Introduction

A measurement of subcutaneous fat is useful for individuals trying to alter their body composition for health, performance, or appearance. Both skinfold calipers and ultrasound can provide measures of subcutaneous fat; however, the calipers give an indirect measure of a fold of compressed fat sandwiched between two layers of skin, and ultrasound offers a direct measure of uncompressed fat thickness (Himes et al. 1979; Müller et al. 2013; Ackland and Müller 2018). The technical principles, procedures, advantages and disadvantages for using ultrasound to measure subcutaneous fat thickness have been reviewed by Wagner (2013). Although ultrasound has been used to measure subcutaneous fat thickness since the 1960s (Booth et al. 1966), it has received little attention as a body composition assessment method until recently. A search of the Scopus database, using the combined search terms of “body composition,” “subcutaneous fat” and “ultrasound” revealed that 44% of all published research on this topic has occurred in just the past 6 y. Improvements in portability and technology combined with user-friendly software have likely fueled this resurgence in the interest of ultrasound as a body composition assessment method.

Ultrasound can be measured with either amplitude modulation (A-mode) or brightness modulation (B-mode). A-mode consists of an x-axis representing depth and a y-axis representing amplitude. A graphical spike in the amplitude of the ultrasound wave at the interface between two different tissues (e.g., subcutaneous fat and muscle) is displayed during A-mode scans (Fig. 1a). In contrast, there are no vertical spikes with B-mode ultrasound, rather a 2-D image of the underlying tissues is mapped. The brightness of the image will change depending on the amplitude or intensity of the ultrasound echo. Thus, the change in brightness indicates a tissue interface (Fig. 1b). Considering the value of viewing an image, there are more clinical applications for B-mode ultrasound than for A-mode ultrasound. However, B-mode ultrasounds are considerably more expensive than A-mode devices, and the interpretation of the
results require more technician skill, training and time.

One device that has contributed to the increased research of ultrasound for body composition assessment is the BodyMetrix BX2000 (IntelaMetrix, Inc., Livermore, CA). Unlike traditional ultrasound machines that have many clinical imaging applications, this low-cost, low-resolution, A-mode ultrasound scanner with accompanying proprietary software was designed and marketed solely for the measurement of subcutaneous fat thickness. Although it has a singular use, the BX2000 is small, portable and less than 5% of the cost of high-resolution B-mode ultrasound machines. Thus, for the fitness professional or clinician who wants to use ultrasound to assess subcutaneous fat, the BodyMetrix BX2000 is an appealing option if it proves to be valid.

Research indicates excellent test-retest reliability (Loenneke et al. 2014; Smith-Ryan et al. 2014; Wagner et al. 2016) and inter-rater reliability (Wagner et al. 2016) for measuring subcutaneous fat thickness at various sites using the BodyMetrix BX2000. However, reliability does not equal validity, and before the BX2000 can be recommended as a body composition assessment tool this low-resolution ultrasound device should be compared with a high-resolution imaging device. We recently made that comparison by pitting the BX2000 against a high-resolution B-mode ultrasound and manually measured dissected thicknesses on six moist human cadavers (Wagner et al. 2019). Correlation coefficients between both ultrasound devices and the dissected measurement exceeded 0.90 at nearly all measurement sites, and there were no significant differences ($p > 0.05$) between the devices and the dissected measurement at any of the sites. Furthermore, the mean difference in fat thickness between the A-mode and B-mode was $<0.7$ mm at all sites except the calf (1.2 mm). Although the direct measurements of subcutaneous fat thicknesses on the dissected cadavers provided a level of concurrent validity that is not typically available, the small sample size precluded the use of more traditional statistical analyses. Using a larger sample of living humans was the logical follow-up study to our recent cadaver analysis. Thus, the purpose of the present study was to compare the subcutaneous fat thicknesses obtained from the BodyMetrix BX2000 A-mode ultrasound and a high-resolution B-mode ultrasound.

**Materials and Methods**

**Participants**

Adults from the general population older than the age of 18 y were invited to participate without regard to their health status or physical fitness level. Being pregnant or missing limbs were exclusion criteria. Using G*Power 3.1 (Informer Technologies, Inc., Los Angeles, CA), a sample size of 34 was recommended to run a two-tailed dependent $t$-test with an $\alpha$ of 0.05, statistical power of 80% and a medium effect size of $d=0.5$, which is considered an
average effect size in behavioral sciences (Cohen 1988; Sedlmeier and Gigerenzer 1989; Lipsey and Wilson 1993). Our sample size was 40 (20 men, 20 women). The Utah State University Institutional Review Board approved the study (protocol #8249), and participants signed a written informed consent before any data collection.

**Procedures**

All testing occurred in the same laboratory. With participants wearing only shorts and a t-shirt, heights were measured to the nearest 0.1 cm with a Seca 869 digital scale. Each individual’s height, weight and both A-mode and B-mode ultrasound measurements were completed in a single session.

The ultrasound measurement sites corresponded to the skinfold sites used for the Jackson and Pollock (1978) and Jackson et al. (1980) formulas. These sites were selected because of their frequent use in body composition research, and also because the Body View Professional software (IntelaMetrix, Brentwood, CA) associated with the BodyMetrix BX2000 automatically calculates an estimate of body fat percentage (%BF) from these sites, using a proprietary formula. This %BF estimate was valuable to the participants and served as a recruitment tool for participation.

