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ABSTRACT

Recently, it was reported that intra-abdominal thickness (IAT) assessments using ultrasound are most reliable if measured from the linea alba to the anterior vertebral column. These two anatomical sites can be simultaneously visualized using a linear-array transducer. Linear-array transducers have different operational characteristics when compared to conventional curved-array transducers, and are more reliable for some ultrasound-derived measures such as abdominal subcutaneous fat thickness (SFT). However, it is unknown whether linear-array transducers facilitate more reliable IAT measurements than curved-array transducers. The purpose of the current study was to (i) compare the reliability of linear- and curved-array transducer assessments of IAT and maximal abdominal ratio (MAR); and (ii) use the findings to update central adiposity measurement guidelines. Fifteen healthy adults (27 y [SD 10], 60% female) with a range of somatotypes (BMI: 24 [SD 4], range: 19 - 33kg/m^2; waist circumference: 75 [SD: 11], range: 61 – 96cm) were tested on three mornings under standardized conditions. IAT was assessed 2cm above the umbilicus (transverse plane), measuring from linea alba to the anterior vertebral column. MAR was defined as the ratio of IAT to SFT. IAT range was 25 - 87mm and MAR 0.15 - 0.77. Between-day intra-class correlation coefficient values for IAT measurements made were comparable (0.96-0.97) for both transducers, as were MAR values (0.95). In conclusion, while both transducers provided equally reliable measurement of IAT, the use of a single linear-array transducer simplifies the assessment of central adiposity.

Key Words: intra-abdominal; visceral; ultrasound; obesity; reproducibility; guidelines.

ABBREVIATIONS

BMI – body mass index

CV- coefficient of variation

IAT- intra-abdominal thickness

ICC- intra-class correlation coefficient

IVC- inferior vena cava

LA- linea alba

MAR- maximum abdominal ratio (SFT/IAT)

RA- rectus abdominus;

RC- reproducibility coefficient

RC%- reproducibility coefficient expressed as a percentage of the mean

SD - standard deviation

SEM- standard error of measurement

SFT- abdominal subcutaneous fat thickness

INTRODUCTION

Obesity has reached pandemic proportions and has become a major global health concern, with the incidence of obesity increasing at a faster rate in low- and middle-income countries than high-income countries [1]. The concern is not limited to the presence of obesity *per se*, but also the associated clustering of cardio-metabolic complications, including hypertension, hypercholesterolemia and type 2 diabetes [2], each of increase cardiovascular disease (CVD) risk, even in children [3-5]. For patients at risk for cardio-metabolic complications, appropriate measurement of obesity may assist with diagnostic and treatment strategies; however, although estimation of whole body adiposity is important, cardio-metabolic complications are more closely associated with central adiposity[6]. Central adipose tissue, in particular the visceral fat surrounding the internal organs, has been linked to whole body metabolic dysregulation, and may be intrinsically toxic to vascular health [7,8]. Unfortunately, obesity is typically classified using the body mass index (BMI), which does not and cannot distinguish adipose type and distribution [5]. Accurate (validity), precise (reliability), cost-effective, and simple tools for classifying central adiposity are therefore likely to be advantageous in the fight against the obesity global health crisis.

Central adiposity can be assessed with a high degree of accuracy and precision using computed tomography (CT) [9,10,11] or magnetic resonance imaging (MRI) [12,13], however these imaging modalities require highly technical equipment, are expensive to set-up and operate, and need skilled technicians. Alternatively, Brightness-mode (B-mode) ultrasound has been demonstrated to be reliable and has been well-validated against CT [9,10,11] and MRI [12,13]. Perhaps more importantly, ultrasound is relatively inexpensive and can be portable, making this apparatus suitable for wide-spread use, including in clinical settings and low- and middle-income countries. Guidelines including standard operating procedures for the measurement of central obesity using B-mode ultrasound have recently been published to facilitate the adoption of standardised ultrasound-derived measurements [14].

