Ultrasound Measurements of Visceral and Subcutaneous Abdominal Thickness to Predict Abdominal Adiposity Among Older Men and Women

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Accurate measures of visceral and abdominal subcutaneous fat are essential for investigating the pathophysiology of obesity. Classical anthropometric measures such as waist and hip circumference cannot distinguish between these two fat depots. Direct imaging methods such as computed tomography and magnetic resonance imaging (MRI) are restricted in large-scale studies due to practical and ethical issues. We aimed to establish whether ultrasound is a valid alternative method to MRI for the quantitative assessment of abdominal fat depots in older individuals. The study population comprised 74 white individuals (41 men and 33 women, aged 67–76 years) participating in the Hertfordshire Birth Cohort Physical Activity trial. Anthropometry included height, weight, waist and hip circumferences. Abdominal fat was measured by ultrasound in two compartments: visceral fat defined as the depth from the peritoneum to the lumbar spine; and subcutaneous fat defined as the depth from the skin to the abdominal muscles and compared to reference measures by MRI (10-mm single-slice image). Ultrasound measures were positively correlated with MRI measures of visceral and subcutaneous fat (visceral: r = 0.82 and r = 0.80 in men and women, respectively; subcutaneous: r = 0.63 and 0.68 in men and women, respectively). In multiple regression models, the addition of ultrasound measures significantly improved the prediction of visceral fat and subcutaneous fat in both men and women over and above the contribution of standard anthropometric variables. In conclusion, ultrasound is a valid method to estimate visceral fat in epidemiological studies of older men and women when MRI and computed tomography are not feasible.

Obesity (2010) **18,** 625–631. doi:10.1038/oby.2009.309

INTRODUCTION

Obesity among older people is a major public health issue due to its association with increased morbidity and reduced quality of life (1,2). In the United Kingdom, between 1993 and 2005, the prevalence of obesity increased by 12.5% in the 65–74 year age group (1). This rapid rise will potentially lead to increased health-care costs and challenging health-care delivery as the proportion of individuals over 65 years in the United Kingdom continues to grow (1).

BMI is generally used to classify obesity. However, BMI is a very crude measure of obesity in that it does not distinguish between tissues, e.g., muscle and fat mass. Notwithstanding this fundamental potential for misclassification, standard BMI cut-off values may not be appropriate to use among those over 70 years due to age-related changes in body composition (3,4). These changes are characterized by a progressive loss of muscle mass and increase in fat mass (5,6). Hence, for any given BMI, loss of muscle mass may mask increased fat (7). Furthermore, with aging a greater proportion of fat tends to accumulate centrally, within the abdominal cavity (5,6) and BMI is a poor indicator of this distribution of fat in older individuals (6). In large-scale population studies, waist circumference, waist-to-hip ratio, and sagittal diameter have been used to estimate abdominal fat (5,8,9). However, these measures do not differentiate visceral fat from abdominal subcutaneous fat (10,11) and these specific depots may have very different metabolic consequences. Excessive visceral adipose tissue (VAT) is related to insulin resistance (10), whereas subcutaneous adiposity

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Received 6 May 2009; accepted 2 August 2009; published online 24 September 2009. doi:10.1038/oby.2009.309

(SCAT) may have an independent antiatherogenic effect (12). Furthermore, a recent study has reported that the ratio between these two abdominal fat depots (VAT:SCAT) is crucial in predicting the development of an unfavorable metabolic profile (13). That study suggested that individuals with a high proportion of visceral fat relative to abdominal subcutaneous fat have more adverse metabolic profiles compared to those with the opposite phenotype (13). Apart from direct imaging methods such as magnetic resonance imaging (MRI) or computed tomography, there are as yet no validated anthropometric indicators of VAT:SCAT ratio. The use of these techniques is limited in large epidemiological studies and for repeated investigations due to practical and ethical constraints (14).

Ultrasonography (US) has been shown to be an alternative, noninvasive, reliable method to estimate these two fat compartments. Its validity compared to MRI or computed tomography has been tested in adult women (15), obese adults (14,16), and diabetic adults (17,18); however, it is important to test its validity in older individuals as fat accumulation in the abdomen increases with age.

