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

Objective: 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, which recruited 94 university and community dwelling adults, to attend a single data collection session. Adipose tissue thickness was quantified in a supine or prone position using: 1) the 4-site protocol, which images 2 anterior sites on each thigh, and 2) the 9-site protocol, which images 9 anterior and posterior sites. Adipose tissue thicknesses from the 4-site protocol were compared against fat mass index derived from dual-energy x-ray absorptiometry scans. Subsequently, we optimized the accuracy of the 4-site protocol with the addition of bedside-accessible adipose tissue thicknesses from the 9-site protocol and easily obtained covariates.

Results: The 4-site protocol was strongly associated (R^2=0.65) with fat mass index, but wide limits of agreement (-3.53, 3.50 kg/m^2) were observed using Bland-Altman analysis. With the addition of the anterior upper arm and abdomen adipose tissue thicknesses, and covariates age, sex, and body mass index, model accuracy improved (R^2=0.93) and Bland-Altman analysis displayed narrower limits of agreement (-1.57, 1.60 kg/m^2).

Conclusions: The optimized protocol developed here can be applied at the bedside and provide accurate assessments of fat mass index.

Keywords: adipose tissue thickness, ultrasound, fat mass, obesity, body composition

List of abbreviations: ADP, air displacement plethysmography; BIA, bioelectrical impedance analysis; BMI, body mass index; computed tomography, CI, confidence interval; CT; dual-energy x-ray absorptiometry, DXA; IQR, inter-quartile range; MRI, magnetic resonance imaging; NHANES, National Health and Nutrition Examination Survey; SEE, standard error of the estimate.

Introduction

The prevalence of overweight and obese adults is increasing in both developed and developing countries, leading to a world-wide pandemic, which requires urgent intervention to mitigate the substantial health risks associated with this condition [1,2]. Not only does obesity increase the risk of developing non communicable diseases such as hypertension, type 2 diabetes, coronary heart disease, stroke, and many cancers [1], it also negatively impacts 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 in indirectly measuring adiposity, due to its simplicity of assessment and interpretation. However, BMI cannot distinguish specific tissues, and changes in body composition can be highly variable amongst individuals and have 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 for quantifying adiposity and tracking changes over time, but these approaches are impractical due to costs, radiation exposure (in the case of CT scans) and

limited accessibility [6]. This challenge in obtaining accurate measures of adiposity has been recognized in the strategic plan for obesity research released by the National Institute of Health, with an emphasis placed on clinically applicable approaches [7].

Ultrasound, 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 (BIA) [12], hydrostatic weighing [13,14], air displacement plethysmography (ADP) [12,15,16], and multi-compartment models [17,18]. However, the majority of these protocols are applied in a non-supine posture and include posterior adipose tissue thicknesses; limiting their clinical application in individuals with reduced mobility or patients 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 1) assessed the agreement between a 4-site protocol (images 4 locations on the anterior thigh compartment) and DXA-based fat mass index, and 2) optimized the accuracy of the 4-site protocol by incorporating additional bedside-accessible adipose tissue thicknesses and easily obtained covariates.

Materials and 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 9-site [19] and 4-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 years 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: 1) had a previous history of neuromuscular disorders, 2) were currently or suspect they may be pregnant, 3) had undergone a barium swallow or nuclear medicine scan within the past three weeks, 4) had a stroke within the past five years, 5) had a prosthetic joint replacement, or 6) had an implantable electronic device. Participants were instructed to refrain from alcohol consumption for 24 hours and moderate to vigorous physical activity for 48 hours prior to their scheduled data collection session.

DXA Imaging Procedures

Height and weight was 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 1-2 whole body DXA scans were performed (Hologic Discovery QDR 4500, Hologic, Toronto, ON). Using Hologic software (version 13.2), whole body scans were segmented into head, trunk, upper limbs, and lower limbs by a single trained

93 investigator, as previously described [22]. Fat mass index was calculated by summing the
94 fat mass of all segments and normalizing by the participants height squared (kg/m^2).

95 **Ultrasound Imaging Procedures**

96 Transverse images of adipose and muscle tissue at predefined sites were obtained
97 using a real-time B-mode ultrasound device (M-Turbo, Sonosite, Markham, ON) equipped
98 with a multi-frequency linear array transducer (L38xi, 5-10 MHz). Adjustable parameters
99 gain, time-gain-compensation, compression, resolution and musculoskeletal setting were
100 held constant throughout the imaging procedure; however, depth was adjusted as necessary
101 to obtain complete images of adipose and muscle tissue. All images were obtained with the
102 participant lying supine or prone, with a neutral wrist and ankle rotation (maintained using
103 an adjustable strap). Landmarks were identified by palpation and specific sites to be
104 imaged were marked using a flexible tape measure and ink. To minimize potential
105 compression of the underlying tissues by the ultrasound transducer during imaging, two
106 criteria were applied: 1) a thick layer of ultrasound gel was maintained between the probe-
107 skin interface, ensuring no direct contact, and 2) the operator visually confirmed that the
108 skin, adipose tissue, and muscle belly maintained a convex shape prior to freezing the
109 image, as previously described [21]. Images were downloaded using the lowest level of
110 compression and analyzed for adipose tissue thickness using ImageJ software (NIH,
111 Bethesda, MD, version 1.6.0_24) [23]. Adipose thickness was analyzed in pixels using the
112 line segment tool and converted to distance (cm) using depth-adjusted pixel/cm ratios.
113 Adipose tissue thickness was taken as the distance between the superficial border of the
114 muscle fascia and the deep border of the skin (Supplemental Figure 1). All images for a

115 single participant were analyzed before moving to the next participant, however, no
116 reference was made to previous measurements until all participants were completed.

