mass index was calculated by summing the fat mass of all segments and normalizing by the participants height squared (kg/m^2).

Ultrasound imaging procedures

Transverse images of adipose and muscle tissue at predefined sites were obtained using a real-time B-mode ultrasound device (M-Turbo, Sonosite, Markham, Ontario, Canada) that was equipped with a multifrequency linear array transducer (L38 xi, 5-10 MHz). Adjustable parameters gain, time-gain-compensation, compression, resolution, and musculoskeletal setting were held constant throughout the imaging procedure; however, depth was adjusted as necessary to obtain complete images of adipose and muscle tissue.

All images were obtained with the participant lying supine or prone with a neutral wrist and ankle rotation (maintained using an adjustable strap). Landmarks were identified by palpation and specific sites to be imaged were marked using a flexible tape measure and ink. To minimize potential compression of the underlying tissues by the ultrasound transducer during imaging, two criteria were applied. A thick layer of ultrasound gel was maintained between the probe-skin interface to ensure no direct contact and the operator visually confirmed that the skin, adipose tissue, and muscle belly maintained a convex shape before freezing the image as previously described [21].

Images were downloaded using the lowest level of compression and analyzed for adipose tissue thickness using ImageJ software version 1.6.0_24 (NIH, Bethesda, MD) [23]. Adipose thickness was analyzed in pixels using the line segment tool and converted to distance (cm) using depth-adjusted pixel/cm ratios. Adipose tissue thickness was taken as the distance between the superficial border of the muscle fascia and the deep border of the skin (Suppl. Fig. 1). All images for a single participant were analyzed before moving to the next participant; however, no reference was made to previous measurements until all participant images were completed.

Ultrasound protocols

The four-site protocol imaged the adipose tissue thickness at the midpoint and distal two-thirds on anterior surface of the left and right thigh between the anterior superior iliac spine and the upper pole of the patella. Each landmark was imaged twice and the average thickness across all sites was calculated. The nine-site protocol imaged anterior and posterior adipose tissue thicknesses on the right side of the body as previously described. Briefly, the landmarks included the anterior and posterior upper arm, the anterior forearm, the abdomen, the subscapular area, the anterior and posterior thigh, and the anterior and posterior lower leg. These landmarks were imaged once.

Statistical analysis

Continuous variables were assessed for normality using the Shapiro-Wilks test and confirmed using quantile-quantile plots. Normality was violated for several variables; therefore, the data are presented as median (interquartile range) and differences between male and female participants were analyzed using the Mann-Whitney U test.

All linear regression analyses were performed using a three-fold cross-validation that was stratified by fat mass index (kg/m^2) to improve the generalizability of the developed models [24]. A three-fold cross-validation divides the participant cohort into three equally distributed groups, where model development occurs using two groups with subsequent testing of the model in the left out group. This process is repeated three times and averaged across all groups to assess model accuracy. A linear regression analysis to predict fat mass index was performed using the average four-site adipose tissue thickness ([right midpoint + right distal third + left midpoint + left distal third]/4) multiplied by limb length (m).

The variables to be included in the optimized model to predict fat mass index were selected using a combination of a priori (four-site adipose thickness, anterior upper arm adipose thickness, age, sex, and BMI) and stepwise regression (abdomen adipose thickness) selected variables. A priori selected variables were chosen to maintain consistency with our previously developed [21] bedside-applicable appendicular lean tissue model (four-site and anterior upper arm) and factors known to influence or be associated with adiposity (age, sex, and BMI). The stepwise regression analysis included the anterior forearm, abdomen, and anterior lower leg adipose tissue thicknesses.