The most widely reported indicator of central adiposity is intra-abdominal thickness (IAT), a marker of visceral fat which has typically been defined as the distance from the linea alba to the anterior aorta, 2 cm above the umbilicus on the xiphoumbilical line [11,15,16]. However, we recently reported [14] that IAT assessments are more reliable if measured between the linea alba and the anterior aspect of the vertebral column – which can be visualized in the same scan using a linear-array transducer. Unlike curved-array transducers, linear array transducers emit an equal number of scan lines at all penetration levels, ensuring consistent lateral resolution [17], potentially permitting more reliable discernment of the vertebral column. Just as important, abdominal subcutaneous fat thickness (SFT) assessments have been reported to be more reliable if made using a linear-array transducer; SFT is required to calculate maximal abdominal ratio (MAR, SFT: IAT) [14]. Patients with a low SFT compared to IFT (low MAR) may not be able to accommodate excess triglycerides and thus prevent the flow of lipid to into the visceral depot and non-adipose tissues [18,19]. Use of a linear-array transducer may permit quicker and more reliable assessments of IAT as well as MAR.

The purpose of the current study was twofold: (i) compare the reliability of B-mode ultrasound-derived recording of IAT and MAR using linear- and curved-array transducers to identify the anterior aspect of the vertebral column as the deep landmark of interest; and (ii) use these findings to inform guidelines and standardized operation procedures for the measurement of central adiposity.

METHODS

Participants and Study Design

Fifteen healthy adults [27 y (SD 10), 60% female], with a range of somatotypes (BMI: 24 (SD 4), range: 19 - 33 kg/m^2; waist circumference: 75 (SD: 11), range: 61 – 96 cm] were tested on three different, non-consecutive days over one week. To minimize the influence of extraneous factors on the abdominal cavity, participants were tested between the hours of 7am and 10am in a fasted state, having refrained from caffeine, alcohol or strenuous exercise for 24 hours. Informed consent was obtained in accordance with the requirements of the XXX University Human Ethics Committee.

Central Adiposity Measurements

B-mode ultrasound measurements (Figure 1) were made using a Terason T3200 (Teratech Corp., Burlington, MA) equipped with a 4-15 MHz linear array transducer and a 1-6 MHz curved array transducer following recently published guidelines on the measurement of central adiposity using B-mode ultrasound [14].

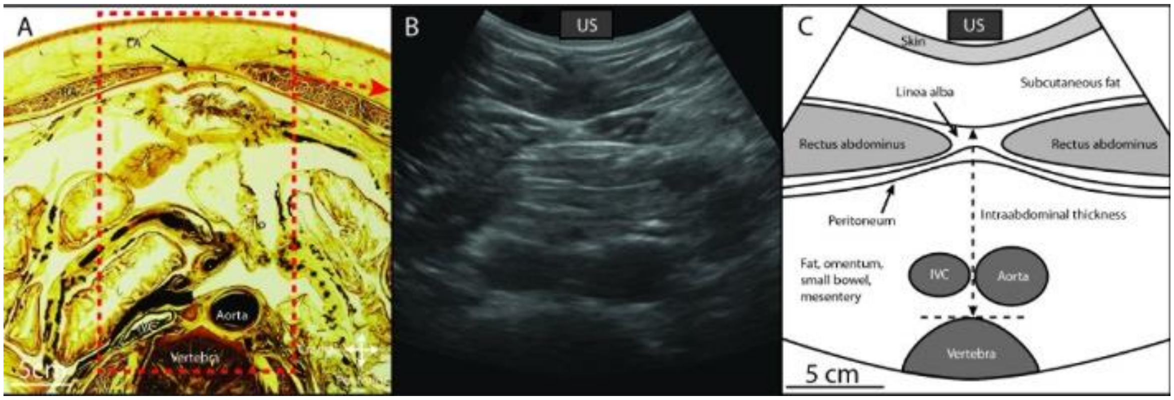

Figure 1.

After 10 minutes supine rest, a single operator (Geoffron) made six scans with each transducer: three to the depth of the vertebral column (Figures 2A and 2B),