Therefore, the aim of this study was to assess the validity of ultrasound measures of visceral and subcutaneous abdominal fat in older men and women, compared to MRI. Secondly, we investigated whether the addition of ultrasound measures to standard anthropometric variables would increase the prediction accuracy of abdominal fat depots.

METHODS AND PROCEDURES

Study population

The study was based on data collected at baseline on 74 healthy adult women (n = 33) and men (n = 41) aged 67–76 years, participating in the Hertfordshire Birth Cohort Physical Activity trial (19). Participants attended the clinical research facilities at the MRC Epidemiology Unit, Cambridge, UK and the Addenbrooke's Centre for Clinical Investigation, Addenbrooke's Hospital, Cambridge, UK, between January 2007 and February 2008. Based on the Bland–Altman method for comparing methods of measurement (20), a sample size calculation indicated that 40 individuals were required to allow confidence intervals for limits of agreement between any two measures to be around half a standard deviation of their differences.

The exclusion criteria applied in the main trial were also use in this validation study. Participants were excluded if unable to cycle unaided for a minimum of 30 min; had contraindications for physical activity; had prevalent diabetes, untreated or unstable ischemic heart disease, pacemakers, metal implants and were suffering from claustrophobia.

Volunteers were instructed to refrain from eating 10 h before their arrival at the research clinic due to glucose tolerance testing undertaken for the main trial and to decrease bowel peristalsis for the imaging procedures.

The study was undertaken with approval of the Hertfordshire Local Research Ethics Committee and performed in accordance with the declaration of Helsinki. All participants gave written informed consent.

Anthropometry

Weight, height, waist and hip circumferences were measured by trained field workers while participants were barefoot and wearing light clothing.

Weight was measured using a calibrated scale (TANITA model BC-418 MA; Tanita, Tokyo, Japan) and recorded to the nearest 0.2 kg. Height was measured using a wall-mounted stadiometer (SECA model 240; Seca, Birmingham, UK) and recorded to the nearest 0.1 cm. BMI was calculated as weight/height^2 (kg/m^2). Waist circumference was measured with a D-loop tape measure (Chasmors, London, UK) at the midpoint between the inferior border of the costal margin and the anterior superior iliac crests and the hip circumference at the widest level over the greater trochanters. Both measures were recorded to the nearest 0.1 cm.

Abdominal fat

Ultrasonography. The visceral and subcutaneous abdominal fat thicknesses were measured with a Logic Book XP ultrasound (GE Healthcare, Bedford, UK), using the 3C MHz-RS abdominal curved array transducer (GE Healthcare). The transducer was placed on the location where the xiphoid line intercepted the waist circumference. The visceral thickness was defined as the depth from the peritoneal boundary to the corpus of the lumbar vertebra on longitudinal scanning at the end of a quiet expiration to avoid tensing and distorting the abdominal cavity (14).

Subcutaneous abdominal fat thickness was measured on the same location, but on a transverse plane, and was defined as the depth from the cutaneous boundary to the linea alba (14). The image was captured when the transducer just had contact with the skin to avoid compressing the subcutaneous adipose fascia. The scans were obtained by three trained sonographers. The relative intraobserver technical error of measurement for the visceral thickness ranged between 1.8 to 2.9% and 0.6 to 3.0% for subcutaneous fat thickness, and the relative interobserver technical error of measurement was 2.4% for visceral thickness and 2.1% for subcutaneous thickness.