Accounting for a priori defined variables, only the abdomen adipose tissue thickness remained significant within the model (Suppl. Table 1). The anterior upper arm, abdomen, and averaged four-site adipose tissue thickness were summed and utilized for model development. One participant was missing adipose tissue thickness for the abdomen, which was predicted using a regression analysis of trunk fat mass and sex (n = 93) against abdomen adipose tissue thickness. A correlation analysis of predicted (trunk fat mass and sex) and ultrasound measured abdomen adipose tissue thickness demonstrated a strong association ($r = 0.80$, $P < 0.001$).

A Bland-Altman plot analysis was used to compare fat mass index that was derived from DXA with that predicted from both the four-site and optimized protocols. Limits of agreement (95% confidence interval [CI] of the differences) were calculated and used for interpretation [25]. A regression analysis of the differences against the averages (assessment of proportional bias–non-constant bias across the range of data) and visually assessing a plot of the residuals against averages (assessment of homoscedasticity–even spread of data across the range of data) was performed to ensure that constant limits of agreement are valid [26]. These assumptions were valid for all Bland-Altman plots.

Regression coefficients were interpreted as weak (0.30–0.50), moderate (0.50–0.70), and strong (0.70–1.00) [27]. All analyses were performed using SPSS version 24.0 (IBM Corp; Chicago, IL) and the level of significance was set at $P \leq 0.05$.

Results

Of the 94 participants who were recruited, 56% were female and when compared with male participants, female participants displayed a significantly lower median BMI (23.7 vs. 25.6 kg/m^2; $P = 0.016$) but higher median body fat percentage (34.7 vs. 24.2 %; $P < 0.001$) and body fat index (7.8 vs. 6.4 kg/m^2; $P = 0.001$; Table 1). Fifty-five of 94 participants were ages <60 y and 39 participants were ages ≥60 y.

When compared with male participants, female participants displayed significantly greater adipose tissue thickness for all

Table 1
Physical description of participant cohort

Variables Median (IQR)	All (n = 94)	Men (n = 41)	Women (n = 53)	*P*-value
Age, y	34.0 (24.0–70.0)	33.0 (25.0–73.0)	34.0 (23.0–68.8)	0.161
Height, m	1.70 (1.62–1.77)	1.77 (1.72–1.81)	1.64 (1.58–1.69)	<0.001
Weight, kg	70.5 (62.6–82.3)	82.3 (71.8–88.5)	64.9 (58.6–70.8)	<0.001
Sex, % female	56	-	-	-
BMI, kg/m^2	24.2 (22.3-27.2)	25.6 (23.7-27.6)	23.7 (21.9-26.4)	0.016
Underweight <18.5 kg/m^2	0	0	0	-
Normal 18.5 to 24.9 kg/m^2	51	16	35	-
Overweight 25.0 to 29.9 kg/m^2	30	19	11	-
Obese ≥30 kg/m^2	13	6	7	-
Body fat index, kg/m^2	7.4 (5.7–9.0)	6.4 (5.0–7.7)	7.8 (6.3–10.2)	0.001
Body fat percent, %	30.2 (23.9–36.7)	24.2 (20.3–30.2)	34.7 (29.2–40.1)	<0.001
Appendicular body fat, kg	9.4 (7.6–11.6)	7.9 (6.4–9.9)	10.8 (8.8–14.0)	<0.001
Trunk fat mass, kg	9.5 (7.0–12.8)	10.4 (7.0–13.0)	9.4 (6.9–9.4)	0.612

BMI, body mass index; IQR, interquartile range

limb-based landmarks ($P < 0.05$) but not trunk-based measures (subscapular and abdomen; $P > 0.05$; Table 2), which corresponded with differences that were observed in appendicular and trunk fat mass between male and female participants (Table 1).

Across the three-fold cross-validation groups, the linear regression analysis using the four-site protocol to predict fat mas index resulted in an average adjusted R^2 of 0.65 and standard error of the estimate (SEE) of 1.73 kg/m^2 ($P < 0.001$; Table 3).