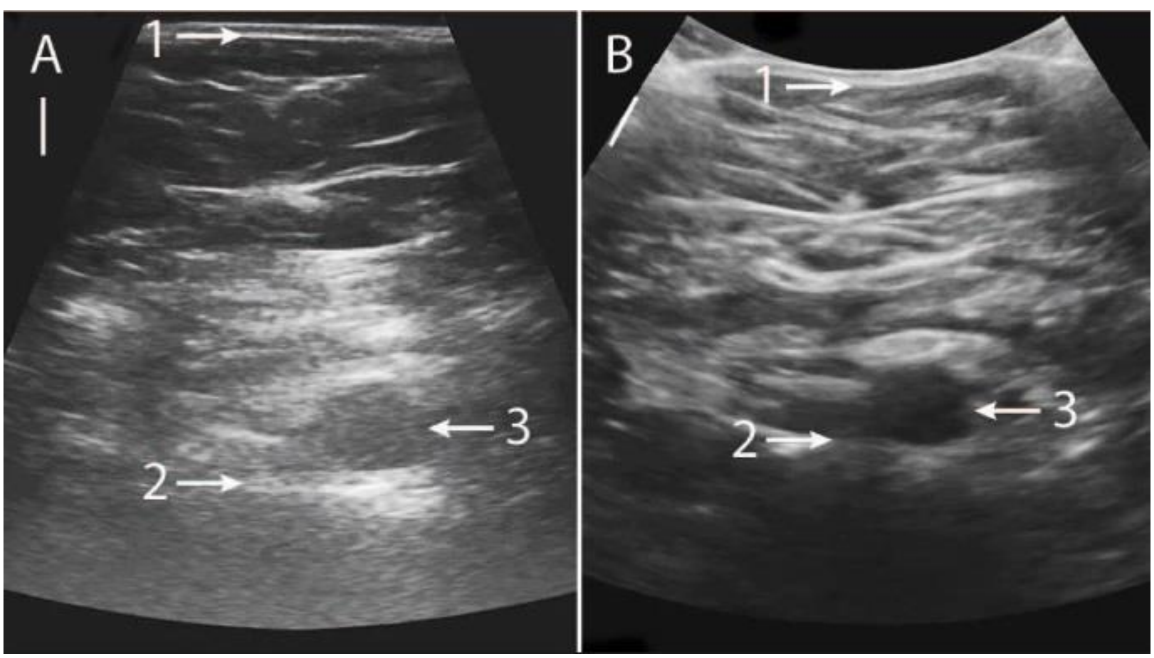

Figure 2.

and then three to the depth of the linea alba (Figures 3A and 3B).

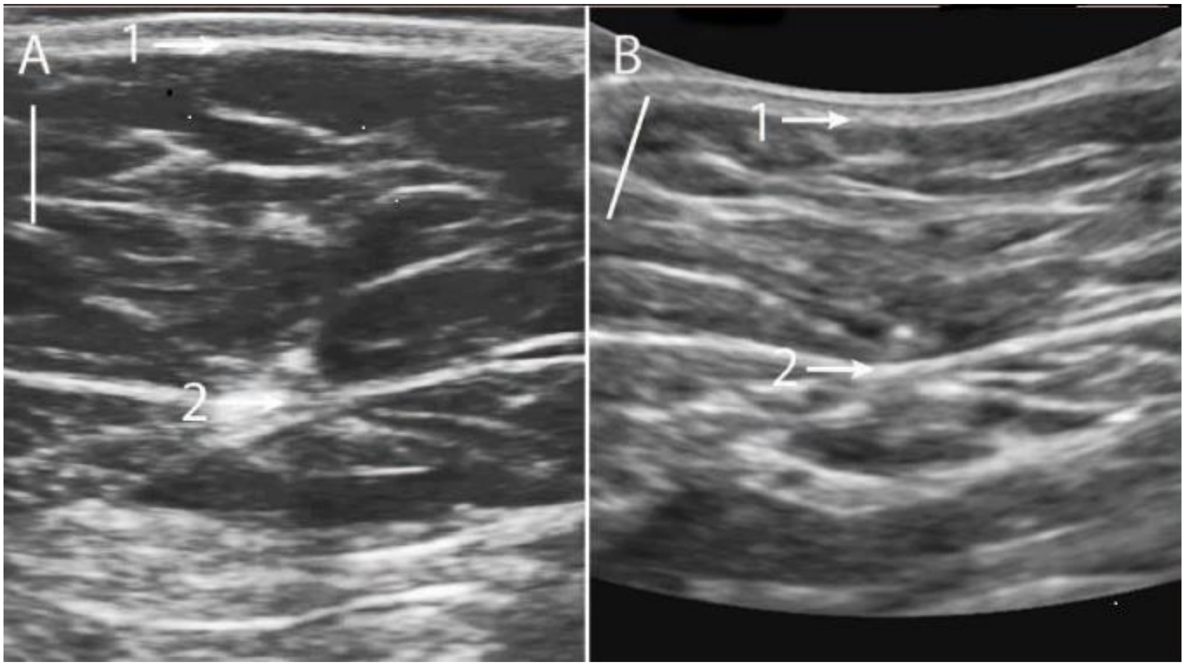

Figure 3.