Magnetic Resonance Imaging. The MRI images were acquired immediately after the participants' arrival at the Wolfson Brain Imaging Centre, Addenbrooke's Hospital, Cambridge, UK. A safety questionnaire was administered before the volunteer entering the MRI scanning area. The volunteer was placed supine in a Siemens 3T Tim trio whole body scanner (Camberley, UK) and a body matrix coil in conjunction with a spine coil was used in acquiring the images. Initial localizer images of the abdomen, acquired in three orthogonal directions, were used to locate the L4 vertebral body, which was subsequently placed at the isocentre using the Tim component of the scanner. A T1-weighted turbo spin echo, water suppressed, transaxial slice, with a thickness of 10 mm, was acquired and centered on the L4 vertebral body by trained radiographers. The in-plane resolution was 1.3 × 1.3 mm, field of view 500 × 500 mm, repetition time = 400 ms, echo time = 21 ms, 2 averages, 3 concatenations. Volumes of VAT and SCAT were calculated using a semiautomated method and a threshold map, in combination with manual input to distinguish between the VAT and SCAT compartments. The software analyze 7.0 (BIR; Mayo Clinic, Rochester, MN) was used for the calculations.

As the US parameters are one-dimensional and the MRI measures are two-dimensional, the precision and validity of US to predict VAT and SCAT cannot be directly assessed. However, to gain some insight into the precision and validity of the US measures, visceral and subcutaneous thicknesses were also determined on the MRI image, using the MRI imaging software analyze 7.0 (BIR; Mayo Clinic, Rochester, MN). The same anatomical landmarks were applied when obtaining the thickness on the MRI slice as were used for the respective US measures. To avoid inter-reader variation, all the images were reviewed and calculations performed by the same physicist.

Statistical analysis

Statistical analysis was performed using STATA version 9.2 (StataCorp, College Station, TX). Means and s.d. of baseline characteristics were presented separately for men and women and differences between them were tested using unpaired t-tests. Spearman rank correlation coefficients were calculated to describe associations between the different measures of abdominal fat. Linear regression analysis was firstly performed to quantify the proportion of variance of VAT and SCAT explained by US measures. Subsequently, to study the added value of ultrasound measures over simple anthropometry, multiple linear regression models were constructed. A hierarchical and pragmatic approach was used to derive the prediction models for VAT and SCAT, using different anthropometric and US measures as possible predictors. The variance inflation factor was used to detect collinearity between the different covariates; if the variance inflation factor was >5 for any two covariates, only one of them was included in the prediction model.

Finally, the level of agreement in visceral and subcutaneous abdominal thickness between US and MRI was assessed using Bland–Altman plots. Mean difference/bias between the two methods was calculated and tested against zero using a paired *t*-test.

RESULTS

Characteristics of the study sample are summarized in **Table 1**. There were no differences between men and women in mean BMI and MRI-derived subcutaneous fat thickness measures. However, men were taller and heavier and tended to have larger waist circumferences, VAT, and visceral thicknesses by US and MRI; whereas women had greater SCAT and subcutaneous fat thickness by US.

Correlations with abdominal fat

Table 2 shows Spearman correlation coefficients between US or anthropometric variables and either VAT, SCAT, or VAT:SCAT ratio by MRI. The measure most highly correlated with VAT was US visceral thickness in both men and women, followed by waist circumference. The weakest correlations with VAT were observed for BMI, weight and hip circumference, particularly in women.

All anthropometric measures and the US subcutaneous fat thickness were moderately correlated with SCAT in both men and women. Hip circumference was the measure most highly correlated with SCAT in both men and women, whereas BMI showed the strongest correlation in women. Correlations between the US parameters and VAT:SCAT ratio, which is the index generally used to describe abdominal fat distribution, were only moderate, but were higher than those between anthropometric variables and VAT:SCAT ratio.

Prediction of abdominal fat by anthropometry and US

Univariate regression analysis was initially performed to quantify the contribution of the different US measures to VAT and SCAT. US visceral thickness explained 69% and 78% of the variance in VAT in men and women, respectively, whereas US subcutaneous thickness explained 30% and 57% of the variance in SCAT in men and women, respectively.