117 **Ultrasound Protocols**

118 The 4-site protocol images the adipose tissue thickness at the mid-point and distal
119 two-thirds on anterior surface of the left and right thigh between the anterior superior iliac
120 spine and the upper pole of the patella. Each landmark was imaged twice and the average
121 thickness across all sites was calculated. The 9-site protocol images anterior and posterior
122 adipose tissue thicknesses on right side of the body, as previously described. Briefly, the
123 landmarks included: the anterior and posterior upper arm, the anterior forearm, the
124 abdomen, the subscapular area, the anterior and posterior thigh, and the anterior and
125 posterior lower leg. These landmarks were imaged once.

126 **Statistical Analysis**

127 Continuous variables were assessed for normality using the Shapiro-Wilks test and
128 confirmed using quantile-quantile plots. Normality was violated for several variables,
129 therefore data is presented as median (inter-quartile range (IQR)) and differences between
130 males and females were analyzed using the Mann-Whitney U test.

131 All linear regression analysis was performed using a 3-fold cross-validation,
132 stratified by fat mass index (kg/m^2), to improve the generalizability of the developed
133 models [24]. A 3-fold cross-validation divides the participant cohort into 3 equally
134 distributed groups, where model development occurs using 2 groups, with subsequent
135 testing of the model in the left out group; this process is repeated 3 times and averaged

across all groups for assessment of model accuracy. Linear regression analysis to predict fat mass index was performed using the average 4-site adipose tissue thickness [(right midpoint + right distal third + left midpoint + left distal third)/4] multiplied by limb length (m).

Variables to be included in the optimized model to predict fat mass index were selected using a combination of a-priori (4-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 (4-site, and anterior upper arm) and factors known to influence or be associated with adiposity (age, sex, and BMI). 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 (Supplemental Table 1). Anterior upper arm, abdomen, and the averaged 4-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 regression analysis of trunk fat mass and sex (n=93) against abdomen adipose tissue thickness. Correlation analysis of predicted (trunk fat mass and sex) and ultrasound measured abdomen adipose tissue thickness demonstrated a strong association ($r=0.80$, $p<0.001$).

Bland-Altman plot analysis was used to compare fat mass index derived from DXA with that predicted from both the 4-site and optimized protocols. Limits of agreement

158 (95% confidence interval (CI) of the differences) were calculated and used for
159 interpretation [25]. Regression analysis of the differences against the averages (assessment
160 of proportional bias – non-constant bias across the range of data) and visually assessing a
161 plot of the residuals against averages (assessment of homoscedasticity – even spread of
162 data across the range of data) was performed to ensure that constant limits of agreement
163 are valid [26]. These assumptions were valid for all Bland-Altman plots.

164 Regression coefficients were interpreted as weak (0.30-0.50), moderate (0.50-0.70
165 and strong (0.70-1.00) [27]. All analysis was performed using SPSS (IBM, Chicago, IL,
166 USA, version 24.0) and the level of significance was set at $p \leq 0.05$.

167 **Results**

168 Of the ninety-four participants recruited, 56 % were female, and compared with
169 males, females displayed significantly lower median BMI (23.7 vs. 25.6 kg/m^2, p=0.016),
170 but higher median body fat % (34.7 vs 24.2 %, p<0.001) and body fat index (7.8 vs 6.4
171 kg/m^2, p=0.001) (Table 1). Fifty-five of the 94 participants were aged <60 years old and 39
172 were aged ≥60 years of age.

173 **Table 1.** Physical description of participant cohort

Variables Median (IQR)	All (n=94)	Males (n=41)	Females (n=53)	p-value
Age, year	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 18.5-24.9 kg/m^2	51	16	35	-
Overweight 25.0-29.9 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, inter-quartile range.

Compared to males, females displayed significantly greater adipose tissue thickness for all limb-based landmarks ($p<0.05$), but not trunk-based measures (subscapular and abdomen) ($p>0.05$) (Table 2); which corresponded with differences observed in appendicular and trunk fat mass between males and females (Table 1).

Table 2. Adipose tissue thickness measured from ultrasound

Variable Median (IQR)	All (n=94)	Males (n=41)	Females (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, inter-quartile range

Across the 3-fold cross-validation groups, linear regression analysis using the 4-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).