The Bland-Altman analysis of the four-site protocol to predict fat mass index demonstrated a non-significant fixed bias of −0.02 kg/m^2 (95% CI, −0.37, 0.34) and limits of agreement of −3.53 and 3.50 kg/m^2 (Fig. 1). Across the three-fold cross-validation groups, the multiple linear regression analysis using the summed anterior upper arm, abdomen, and average four-site adipose tissue thickness alongside age, sex, and BMI to predict DXA fat mass index resulted in an average adjusted R^2 of 0.93 and SEE of 0.75 kg/m^2 (Table 4).

The *P*-value that indicated significance of the model in validation cohort was X_2 = four-site average + abdomen + anterior upper arm (cm), X_3 = age (y), X_4 = sex (male = 0, female = 1), X_5 = BMI (kg/m^2).

The Bland-Altman analysis of the optimized protocol to predict fat mass index demonstrated a nonsignificant fixed bias of 0.01 kg/m^2 (95% CI, −0.15, 0.17) and limits of agreement of −1.57 and 1.60 kg/m^2 (Fig. 2).

Discussion

The objective of this study was to develop and internally validate a bedside-viable ultrasound protocol to estimate the DXA-derived fat mass index. We demonstrated that a four-site protocol that images the anterior thigh compartment (often utilized for muscle thickness quantifications [20,28]) is strongly associated ($R^2 = 0.65$) with fat mass index; however, wide limits of agreement were observed for the Bland-Altman analysis. The addition of adipose tissue thicknesses of the anterior upper arm and abdomen, along with age, sex, and BMI improved the predictive accuracy

Table 2
Adipose tissue thickness measured from ultrasound

Variable Median (IQR)	All (n = 94)	Men (n = 41)	Women (n = 53)	*P*-value
Anterior upper arm, cm	0.42 (0.27–0.65)	0.27 (0.17–0.42)	0.56 (0.40–0.80)	<0.001
Posterior upper arm, cm	0.82 (0.46–1.29)	0.45 (0.31–0.64)	1.10 (0.85–1.59)	<0.001
Anterior forearm, cm	0.37 (0.27–0.52)	0.28 (0.22–0.38)	0.47 (0.34–0.60)	<0.001
Abdomen, cm	2.22 (1.68–3.10)	1.89 (1.63–2.88)	2.45 (1.76–3.13)	0.076
Subscapular, cm	0.62 (0.46–1.00)	0.60 (0.47–0.82)	0.65 (0.45–1.13)	0.206
Anterior upper leg, cm	0.99 (0.57–1.43)	0.55 (0.45–0.80)	1.33 (1.04–1.74)	<0.001
Posterior upper leg, cm	1.01 (0.65–1.47)	0.65 (0.51–0.91)	1.21 (0.99–1.86)	<0.001
Anterior lower leg, cm	0.13 (0.07–0.24)	0.089 (0.06–0.16)	0.18 (0.09–0.27)	0.001
Posterior lower leg, cm	0.60 (0.39–0.82)	0.35 (0.26–0.55)	0.76 (0.57–0.96)	<0.001
Average 4-site, cm	1.07 (0.65–1.52)	0.61 (0.53–0.84)	1.40 (1.10–1.87)	<0.001

IQR, interquartile range

Table 3
Linear regression to predict fat mass index using the four-site protocol

Model development	Fat mass index prediction (kg/m^2)	Validation group	Adjusted R^2	SEE (kg/m^2)	*P*-value*
Groups 1 + 2	0.079 X_1 + 3.613	3	0.61	2.16	<0.001
Groups 1 + 3	0.078 X_1 + 3.500	2	0.53	1.72	<0.001
Groups 2 + 3	0.074 X_1 + 3.607	1	0.81	1.30	<0.001
Average	0.077 X_1 + 3.573	-	0.65	1.73	-

SEE, standard error of the estimate
* *P*-value indicates significance of the model in validation cohort. X_1 = average four-site adipose tissue thickness ([right midpoint + right distal third +left midpoint + left distal third]/4) (cm) multiplied by limb length (m).