Results of the multivariate regression analyses are shown in **Tables 3** and **4**. The addition of US visceral thickness to BMI and waist circumference improved the explained variance in VAT from 64% to 75% in men, and from 63% to 81% in women. This was reflected in the 15% and 28% reduction in root mean squared error, respectively. Similarly, the addition of US subcutaneous thickness to BMI and waist circumference improved

Table 1 Characteristics of the study sample

	Men (*n* = 41)	Women (*n* = 33)	Total (*n* = 74)	Range	*P* value[a]
Age (year)	71 ± 2.2	71 ± 2.6	71 ± 2.4	67–76	0.5
Anthropometric measures					
Weight (kg)	83.6 ± 13.1	69.4 ± 10.8	77.2 ± 13.9	48.6–123.3	<0.0001
Height (cm)	174.3 ± 6.6	161.4 ± 5.4	168.5 ± 8.9	148.8–186.4	<0.0001
BMI (kg/m^2)	27.4 ± 3.6	26.7 ± 4.5	27.1 ± 4.0	20.7–40.4	0.4
Waist circumference (cm)	103.2 ± 10.1	91.0 ± 11.3	98.0 ± 12.2	72.2–131	<0.0001
Hip circumference (cm)	106.3 ± 7.3	106.0 ± 9.9	106.1 ± 8.4	89.7–132.3	0.9
Waist:hip ratio	1.0 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.7–1.1	<0.0001
MRI measures					
VAT (cm^2)	155.1 ± 73.6	105.3 ± 59.8	132.6 ± 71.8	29.2–393.7	0.001
SCAT (cm^2)	235.6 ± 75.1	278.2 ± 95.9	254.5 ± 87.1	105.9–533.9	0.04
VAT:SCAT ratio	0.7 ± 0.2	0.4 ± 0.2	0.5 ± 0.3	0.1–1.3	<0.0001
Visceral thickness (cm)	7.2 ± 2.4	5.3 ± 2.3	6.3 ± 2.6	2.3–13.6	0.0007
Subcutaneous fat thickness (cm)	2.4 ± 0.8	2.2 ± 0.8	2.3 ± 0.8	1–4.5	0.3
US measures					
Visceral thickness (cm)	7.7 ± 2.4	5.6 ± 2.7	6.7 ± 2.8	2.6–14.8	0.001
Subcutaneous fat thickness (cm)	2.6 ± 1.0	3.1 ± 0.9	2.8 ± 0.9	0.9–4.9	0.01
Visceral: subcutaneous thickness ratio	3.3 ± 0.3	1.9 ± 1.1	2.7±1.6	0.7–8.7	0.0001

Data are presented as mean ± s.d.
MRI, magnetic resonance imaging; SCAT, subcutaneous adipose tissue; US, ultrasonography; VAT, visceral adipose tissue.
[a]Sex differences by *t*-test.

Table 2 Spearman rank correlation coefficients between ultrasound, anthropometry, and abdominal adiposity areas measured by MRI

	MRI measures					
	Men			Women		
	VAT (cm^2)	SCAT (cm^2)	VAT:SCAT ratio	VAT (cm^2)	SCAT (cm^2)	VAT:SCAT ratio
Anthropometry						
Weight (kg)	0.69	0.63	0.36	0.54	0.72	0.1
BMI (kg/m^2)	0.68	0.62	0.38	0.51	0.79	0.12
Waist circumference (cm)	0.72	0.69	0.36	0.7	0.66	0.32
Hip circumference (cm)	0.69	0.71	0.37	0.38	0.73	0.1
Waist:hip ratio	—	—	0.27	—	—	0.43
US measures						
Visceral thickness (cm)	0.82	0.7	—	0.8	0.52	—
Subcutaneous fat thickness (cm)	0.1	0.63	—	0.25	0.68	—
Visceral:subcutaneous thickness ratio	—	—	0.65	—	—	0.62

MRI, magnetic resonance imaging; SCAT, subcutaneous adipose tissue area; US, ultrasonography; VAT, visceral adipose tissue area.