The transducer was placed 2 cm above the umbilicus on the xiphoumbilical line (transverse plane). Each scan was recorded as a 10 second high resolution video clip, during which the participants were asked to position their hands over their heads and breath-hold following quiet exhalation. A copious amount of ultrasound gel was applied to the transducer and care was taken to exert minimal transducer pressure so as not to displace abdominal contents and affect measurement. Magnification and focal zone settings were adjusted to optimize the boundaries of interest, and the settings recorded to enable replication between visits. All other ultrasound global and transducer-dependent settings were standardized.

Intra-Abdominal Fat Thickness

IAT was measured using both transducers. The distance to the linea alba was measured using the scans taken to the depth of the linea alba (Figures 3A and 3B). The distance to the anterior aspect of the vertebral column was measured using the scans taken to the depth of the vertebral column (Figures 2A and 2B). IAT was calculated by subtracting the two distances.

Abdominal Subcutaneous Fat Thickness

SFT was measured with the linear-array transducer using the scans taken to the depth of the linea alba (Figure 3A and 3B). The distance to the fat-skin interface and linea alba were independently measured, and SFT calculated by subtracting the two measurements.

Maximum Abdominal Ratio

MAR was defined as the ratio of IAT to SFT, with SFT measured using a linear array transducer in accordance with previously reported recommendations [16].

Image Analysis

The ultrasound scans were directly captured as high definition (1920*1080) AVI clips using screen capture software (liteCam HD, Rsupport Co., Seoul, Korea). Using open source video editor software (Avidemux v2.6.8, Avidemux, Paris, France), three diastolic images were extracted per AVI file. The images were extracted during diastole to ensure consistent displacement of the abdominal cavity. Measurements were made on the JPEG images using open source image analysis software (ImageJ, National Institutes of Health, MD). All images, for each measurement day, were analyzed by the ultrasound operator (Geoffron). To determine between-observer reliability one set (one day) of images per participant was measured by three observers (Geoffron, Stoner, Gram); the operator/observer (Geoffron) extracted a set of images for each participant and did not provide the other two operators (Stoner, Gram) with the identity of the participant.

Data analysis

Statistical analyses were performed using Statistical Package for Social Sciences version 21 (SPSS Inc., Chicago, Il). All data are reported as means (SD), unless otherwise specified. Statistical significance was defined as $p<0.05$ (two tailed). Between-day and between-observer reliability of parameters was assessed by calculating the intra-class correlation coefficient (ICC), standard error of measurement (SEM), and reproducibility coefficient (RC). The ICC was calculated according to the formula: SD_b^2 / SD_b^2+SD_w^2, where SD_b^2 and SD_w^2 are the between and within-subject variance. In general, ICC values above 0.75 are considered to indicate excellent reproducibility [20]. The reproducibility coefficient (RC) is defined as the critical difference in a parameter that must be exceeded between two sequential results in order for a statistically significant change to occur in an individual [21]. Absolute RC was calculated using the formula: 1.96 x SEM x √2, where 1.96 corresponds to 95% confidence interval, and SEM was calculated using the equation: SD_b* √(1-ICC). The RC was also expressed as a percentage of mean (RC%).

Agreement between methodologies (linear- vs. curved-array) was assessed by measuring the Pearson's product-moment correlation coefficient. The uniformity of error was assessed by visual analysis of regression plots, and the standard error of estimate (SEE) was derived from the regression analysis to provide an estimation of random error [22]. In addition, the SSE was divided by the SD of the criterion (curved-array) to provide a standardized indicator of error, whereby <0.20 is considered a trivial difference, 0.2-0.6 small, 0.6-1.2 moderate, 1.2-2.0 large, and >2.0 very large difference. The relative standard error (RSE) was also calculated by expressing SSE relative to the mean of the criterion.

