European Journal of Clinical Nutrition (2019) 73:518–523
https://doi.org/10.1038/s41430-018-0085-2

ARTICLE

A-mode and B-mode ultrasound measurement of fat thickness: a cadaver validation study

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Received: 21 August 2017 / Revised: 4 November 2017 / Accepted: 19 December 2017 / Published online: 1 February 2018

Abstract

Background/Objectives With technological advances, there has been a resurgence in ultrasound as a method to measure subcutaneous fat thickness. Despite the increased interest in this methodology, research comparing A-mode and B-mode ultrasound devices is lacking. Subcutaneous fat thickness measured by a low resolution (2.5 MHz) A-mode ultrasound and a high resolution (12 MHz) B-mode ultrasound were compared to the actual fat thickness in dissected cadavers.

Subjects/Methods Subcutaneous fat thickness of six cadavers was measured at the abdomen, thigh, triceps, and calf (plus chest for males and suprailiac for females) with both ultrasound devices before the cadavers were dissected and site-specific thickness was measured.

Results Correlations between both ultrasounds and the dissected measurement exceeded 0.90 at all sites with a few exceptions. At the abdomen, the relationship between the ultrasounds was 0.76, and the B-mode and dissected measurement was also 0.76. The correlation between dissection and A-mode was 0.75 for the suprailiac site, but it was not possible to discern the separation of tissue at this site when using the B-mode device. There were no significant differences ($P > 0.05$) between the devices and the dissected measurement at any of the six sites. The mean difference in fat thickness between A-mode and B-mode was <0.7 mm at all sites except the calf (1.2 mm)

Conclusion With the exception of the suprailiac site, both A-mode and B-mode ultrasound are equally capable of providing measurements of subcutaneous fat thickness with an accuracy of <1 mm at most sites.

Introduction

Ultrasound is traditionally used in clinical settings to provide an image of soft tissue structures, but it can also be used as a body composition tool to measure the thickness of subcutaneous fat. Ultrasound offers several advantages over other methods of body composition assessment. It is less expensive and more portable than hydrodensitometry, the Bod Pod, or dual-energy X-ray absorptiometry (DXA). Hydrodensitometry and the Bod Pod provide whole body measurements only, but ultrasound can provide site-specific measurements. Ultrasound is noninvasive, and unlike DXA there is no ionizing radiation exposure. Measurements with skinfold calipers require pinching, and thus compression of the underlying tissue; in contrast, ultrasound can measure tissue thickness without compression. Furthermore, for measuring subcutaneous fat, the interrater reliability of the ultrasound method is superior to the skinfold method [1].

Ultrasound measurements can be made with amplitude modulation (A-mode) or brightness modulation (B-mode). B-mode scans produce a two-dimensional image of the underlying tissue, while A-mode scans result in a waveform with spikes or peaks at the interface of two different tissues (e.g., where subcutaneous fat and muscle meet). Both A-mode and B-mode ultrasound have been used to measure subcutaneous fat thickness. The technical principles underlying the ultrasound method as well as the technique and measurement procedures for using ultrasound to measure subcutaneous fat thickness have previously been reviewed [2].

As a clinical imaging device, B-mode ultrasound is far more commonplace than A-mode ultrasound; however, B-mode devices are considerably more expensive and require

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more technical experience for successful operation as well as image analysis. For the simple measure of tissue thickness, a peak on a graph is just as useful as an image on a computer screen. Thus, A-mode ultrasound provides a lower cost alternative to B-mode ultrasound for body composition assessment. Using A-mode ultrasound to measure subcutaneous fat thickness is not new. In fact, Booth et al. [3] reported using this method in the mid-1960s. Nevertheless, relative to other body composition assessment methods, ultrasound has not received much attention. However, technological advances, improved portability, new low-cost devices, and user-friendly software designed specifically for measuring subcutaneous fat thickness have led to a resurgence in the interest of ultrasound, especially A-mode ultrasound, as a method of body composition assessment.