Table 3 Prediction models for VAT using anthropometry and ultrasound visceral thickness

			$B^b \pm$ s.e				
		Model[a]	BMI (kg/m^2)	WC (cm)	US visceral (cm)	R^2%	RMSE
Men	VAT (cm^2)	1	15.9 ± 2.0	—	—	60	45.6
		2	—	5.8 ± 0.6	—	65	43.7
		3	6.1 ± 4.6	3.8 ± 1.6	—	64	43.4
		4	3.2 ± 4.2	1.7 ± 1.6	14.6 ± 4.2	75	37
Women	VAT (cm^2)	1	8.8 ± 1.8	—	—	42	45.7
		2	—	4.3 ± 0.7	—	64	37
		3	−0.6 ± 2.7	4.5 ± 1.1	—	63	37.7
		4	−1.8 ± 1.9	1.8 ± 0.9	15.9 ± 3.1	81	27

All coefficients were $P < 0.001$.
RMSE, root mean squared error; US, ultrasonography; VAT, visceral adipose tissue; WC, waist circumference.
[a]Model: 1 included BMI; 2 included waist; 3 BMI and the covariate waist circumference; 4 included BMI and the covariates waist circumference and US visceral thickness.
[b]B represents expected change in VAT per unit increase in the covariate.

the prediction of SCAT from 75% to 87%, in men, and from 76% to 81% in women, with corresponding root mean squared error reductions of 24% and 8%, respectively.

All models satisfied the multicollinearity requirement (variance inflation factor <5). Age was rejected as a predictor in all models ($P = 0.1$ in men and $P = 0.7$ in women). The independent variable height was also investigated, but was not included in the final models as the results were similar to those with BMI in the model.

Precision of ultrasound measures

US subcutaneous and visceral thicknesses were compared to the corresponding MRI thicknesses. Visceral thickness by US and MRI were highly correlated ($r = 0.90$ in men and women; $r = 0.84$ in men; $r = 0.85$ in women). Subcutaneous thickness by US and MRI were also positively related ($r = 0.73$ in men and women; $r = 0.73$ in men; $r = 0.71$ in women). For the visceral thickness, the Bland–Altman analysis (**Figure 1a,b**) revealed a small nonsignificant mean difference of 0.34 ± 1.3 cm ($P = 0.1$) in men and 0.33 ± 1.1 cm ($P = 0.09$) in women. However, for subcutaneous thickness (**Figure 2a,b**) significant mean biases were observed in both sexes. The mean difference between the two methods for estimating subcutaneous thickness was 0.25 ± 0.56 cm ($P = 0.007$) in men and 0.69 ± 0.65 cm ($P < 0.0001$) in women. The differences (relative to the mean) between the two methods for measuring visceral thickness were 4.7% in men and 6.6% in women, and for estimating subcutaneous fat were 10% in men and 31% in women.

DISCUSSION

In this study, we found that ultrasound is a valid method for the assessment of visceral and abdominal subcutaneous fat in

Table 4 Prediction models for SCAT area using anthropometry and ultrasound subcutaneous thickness

			$B^b \pm$ s.e				
		Model[a]	BMI (kg/m²)	WC (cm)	US subcutaneous (cm)	R^2%	RMSE
Men	SCAT (cm²)	1	15.8 ± 2.0	—	—	60	46.5
		2	—	6.2 ± 0.6	—	75	37
		3	−1.7 ± 4.0	6.7 ± 1.4	—	75	37.2
		4	3.6 ± 3.2	4.5 ± 1.2	29.4 ± 5.4	87	28.2
Women	SCAT (cm²)	1	19.5 ± 1.9	—	—	75	48.3
		2	—	6.0 ± 1.2	—	44	73.1
		3	22.0 ± 3.4	−1.2 ± 1.3	—	76	48.1
		4	17.5 ± 3.6	−1.3 ± 1.3	30.2 ± 11.6	81	44.1

All coefficients were $P < 0.001$.
RMSE, root mean squared error; SCAT, subcutaneous adipose tissue; US, ultrasonography; WC waist circumference.
[a]Model: 1 included BMI; 2 included waist; 3 included BMI and the covariate waist circumference; 4 included BMI and the covariates waist circumference and US subcutaneous thickness. [b]B represents expected change in VAT per unit increase in the covariate.