RESULTS

Intra-Abdominal Fat Thickness

The criterion ICC of 0.75 was exceeded for measurements obtained using both transducers (Table 1), and the RC% values were comparable. Measurements made to the linea alba and vertebra were also comparable for the two transducers. The SEE indicated the difference between the two measurements was small, and the correlation plot (Figure 4A) indicated no systematic bias given the findings of an intercept of near zero and random scatter along the line of identity.

MAXIMUM ABDOMINAL RATIO

Similar to intra-abdominal fat thickness, the criterion ICC of 0.75 was exceeded for measurements obtained using both transducers (Table 1), with RC% values being comparable. The SEE indicated the difference between the two measurements was small, and the correlation plot (Figure 4B) indicated no systematic bias.

DISCUSSION

Our findings suggest that B-mode ultrasound assessments of IAT and MAR can be obtained with comparable reliability and precision using either linear- or curved-array transducers. We previously reported that SFT measurements are more reliable when obtained with a linear-array transducer [14]; as SFT is required to calculate MAR, these findings support the use of a linear-array transducer to simultaneously and reliably obtain all (IAT, SFT, MAR) measurements to simplify the technical process of image acquisition.

TECHNICAL CONSIDERATIONS

Prior to discussing the merits of the current study, a number of technical considerations should be considered. This study assessed the precision (reliability) but not the accuracy (validity) of ultrasound-central adiposity parameters, however a number of previous studies have demonstrated these parameters to be accurate [9,10,11,13,23] and our findings therefore have validity in the context of the current literature. In addition, to determine the upper-limit of reliability for this procedure, this study assessed relatively young, healthy individuals. We did recruit a range of somatotypes, with the BMI of the recruited participants ranging from 19 kg/m^2 (normal weight) to 33 kg/m^2 (obese), waist circumference from 61 cm to 96 cm, and IAT from 25 mm to 87 mm. Further, it was found that neither BMI nor waist circumference were associated with the residual error of the measurements (data not shown), suggesting that ultrasound-derived measurements are suitable for a range of somatotypes. Nonetheless, future studies are warranted to determine the specific influence of BMI and waist circumference on the reliability of IAT measurements, and to determine whether reliable measurements can be obtained in persons with a BMI greater than 33 kg/m^2. Further, it is recommended that clinicians and research academics determine the reliability of measurements collected in their specific setting.

INTRA-ABDOMINAL FAT THICKNESS

The between-day ICC values observed for IAT measurements made with the linear array (0.96) and curved array (0.97) transducers were comparable to the value we previously reported using a curved-array transducer (0.96), and similar to the value (0.97) previously reported by Bazzocchi et al [16]. Furthermore, the SEM values indicated that both the linear-array (3.17 mm) and curved-array (3.04 mm) transducers provide similar levels of precision.

The between-observer SEM values were excellent for both linear-array (0.50 mm) and curved-array (0.63 mm) transducers, and the absolute SEE (4.44 mm) for the between-transducer comparison was small. While it is recommended that the same operator and same transducer are used for a given study or patient, these findings suggest that comparisons can be made between clinics or between sites for multi-site studies, even if different operators or transducers are used. However, it should be recognised that while the two transducers provided similar measurement values, the same ultrasound device was used and it is therefore unclear whether similar reliability exists when more than one machine is used.

Intra-abdominal fat thickness independent of age, sex, and BMI has been found to be a significant predictor of the presence of metabolic syndrome [23], and a significant marker of CVD in both sexes [11]. Using measurements made to the posterior aorta, cut off values of 7 and 9 cm successfully differentiated men at moderate and high risk of CVD, with corresponding values for women of 7 and 8 cm [11]. Based on findings from the current study, the RC values for both transducers (8.4 – 8.8 mm) indicate that, for a given individual, a difference of less than 1 cm can be detected between visits. Therefore, while further study is warranted to determine optimal cut-off points for measurements made to the anterior aspect of the vertebral column, there is real potential for clinical application of ultrasound-derived measurements to this anatomical landmark.

MAXIMUM ABDOMINAL RATIO

The between-day ICC values observed for MAR measurements made with the linear-array (0.95) and curved-array (0.95) transducers were comparable to the value we previously reported using a curved-array transducer (0.99), and superior to the value (0.83) previously reported by Bazzocchi et al [16]. Furthermore, the between-day SEM values (0.11 – 0.12) indicate similar levels of precision, the between-observer SEM values (0.01) were trivial, and the absolute SEE (0.04) for the between-transducer comparison was small.