A commercially available A-mode ultrasound device that has contributed to the resurgence in this method of body composition assessment is the BodyMetrix BX2000 (IntelaMetrix, Inc., Livermore, CA, USA). This economical ultrasound device attaches to a laptop computer. The associated software (Body View Professional) converts subcutaneous fat thickness measurements from common skinfold site locations into an estimate of total body fat percentage (%BF). The %BF estimates from the BodyMetrix ultrasound have been evaluated against the %BF estimates from skinfolds [1, 4–6], hydrodensitometry [6], air displacement plethysmography [1, 7, 8], DXA [9–12], and a three component model [13]. However, these are fundamentally validation studies of the algorithms or prediction equations used to estimate %BF rather than a validation of what is purported to be measured by the ultrasound method, namely subcutaneous fat thickness. The fat thicknesses obtained by the BodyMetrix device at individual sites have been compared to skinfolds at those sites [1, 5], but this is not an equivalent comparison. Ultrasound measures fat thickness, while skinfolds measure a compressed fold of fat between two layers of skin. A more appropriate validation comparison would include subcutaneous fat thickness measurements at various sites using another imaging device capable of measuring fat thickness, such as B-mode ultrasound. Additionally, a direct measurement of subcutaneous fat from dissected cadavers would further validate thickness measurements obtained from both the BodyMetrix BX2000 and a B-mode ultrasound. Thus, the purpose of this study was to validate the BodyMetrix BX2000 A-mode ultrasound for measuring subcutaneous fat thickness by comparing the values obtained by this device at various body sites to thicknesses obtained by a high-resolution B-mode ultrasound and direct measurements on cadavers.

Methods

Participants and measurement sites

The university's Institutional Review Board approved the study (protocol #7560). The subcutaneous fat thickness of a cohort of 6 cadavers (3 male, 3 female), aged 80.8 ± 8.9 y at the time of death, was measured at four sites (abdomen, thigh, triceps, and calf). Additionally, chest and suprailiac measurements were made on males and females, respectively. All measurements were taken on the right side of the body. These sites were selected as they are commonly used skinfold sites in the equations of Jackson and Pollock [14] and Jackson et al. [15]. The anatomical landmarks of these sites have been previously described and illustrated [16]. Alternative sites specific for ultrasound measurement of subcutaneous fat have recently been recommended [17]. These sites were not used, however, because they require the subject to actively move into various positions, which is not possible with cadavers. Sample size was limited to the number of cadavers available to the university. The cadavers were at the university for just over one month, in their moistened hospital gown, wrapped in plastic, and inside a heavy duty body bag prior to the study. No appreciable desiccation took place.

Procedures

All measurements took place in the university's cadaver laboratory. Measurement sites were marked with a surgical marker. Ultrasound measurements were made with the BodyMetrix BX2000 in A-mode, and the B-mode measurements were made with a NextGen LOGIQ eR7 ultrasound with a 12L-RS linear array transducer with a 38.4 mm field-of-view (GE Healthcare, Milwaukee, WI, USA). The BodyMetrix device operates at 2.5 MHz. The B-mode ultrasound was set at 12 MHz (gain = 50, dynamic range = 72). A thickness value for the A-mode measurements was obtained automatically using the BodyView software that accompanies the BodyMetrix device. This software requires multiple measurements to be taken at each site before providing a thickness value. Three images were taken at each site using the B-mode and saved for later analysis. The image with the clearest adipose-muscle aponeurosis, and thus the most distinct transition between tissues, was measured. The depth of the B-mode was optimized (gain and dynamic range setting adjustments) for adipose tissue resolution at each site and cadaver. A generous amount of water-soluble transmission gel was applied to the skin to enhance acoustic coupling. Gel was wiped off and reapplied when switching from one device to the other. The B-mode used high-resolution images to determine the subcutaneous

Table 1 Means ± SD (mm) for each method at each measurement site

Site and method	Means ± SD	*F*	*P*
Chest[a]		.031	.970
Measured	12.7 ± 2.1		
A-mode	12.7 ± 4.3		
B-mode	12.9 ± 3.6		
Abdomen		.965	.421
Measured	15.4 ± 2.7		
A-mode	16.2 ± 2.5		
B-mode	16.7 ± 3.8		
Triceps		.097	.909
Measured	18.0 ± 10.1		
A-mode	17.4 ± 9.4		
B-mode	17.4 ± 6.4		
Thigh		2.034	.210
Measured	13.7 ± 5.6		
A-mode	12.8 ± 4.9		
B-mode	12.2 ± 4.2		
Suprailiac[b]		.040	.861
Measured	10.0 ± 7.6		
A-mode	10.6 ± 4.1		
B-mode	NM[c]		
Calf		.574	.581
Measured	16.2 ± 11.0		
A-mode	16.0 ± 12.8		
B-mode	14.8 ± 11.3		