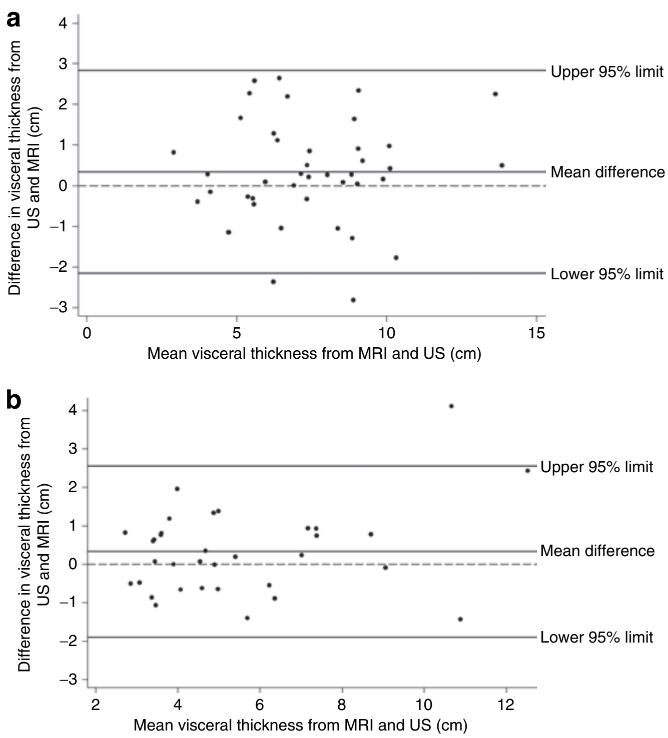

Figure 1 Bland–Altman analysis for (**a**) men and (**b**) women. In both plots, the *y* axis represents the difference between visceral thickness from US and visceral thickness from MRI. The *x* axis represents the mean visceral thickness from the two methods. The plots show a mean difference/bias of 0.34 ± 1.3 cm (4.7% of mean MRI) in men and 0.33 ± 1.1 cm (6.6% of mean MRI) in women and 95% limits of agreement of −2.26; 2.94 cm (−31; 41% of mean MRI) in men and −1.87; 2.53 cm (−35;48% of mean MRI) in women. MRI, magnetic resonance imaging; US, ultrasound.

older men and women when compared to MRI. The correlations between the two methods were strong particularly for visceral fat in women. Furthermore, ultrasound parameters showed much stronger correlations than anthropometry with the MRI visceral: subcutaneous ratio, the index of abdominal fat distribution used in the prediction of the metabolic consequences (13). Despite the strong correlations, US seemed to slightly overestimate visceral and subcutaneous thicknesses in both men and women. Our results also showed that the addition of ultrasound measures significantly improved the prediction of visceral and subcutaneous fat over and above the contributions of the anthropometric variables BMI and waist circumference. The precision of our final models was significantly improved as the root mean squared error for VAT and SCAT substantially decreased in both men and women.

Our results are consistent with the previous studies, which reported strong correlations between visceral thickness and MRI VAT ranging from 0.75 to 0.81 (14–18). Our correlations with SCAT were slightly lower than in other reports (18). Reproducibility of our ultrasound measurements was acceptable, indicated by the relatively low intra- and interobserver errors. This error likely stems from the difficulty in standardizing the amount of decompression applied by the ultrasound operator when lifting the transducer from the abdomen when assessing the subcutaneous fat thickness.

BMI showed the weakest correlations with VAT, suggesting that this index is not an optimal indictor of this adiposity. Similar observations have been reported in studies investigating body composition in older populations (5,6). However, BMI seems to be an appropriate marker of subcutaneous fat in this age group, particularly in women.

Limitations of our study include the use of a single-MRI slice which can only assess VAT and SCAT areas; a more relevant criterion would be the volume of these adiposities in the whole abdomen, the variance of which may not be fully captured by a single slice of the abdomen. Indeed, there may be substantial interindividual variation in the distribution of VAT and SCAT across the abdominal area (21,22). Other studies have also reported that images taken at L1–L2 or L2–L3 might be more suitable for the quantification of these adiposities (23,24). The most accurate method for the estimation of VAT and SCAT would be to derive volumes of these adiposities from a series of MRI multislices. Future research should use MRI volume estimates in validation studies.