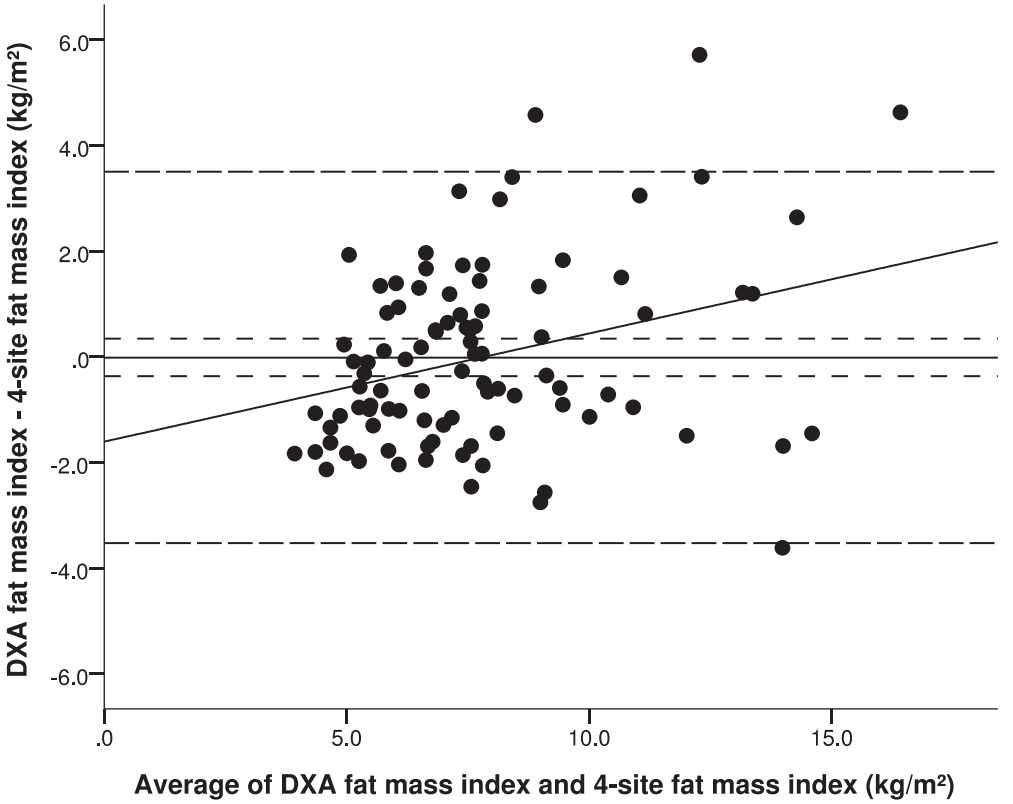

Fig. 1. Bland-Altman plot comparing DXA derived and 4-site predicted fat mass index. A non-significant fixed bias [95% CI] of −0.02 [−0.37, 0.34] kg/m^2 and limits of agreement of −3.53 and 3.50 kg/m^2 were observed. Solid black line – average fixed bias, inner dashed lines – 95% CI for fixed bias, outer dashed lines – limits of agreement (95% CI for differences). CI, confidence interval; DXA, dual-energy x-ray absorptiometry.

(R^2 = 0.93) of the model and the Bland-Altman analysis exhibited narrower limits of agreement.