[a]Chest measurements are for male cadavers only

[b]Suprailiac measurements are for female cadavers only

[c]NM = No measurement, the technician was not able to determine the thickness of the subcutaneous fat at the suprailiac site using B-mode ultrasound

fat thickness from the clearest image for each site. The manufacturer on-screen calipers were used to attain the thickness measures, which were determined specifically from the superficial epidermis surface to the edge of the muscle aponeurosis. One technician took all of the A-mode measurements, while another experienced technician took all of the B-mode measurements. Technicians were blinded to each other's results. Following the ultrasound measurements, a third technician dissected the cadavers at the marked sites so that a physical measurement of subcutaneous fat thickness could be made with the ruler portion of a digital caliper (ABS Digimatic, Mitutoyo Corp., Aurora, IL, USA). The epidermis and dermis were included in the thickness comparisons of both ultrasounds and the physical measurement. All of the measurements on an individual cadaver were completed in a single session; all 6 cadavers were studied over a 2-day period.

Statistical analyses

Means and standard deviations were calculated for each measurement. Mean differences between the A-mode, B-mode, and the physical measurement were evaluated by repeated-measures ANOVA. Pearson correlation coefficients were used to describe the relationship between methods, and intraclass correlation coefficients (ICC) were used to describe the absolute agreement among methods. Considering the small sample size and that the study was underpowered to adequately rely on the previously mentioned statistical analyses, plots for each subject were also constructed to visually inspect the data. Analyses utilized SPSS version 24 (IBM, Inc., Armonk, NY, USA), and plots were constructed using the ggplot2 package in R 3.3.3 (Foundation for Statistical Computing, Vienna, Austria).

Results

Due to the anatomical structure of the body region, the technician was unable to confidently discern the subcutaneous fat thickness for the suprailiac site using the B-mode ultrasound; thus, there are no B-mode data for that site. The means and standard deviations for each method at each measurement site are in Table 1. A 4 × 3 two-way repeated measures ANOVA yielded no significant effect for the site ($P = 0.630$), the method ($P = 0.970$), nor their interaction ($P = 0.217$). There were no significant differences between methods ($P > 0.05$). The mean difference in fat thickness between A-mode and B-mode was <0.7 mm at all measured sites, with the exception of the calf at 1.2 mm. Pearson correlations between all three pairs of methods were ≥0.90 at nearly all of the sites (Table 2). The weakest relationship between methods occurred at the suprailiac and abdomen. The ICC was >0.90 at all measurement sites except the suprailiac.

Individual measurements are represented in Fig. 1. Visual inspection of the data reveals no noticeable pattern of one method consistently overestimating or underestimating the fat thickness relative to the other methods. Additionally, there is no noticeable pattern for a particular measurement site or for an individual. Some inter-individual variability in the tightness of agreement among the three measurement methods exists. For example, the variability among the three methods is small for Male-1 and Male-2, with <2 mm difference across all three methods at nearly every site. However, the spread between the three methods is ≥5 mm at three sites for Female-2.

Table 2 Intraclass correlation coefficients (ICC) for agreement between dissected measured value, A-mode, and B-mode (left side of the table)

		A-mode	B-mode
Chest	Measured	.913	.892
ICC = .962	A-mode		.999
Abdomen	Measured	.970	.764
ICC = .911	A-mode		.757
Triceps	Measured	.968	.948
ICC = .970	A-mode		.912
Thigh	Measured	.998	.921
ICC = .973	A-mode		.926
Suprailiac	Measured	.752	NM[a]
ICC = .834	A-mode		NM[a]
Calf	Measured	.974	.954
ICC = .987	A-mode		.966

Pearson correlation coefficients (right side of the table)

[a]NM = Not measured; the technician was not able to determine the thickness of the subcutaneous fat at the suprailiac site using B-mode ultrasound