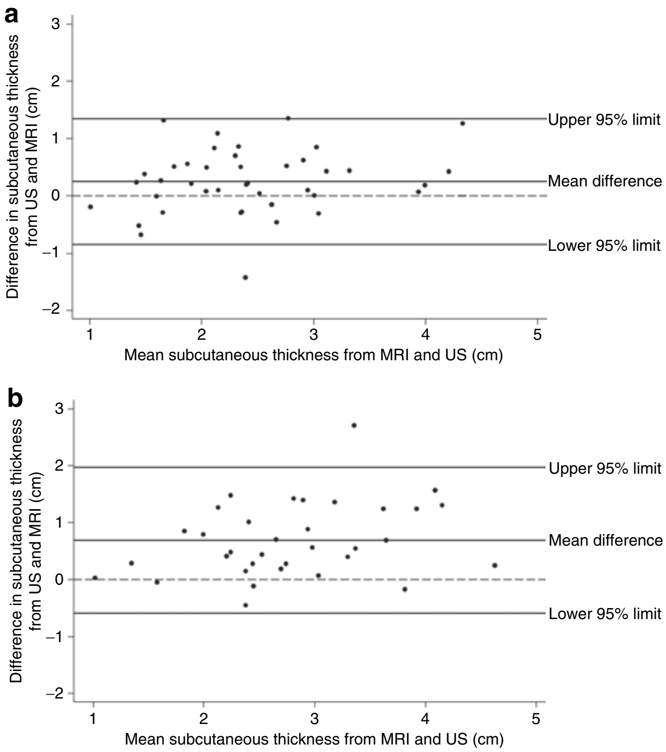

Figure 2 Bland–Altman analysis for (**a**) men and (**b**) women. In both plots, the *y* axis represents the difference between subcutaneous thickness from US and subcutaneous thickness from MRI. The *x* axis represents the mean of subcutaneous thickness from the two methods. The plots show a mean difference/bias of 0.25 ± 0.56 cm (10% of mean MRI) in men and 0.69 ± 0.65 cm (31% of mean MRI) in women and 95% limits of agreement of –0.87; 1.37 cm (– 36; 57% of mean MRI) in men and –0.61; 1.99 cm (–28; 90% of mean MRI) in women. MRI, magnetic resonance imaging; US, ultrasound.

Secondly, we were unable to test "true" agreement between the two methods as the MRI and US measurements have different units. However, there was relatively good agreement between the surrogate thickness measures from the two techniques.

Finally, the use of our prediction models may be limited to populations similar to that examined. These prediction equations will need to be validated in independent studies in different age and ethnic groups. Further research in this area would be valuable.

Obesity is a complex multifactorial disease (25) and large sample sizes are generally required to identify the effects of specific genetic and environmental factors and their interactions (26). The addition of precise and accurate measurements greatly increases the statistical power of studies to identify the aetiology of obesity and also of studies that evaluate the effectiveness of interventions to treat or prevent obesity (26).

In summary, our data in older adults add to the growing evidence that US is a robust method for the estimation of abdominal fat compartments. US improved the prediction of VAT and greatly enhanced the ability to assess the relative quantities of VAT and SCAT. Its application in large epidemiological studies is feasible and should greatly increase the power to demonstrate the determinants and potential different metabolic sequelae of visceral and abdominal subcutaneous fat depots.

ACKNOWLEDGMENTS

We are grateful to all the volunteers who participated in the study. We thank Cheryl Kidney, Alice Thompson, and the radiographers for assisting in data collection, Jessica Horton for coordinating the study, Richard Powell for assisting with data cleaning and Kirsten Corder for her comments. The study was funded by the Medical Research Council.

DISCLOSURE

The authors declared no conflict of interest.

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