Accurate and reliable assessments of body fat are vital to identify health risks on the extreme high and low ends of body fat, track changes in adiposity over time, and determine the effectiveness of targeted interventions with the goal of promoting a healthy body composition [7]. Several studies have previously demonstrated that ultrasound-based measures of adipose tissue thickness are reliable and strongly associated (r = 0.78–0.99) with measures of adiposity from hydrostatic weighing, air displacement plethysmography, DXA, skin fold thickness, bio-electrical impedance analysis, and the four-compartment model in a wide range of cohorts with young, old, athletic, and obese subjects [9–11,14–16,29,30]; however, strong associations are not always observed [31,32]. The majority of these protocols are performed in an upright posture and require posterior landmarks such as the subscapular or posterior upper arm, which limits the application to individuals with reduced mobility (i.e., critically ill, older adults who have difficulty standing for extended periods of time). The development of an ultrasound protocol (i.e., identifying key landmarks) to quantify adiposity, which can be applied bedside, would greatly increase the utility of this tool to quantify body composition.

To the best of our knowledge, a single study has developed and assessed a protocol bedside-applicable ultrasound protocol to estimate whole body adiposity. Eston et al. [13] assessed several anterior and posterior sites in a supine or prone position and observed that the adipose tissue thickness of the anterior thigh and abdomen were strongly associated with body fat percentage that was obtained using hydrostatic weighing in Chinese (r = 0.89) and English (r = 0.80) men. Interestingly, similar anatomic adipose thicknesses that were identified as critical for the assessment of adiposity by Eston et al. were also utilized here in the development of the optimized protocol that involved a much more heterogeneous cohort of participants. Furthermore, several ultrasound protocols have previously demonstrated that the abdomen and anterior thigh adipose tissue thicknesses are strongly associated with whole body measures of adiposity [9,13,14,19,33], which adds additional evidence that these landmarks are critical for accurate predictions within our model.

Although we did observe strong associations for both the four-site and optimized protocols, the evaluation of limits of agreement from the Bland-Altman analysis enables the determination of the error of prediction that is associated with 95% of participants. There

Table 4
Multilinear regression analysis to predict fat mass index using the optimized protocol

Model development	Fat mass index prediction (kg/m^2)	Validation group	Adjusted R^2	SEE (kg/m^2)	*P*-value
Groups 1 + 2	0.742 X_2 + 0.023 X_3 + 1.473 X_4 + 0.302 X_5-4.815	3	0.97	0.59	<0.001
Groups 1 + 3	0.805 X_2 + 0.025 X_3 + 1.270 X_4 + 0.340 X_5 -6.081	2	0.88	0.86	<0.001
Groups 2 + 3	0.695 X_2 + 0.023 X_3 + 1.642 X_4 + 0.367 X_5-6.328	1	0.93	0.80	<0.001
Average	0.747 X_2 + 0.024 X_3 + 1.461 X_4 + 0.336 X_5-5.741	-	0.93	0.75	-