Discussion

At least 11 studies have evaluated the BodyMetrix BX2000 prediction algorithms to estimate total %BF against other body composition methods with equivocal results [1, 4–13]. Rather than evaluate the prediction algorithms, the purpose of the present study was to assess the ability of this A-mode ultrasound device to measure the thickness of subcutaneous fat at various individual sites. There was close agreement, with few exceptions, between the BodyMetrix BX2000 A-mode device, a high-resolution B-mode ultrasound, and a ruler measurement of actual fat thickness from dissected cadavers. The greatest divergence among measurement results occurred in Female-2, and this subject also had the largest subcutaneous fat thicknesses. Given the small sample size, it is not possible to conclusively determine if larger fat thicknesses necessarily result in larger errors (heteroscedasticity) or if this was just happenstance, but heteroscedasticity could be a topic of inquiry for future studies with larger, heterogeneous samples. Additionally, for this particular subject, the A-mode and dissected measurement

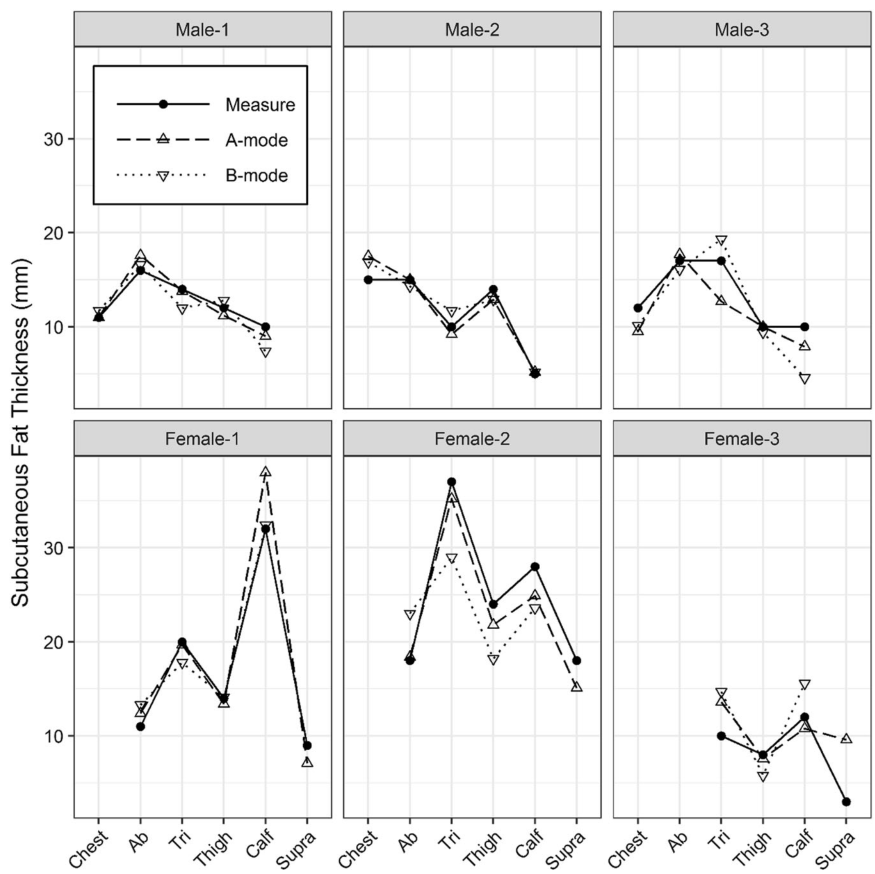

Fig. 1 Comparison of subcutaneous fat thicknesses for each method at each measurement site on each cadaver. Chest measurements were limited to the male cadavers, and suprailiac measurements to the female cadavers. B-mode measurements were unavailable at the suprailiac site due to challenges identifying the underlying structures. *Ab* abdomen, *Tri* triceps, *Supra* Suprailiac

were reasonably similar at each site; thus, it could be that there is greater difficulty interpreting the B-mode images as subcutaneous fat thickness increases.