While further research is required to determine optimal cut-points, MAR may provide important information in addition to IAT. While it is accepted that visceral adipose tissue is a major risk factor for cardio-metabolic complications, the role of subcutaneous adipose tissue remains controversial [6,24]: subcutaneous adipose tissue can accommodate excess triglycerides and thus prevent the flow of lipid into the visceral depot and non-adipose tissues [18], and patients with a relatively high proportion of visceral adipose tissue have been demonstrated to exhibit adverse metabolic profiles [18]. The clinical importance of subcutaneous adipose tissue therefore requires further scrutiny to clarify its impact on cardio-metabolic disorders.

GUIDELINES FOR MEASUREMENT OF CENTRAL ADIPOSITY

Based on the current observations, either linear- or curved-array transducers can reliably be utilised for the measurement of IAT using the anterior aspect of the vertebral column as a deep landmark of interest. However while the linear-array transducer did not provide more reliable measurements, there is reason to suggest preferential use of a linear- rather than curved-array transducer. This is because of the better reliability of this transducer type in other measures of central obesity [14] and the fact that all measurements (IAT, SFT, MAR) can be taken with this transducer, thereby removing the necessity to change between transducer types while undertaking scanning procedures. Based on the observations from the current study previously published guidelines [14] for measuring IAT, SFT and MAR with B-mode ultrasound have been amended and updated (Table 3).

CONCLUSIONS

While the linear- and curved-array transducers provided equally reliable measurement of IAT, previous research and our technical observations support utilisation of a single linear-array transducer for assessment of IAT, SFT and MAR. Based on these observations the recently published guidelines for the measurement of central adiposity using B-mode ultrasound have been updated, with these modifications providing further evidence-based support and development of a framework that facilitates more reliable clinical and investigative application of ultrasound-derived measurements of central adiposity.

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FIGURE LEGENDS

Figure 1. Descriptions of anatomy, images from ultrasound scans and schematic diagrams for measurement of central adiposity, with columns (a) E12 sheet plastinated section (cadaver), (b) ultrasound scan, and (c) schematic diagram of the ultrasound scan. The ultrasound transducer is placed 2 cm above the umbilicus at the xiphoumbilical line. Red box in column (a) indicates an outline of the area of interest. Abbreviations: US-ultrasound transducer; LA- linea alba; RA- rectus abdominus; IVC- inferior vena cava.

(double column)

Figure 2. Exemplar ultrasound scans for measurement of intra-abdominal thickness. Linear- (A) and curved-array (B) transducer scans to optimize the anterior aspect of the vertebra column.

1. Fat-skin interface; 2. Anterior border of the vertebra; 3. Abdominal aorta. Scale bars – 1 cm depth.

(1.5 column)

Figure 3. Exemplar ultrasound scans for measurement of maximum subcutaneous fat thickness. Linear- (A) and curved-array (B) transducer scans to optimize the fat-skin interface and the linae alba.

1. Fat-skin interface; 2. Linea alba. Scale bars – 1 cm depth.

(1.5 column recommended)

Figure 4. Correlation of linear- and curved-array measurements of intra-abdominal fat thickness

Line of best fit (solid line) and perfect agreement (dashed line) are shown.

(single column recommend)

TABLE LEGENDS

Table 1. Between-day reliability of central adiposity parameters.

Abbreviations: IAT- intra-abdominal thickness, MAR- maximum abdominal ratio, (SFT/IAT); SFT- abdominal subcutaneous fat thickness, (SD)- standard deviation; CV- coefficient of variation; ICC- intra-class correlation coefficient; SEM- standard error of measurement; RC- reproducibility coefficient ; RC%- reproducibility coefficient expressed as a percentage of the mean.