SEE, standard error of the estimate

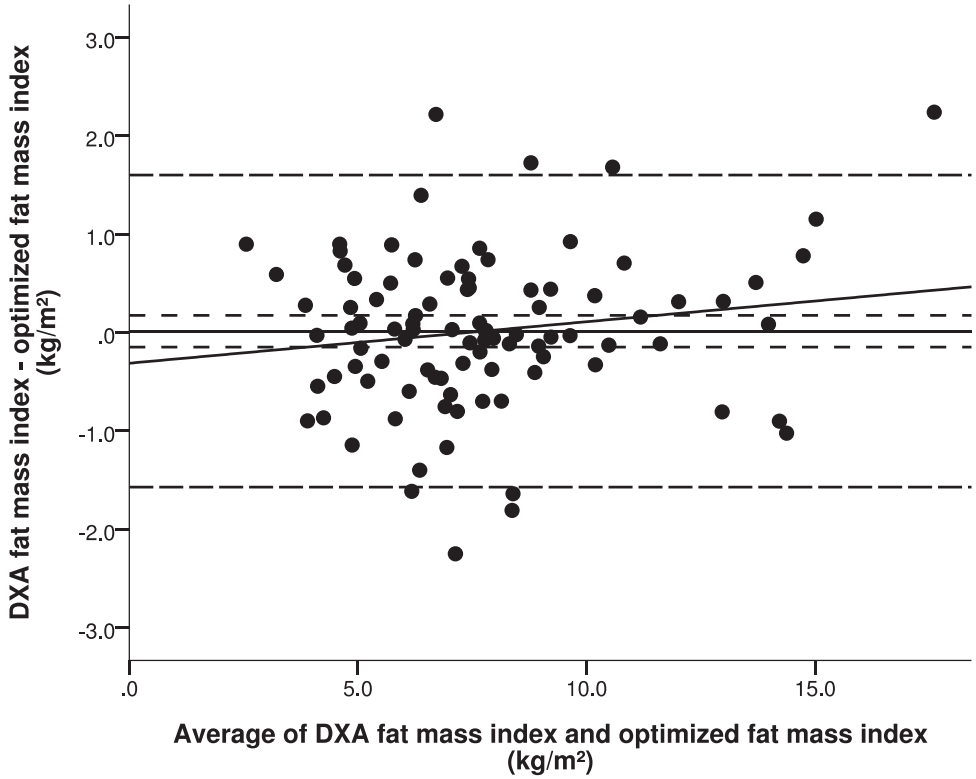

Fig. 2. Bland-Altman plot comparing DXA derived and optimized protocol predicted fat mass index. A non-significant fixed bias [95% CI] of 0.01 [−0.15, 0.17] kg/m^2 and limits of agreement of −1.57 and 1.60 kg/m^2 were observed. Solid black line – average fixed bias, inner dashed lines – 95% CI for fixed bias, outer dashed lines – limits of agreement (95% CI for differences). CI, confidence interval; DXA, dual-energy x-ray absorptiometry.

is currently no standard accepted level of error for measures of adiposity; however, sex-specific fat mass index reference values from the National Health and Nutrition Examination Survey can be used to interpret how a given level of error can alter the classification of an individual's adiposity [34]. For example, the transition from mild fat deficit through normal fat mass to excess fat mass is approximately 3 kg/m^2 for women and 4 kg/m^2 for men [34]. Therefore, the limits of agreement for the four-site protocol (−3.53 and 3.50 kg/m^2) can be reasonably contented as too large to be considered acceptable because they represent a transition from a fat deficit to excess adiposity for women and nearly a similar transition for men. The narrower limits of agreement for the optimized protocol (1.57 and 1.60 kg/m^2) represent less substantial difference in adiposity classification. Furthermore, even though this level of error may still result in different fat deficit classifications (due to very narrow ranges between the groups: 0.3 to 0.7 kg/m^2), ranges for excess adiposity and classes of obesity are larger than the observed limits of agreement (3 kg/m^2 for women and 4 kg/m^2 for men per group), which suggests that this protocol may be useful to estimate fat mass index.

This study has several limitations that may impact the validity and applicability of our results. We quantified subcutaneous adipose tissue thicknesses and related these measures to whole body fat mass index, which includes both visceral and intermuscular adipose tissue. Large differences in these adipose tissue depots between participants may result in additional variability. Our criterion method, DXA, may have increased variability in obese individuals because a process that is known as beam hardening can occur and alter fat tissue quantifications [35]. Although we did recruit sample sizes that were comparable to other investigations that developed ultrasound prediction equations [11,19,36], our sample cohort limited our ability to develop age- and sex-specific equations. Lastly, the use of the optimized model requires BMI, which may be difficult to obtain depending on an individual's mobility or equipment available.

Conclusions

We demonstrated that the four-site protocol adipose tissue thicknesses may be strongly associated with a whole body measure of adiposity, but wide limits of agreement that were observed on Bland-Altman plots suggest that this protocol alone does not accurately predict fat mass index. However, the addition of the anterior upper arm and abdomen adipose tissue thickness alongside age, sex, and BMI significantly improves the associations with fat mass index and reduces the limits of agreement, which suggests that this protocol may be useful to assess adiposity at the bedside.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.nut.2018.04.012.

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