Although this is the first study to compare the BodyMetrix ultrasound to a B-mode ultrasound and cadavers, previous investigators made these comparisons using other A-mode ultrasounds in the mid-1980s. Jones et al. [18] reported excellent agreement between a 5 MHz transducer in A-mode and depth gauge measurements of subcutaneous adipose tissue at 24 sites on a single cadaver. The correlation between the 24 paired measurements was $r = 0.99$ with a standard error of estimate of ±0.65 mm. This bodes well for the validity of the A-mode ultrasound method. In contrast, comparisons of older A-mode ultrasounds to B-mode ultrasounds resulted in poor agreement. Weiss and Clark reported only moderate correlations of 0.39–0.63 between ultrasound modes for measurements of anterior and posterior upper arm sites [19] and 0.50–0.91 for leg sites [20]. Furthermore, the mean adipose thicknesses from the B-mode were significantly smaller than the values from the A-mode. However, they noted that there was frequent "guesswork" by the operator interpreting the A-mode signal because multiple light-emitting diodes, rather than a single one, would appear at a tissue interface making it difficult to identify the fat-muscle interface. Thirty years later, the averaging technique (moving the transducer head slightly above and below the measurement point several times) and the body composition-specific software that accompanies the BodyMetrix device largely eliminates "guesswork" interpreting the scan. However, it is still possible to select the wrong peak as the fat-muscle interface as fibrous tissue can confound the interpretation. Müller et al. [21] described novel ultrasound imaging software that identifies and filters out embedded structures within the subcutaneous fat layer. Presently, this software is specific to analysis of images from high resolution B-mode ultrasound. Thus, a limitation of A-mode ultrasound is that fibrous tissue embedded within the subcutaneous fat layer could create a spike or peak that could be misinterpreted as the fat-muscle interface.

The suprailiac site was the most difficult site to measure with ultrasound. This was the only site of the six measured that did not have a correlation > 0.90 between the A-mode and dissected measurement. The scan went unmeasured at this site with B-mode ultrasound because it was too difficult to discern the underlying tissues. Similarly, Müller et al. [21], in their study to develop ultrasound software to measure subcutaneous adipose tissue, noted the iliac crest, along with the supraspinale and abdomen sites, accounted for the majority of images that could not be measured due to difficulty identifying the structures. Consequently, the suprailiac site is not included among the newly recommended sites for ultrasound measurement of subcutaneous fat [17]. In short, the sites that are commonly used for skinfold measurements are not necessarily the best sites for measuring subcutaneous fat using the ultrasound method because they do not allow for the clearest image of the underlying tissue [17, 21].

Directly measuring the subcutaneous fat thickness on cadavers provides a certain amount of concurrent validity that is not typically available in body composition research, and it is implied that this measurement serves as the criterion. However, the subcutaneous fat layer is not uniform. Additionally, the plasticity of fat adds to the difficulty of getting an accurate physical measurement of subcutaneous fat thickness. Müller et al. [21] acknowledged this point when describing the difficulty of comparing caliper measurements to ultrasound measurements on excised pig tissue. Thus, our ability to accurately measure the dissected subcutaneous fat layer was limited by the plasticity and irregularity of the tissue. This likely contributed as much to the error or difference between methods as either ultrasound device.

The imaging capability and higher resolution make the B-mode ultrasound a more versatile instrument than the A-mode ultrasound. Additionally, software has been developed to be used with high-resolution B-mode ultrasound that recognizes structures such as fibrous tissue embedded within the subcutaneous fat layer, allowing fat thickness to be measured with these fibrous tissues included or excluded [21]. However, the cost differential between A-mode and B-mode ultrasounds is considerable. For example, in the present study, the BodyMetrix BX2000 was less than 6% of the cost of the B-mode ultrasound. Therefore, if ultrasound will be used for a variety of clinical applications in which high-resolution imaging is important then B-mode ultrasound is needed. However, if the sole purpose is to measure subcutaneous fat thickness, the BodyMetrix BX2000 A-mode ultrasound provides a measurement similar to B-mode ultrasound for a fraction of the cost.

In summary, both A-mode and B-mode ultrasound are equally capable of providing measurements of subcutaneous fat thickness with an accuracy of <1 mm at most sites. The suprailiac site, which is a common skinfold site, is not recommended for ultrasound measurement because it is difficult to discern structures. The BodyMetrix BX2000 provides a measurement of subcutaneous fat thickness that is similar to B-mode ultrasound and the dissected measurement at individual sites. More research is needed to determine if the manufacturer-provided prediction equations to convert these individual site measurements into total % BF are valid.

Conflict of interest The authors declare that they have no competing interests.

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