Table 2. Comparison of linear- and curved-array central adiposity measurements

Abbreviations: linae alba; IAT- intra-abdominal thickness, MAR- maximum abdominal ratio, (SFT/IAT)

Table 3. Guidelines for assessing central adiposity with B-mode ultrasound

Table 1

				Between-Day					Between-Observer				
		X	SD	CV	ICC	SEM	RC	RC%	CV	ICC	SEM	RC	RC%
Linear	Skin (mm)	2.75	0.44	7.30	0.83	0.18	0.51	18.4	3.95	0.94	0.10	0.28	10.6
	LA (mm)	22.7	6.88	4.10	0.98	0.92	2.56	11.3	1.47	1.00	0.34	0.93	4.07
	Vertebra (mm)	71.5	18.3	3.82	0.98	2.71	7.50	10.5	0.73	1.00	0.52	1.43	2.02
	SFT (mm)	20.0	6.72	4.59	0.98	0.91	2.51	12.6	1.56	1.00	0.32	0.87	4.33
	IAT (mm)	48.8	15.7	6.35	0.96	3.04	8.42	17.3	1.30	1.00	0.63	1.73	3.59
	MAR	0.45	0.19	9.37	0.95	0.04	0.11	25.4	2.72	1.00	0.01	0.03	7.53
Curved	LA (mm)	21.4	7.63	4.83	0.98	1.02	2.83	13.3	1.51	1.00	0.31	0.86	4.17
	Vertebra (mm)	72.0	21.0	4.17	0.98	2.97	8.23	11.4	0.60	1.00	0.43	1.19	1.67
	IAT (mm)	50.6	17.7	6.37	0.97	3.17	8.80	17.4	0.86	1.00	0.43	1.20	2.38
	MAR	0.44	0.20	9.92	0.95	0.04	0.12	26.8	2.26	1.00	0.01	0.03	6.25

Table 2

	Linear	Curved	Mean Diff.	*r*	SEE Absol.	SSE Stand.	RSE %
LA (mm)	22.6	21.2	1.32	0.97	1.85	0.27	8.20
Vertebra (mm)	72.2	72.7	-0.50	0.98	3.63	0.20	5.02
IAT (mm)	49.7	51.3	-1.59	0.97	4.44	0.29	8.94
MAR	0.43	0.43	0.00	0.98	0.04	0.21	8.84

Table 3

Parameter	Recommendations
Participant Preparation	− Ensure participant reports adequately hydrated and overnight fasted, to avoid abdominal swelling. − Avoid exercise during the preceding 24 hrs. − Women should be tested on day 1-7 of the menstrual cycle (i.e., day 7-14 of the ovarian cycle). − Test conducted with subjects in the supine position. − Rest supine for at least 10 mins in a quiet, temperature controlled room at 21 ^{o}C. − For successive tests, subjects should report at the same time of day to reduce error associated with circadian variation, e.g. abdominal swelling
Transducer Selection	− IAT: convex (curved) 6-1 MHz or linear 15-7 MHz. − SFT: linear 15-7 MHz. − The same transducer(s) should be used for all subjects in a given study.
Ultrasound Settings	− Standardize ultrasound global (e.g., acoustic output, gain, dynamic range, gamma, rejection) and transducer-dependent (e.g., edge enhancement, frame averaging, target frame rate) settings.
Transducer Placement	− 2 cm above the center of umbilicus, transverse plane. − Place transducer perpendicular to the skin, i.e., avoid angling the transducer. − Mark anatomical placement for studies with repeated measurements.
Imaging	− IAT: Posterior edge of linea alba to anterior aspect of vertebra. Ensure linea alba and vertebra appear in the center of the image. − SFT: Fat-skin interface to linea alba. − Apply copious gel to the transducer and exert minimal pressure to avoid displacement of abdominal contents.
Image Capture	− Participant holds hands over head. − Participant quietly expires and then holds breath to minimize chest movement. − Capture 10 second video while holding breath. − Take 6 scans: 3 to the depth of the linea alba (measurements: skin interface, linea alba), and 3 to the depth of the vertebra (measurement: anterior vertebra).
Image Analysis	− Frame Extraction: use video editing software (e.g., Avidemux, www.avidemux.org) to extract 3 diastolic frames. Ensure diastole by manually monitoring aorta. − Measurement software: use dedicated image processing software (e.g., ImageJ, http://imagej.nih.gov/ij) to enable off-site analysis and multi-site collaboration. − Measurement (linea alba clip): for each frame measure the depth of the fat-skin interface and linea alba, and average across frames. − Measurement (vertebra clip): for each frame measure the depth of the vertebra, and average across frames. − Calculation: IAT (vertebral– linea alba); SFT (linea alba depth – fat-skin interface); MAR (SFT/IAT).