Table 3. Linear regression to predict fat mass index using the 4-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	$0.079X_1+3.613$	3	0.61	2.16	<0.001
Groups 1+3	$0.078X_1+3.500$	2	0.53	1.72	<0.001
Groups 2+3	$0.074X_1+3.607$	1	0.81	1.30	<0.001
Average	$0.077X_1+3.573$	-	0.65	1.73	-

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

Bland-Altman analysis of the 4-site protocol to predict fat mass index demonstrated 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 (Figure 1).

Figure 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

Across the 3-fold cross-validation groups, multiple linear regression analysis using the summed anterior upper arm, abdomen, and average 4-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=0.75 kg/m^2 (Table 4).

Table 4. Multi-linear 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	$0.742X_2+0.023X_3+1.473X_4+0.302X_5-4.815$	3	0.97	0.59	<0.001
Groups 1+3	$0.805X_2+0.025X_3+1.270X_4+0.340X_5-6.081$	2	0.88	0.86	<0.001
Groups 2+3	$0.695X_2+0.023X_3+1.642X_4+0.367X_5-6.328$	1	0.93	0.80	<0.001
Average	$0.747X_2+0.024X_3+1.461X_4+0.336X_5-5.741$	-	0.93	0.75	-

p-value indicating significance of the model in validation cohort. X_2 = 4-site average + abdomen + anterior upper arm (cm), X_3 = age (years), X_4 = sex (male=0, female=1), X_5 = BMI (kg/m^2). SEE, standard error of the estimate.

Bland-Altman analysis of the optimized protocol to predict fat mass index demonstrated 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 (Figure 2).

Figure 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.

Discussion

The objective of this study was to develop and internally validate a bedside viable ultrasound protocol to estimate DXA derived fat mass index. We demonstrated that a 4-site

protocol that images the anterior thigh compartment, which is 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 (R^2=0.93) of the model and Bland-Altman analysis exhibited narrower limits of agreement.

Accurate and reliable assessments of body fat are vital for identifying health risks on the extreme high and low ends of body fat, for tracking changes in adiposity over time, and for determining 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, ADP, DXA, skin fold thickness, BIA, and the 4 compartment model in a wide range of young, old, athletic, and obese cohorts [9–11,14–16,29,30]; but strong associations are not always observed [31,32]. However, the majority of these protocols are performed in an upright posture, and require posterior landmarks such as the subscapular or posterior upper arm, limiting their application to individuals with reduced mobility (i.e. critically ill, older adults who have difficulties standing for extended periods). Development of an ultrasound protocol (i.e. identifying key landmarks) to quantify adiposity, which can be applied at the bedside, would greatly increase the utility of this tool for quantifying body composition.

To the best of our knowledge, a single study has developed and assessed a protocol, which is applicable at the bedside. Eston et al. [13], assessed several anterior and posterior

227 sites in a supine or prone position and observed that the adipose tissue thickness of the
228 anterior thigh and abdomen were strongly associated with body fat percentage obtained
229 using hydrostatic weighing in Chinese (r=0.89) and English (r=0.80) men. Interestingly,
230 similar anatomical adipose thicknesses identified as critical for assessment of adiposity by
231 Eston and colleagues, were also utilized here in development of the optimized protocol,
232 which involved a much more heterogeneous cohort of participants. Furthermore, several
233 ultrasound protocols have previously demonstrate that the abdomen and anterior thigh
234 adipose tissue thicknesses are strongly associated with whole body measures of adiposity
235 [9,13,14,19,33], adding additional evidence that these landmarks are critical for accurate
236 predictions within our model.

237 However, while we did observe strong associations for both the 4-site and
238 optimized protocols, evaluating limits of agreement from Bland-Altman analysis enables
239 determination of the error of prediction associated with 95% of participants. There is
240 currently no standard accepted level of error for measures of adiposity, however, sex
241 specific fat mass index reference values from NHANES can be used to interpret how a
242 given level of error can alter the classification of an individual's adiposity [34]. For
243 example, the transition from mild fat deficit through normal fat mass to excess fat mass is
244 approximately 3 kg/m^2 for females and 4 kg/m^2 for males [34]. Therefore, it is reasonable
245 to contend that the limits of agreement for the 4-site protocol (-3.53 and 3.50 kg/m^2) are
246 too large to be considered acceptable, as they representation a transition from a fat deficit
247 to excess adiposity for females, and nearly a similar transition for males. The narrower
248 limits of agreement for the optimized protocol (-1.57 and 1.60 kg/m^2) represent less

substantial differences in adiposity classification. Furthermore, while this level of error may still result in different fat deficit classifications (due to very narrow ranges between 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 females and 4 kg/m^2 for males per group); suggesting that this protocol may be useful for estimating of fat mass index.

This study has several limitations which 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, as a process known as beam hardening can occur, altering fat tissue quantifications [35]. While we did recruit comparable sample sizes to other investigations developing 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 4-site protocol adipose tissue thicknesses may be strongly associated with a whole body measure of adiposity, but wide limits of agreement 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 reduce the limits of agreement, suggesting that this protocol may be useful for assessing adiposity at the bedside.

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Conflict of Interest: Michael T. Paris, and Marina Mourtzakis declare they have no conflict of interest.

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