A hypoallergenic surgical marking pen was used to mark the seven sites: chest, midaxilla, triceps, subscapula, abdomen, anterior suprailiac and thigh. The anatomic landmarks for these sites were described and illustrated byHeyward and Wagner (2004). All measurements were taken on the right side of the body. Once marked, a liberal amount of water-soluble gel was placed on both the marked sites and the ultrasound transducer head to minimize tissue compression. The transducer head was essentially hydroplaning across the skin. Gel was wiped off and reapplied when switching from one device to the other.

A-mode measurements were taken with the BodyMetrix BX2000 according to the manufacturer’s guidelines. This includes moving the transducer wand about 1 cm above and below the marked measurement site several times. This ultrasound operates at a set frequency of 2.5 MHz. Subcutaneous fat thickness was obtained with the Body View Professional software that accompanies the BodyMetrix ultrasound. Multiple measurements were taken according to the software prompts.

B-mode measurements were taken using a NextGen LOGIQ eR7 (GE Healthcare, Milwaukee, WI) with a linear array transducer (model 12 L-RS). The frequency was set at 12 MHz. Three images were taken at each site and saved for later analysis. Multiple technicians were used to collect the A-mode and B-mode measurements on the 40 participants; however, for consistency, the same technician interpreted all the B-mode scans and selected the best (clearest transition between tissues) of the three for analysis. The on-screen calipers were used to attain the thickness measures. The technician interpreting the B-mode scans was blinded to the A-mode results. The epidermis and dermis were included in the thickness comparison of both ultrasounds.

**Statistical analyses**

Stata/MP v. 16 (StataCorp LLC, College Station, TX) was used for all analyses. Statistical significance was accepted as $p < 0.05$. The accuracy of the A-mode ultrasound for measuring subcutaneous fat thickness was evaluated against the B-mode ultrasound measurements. The following statistical tests and evaluative criteria were used to make a decision regarding the validity of the A-mode device:

- Equivalence tests of means with 1 mm difference as delta reveal no significant mean difference between the A-mode and B-mode measurements (Schuirmann 1987; Goldstein 1994; Walker and Nowacki 2011),
- Intra-class correlations (ICCs) are excellent (ICC ≥ 0.75) between the two devices (Shrout and Fleiss 1979; Cicchetti 1994; McGraw and Wong 1996), and
- Bland and Altman (1999) plots of residual scores (average of A-mode and B-mode plotted against each participant’s error score) are random with nonsignificant correlation, indicating no systematic bias.

**Results**

A total of 40 volunteers (20 males, 20 females) completed the study. They ranged in age from 20-57 years (29.7 ± 11.1 y) and in body mass index from 17.3–40.2 kg/m² (24.9 ± 4.5 kg/m²). Estimates of %BF were obtained from the BX2000’s proprietary conversion of the Jackson and Pollock (1978) and Jackson et al. (1980) formulas for men and women, respectively. The %BF for the sample ranged from 6.4%–32.4% (19.4 ± 6.6%).

The mean differences in subcutaneous fat thickness between the 2 ultrasound devices were <0.6 mm at all 7 sites. The Table summarizes the analysis of the mean differences. ICCs exceeded 0.75 at all sites. Equivalence tests of means showed no significant differences in fat thickness between the 2 modes at all sites ($p > 0.05$). According to the equivalence plot (Fig. 2), the 2 measurements, A- and B-modes, were found to be equivalent at all sites except at the abdomen. The Bland and Altman (1999) plots of residual scores at each of the 7 measured sites are presented in Figures 3a–g. Correlation coefficients between the error scores and the average scores ranged from ~0.02 to ~0.306.

None of these were statistically significant ($p > 0.05$), indicating that the errors were evenly distributed with no systematic bias. However, careful visual inspection of the plots suggests heteroscedasticity such that the differences between the 2 ultrasound devices tend to increase as the fat thicknesses increase. This is more noticeable on the trunk measurements (e.g., abdomen and suprailiac) and not apparent for the limb sites (e.g., thigh and triceps). The variability between the 2 devices was greatest at the abdomen, the site with the greatest fat thicknesses. The 95% limits of agreement at the abdomen were -6.61 mm to +7.14 mm. In contrast, the 95% limits of agreement were less...
than ± 4 mm at all other measurement sites.

**Fig. 2** Plot for equivalence tests of means for fat thickness measured with A-mode minus B-mode ultrasound devices.
Discussion

The BodyMetrix BX2000 has undergone considerable validity testing since it became commercially available. Estimates of %BF from this device have been compared with %BF estimates from hydrodensitometry (Utter and Hager 2008), air displacement plethysmography (Johnson et al. 2012; Wagner et al. 2016; Schoenfeld et al. 2017), dual-energy X-ray absorptiometry (Johnson et al. 2014, 2017; Loenneke et al. 2014; Ripka et al. 2016; Baranauskas et al. 2017) and a three-component model (Smith-Ryan et al. 2014). However, these validation studies evaluated the algorithms or proprietary formulas within the BX2000 for estimating the total %BF from the ultrasound scans rather than the ability of the device to measure subcutaneous fat thickness. To our knowledge, the only previous study to evaluate the accuracy of this A-mode ultrasound for measuring subcutaneous fat thickness is our recent cadaver analysis (Wagner et al. 2019).

Fig. 3 Bland-Altman plots of error scores (B-mode minus A-mode) for the (a) chest, (b) midaxilla, (c) triceps, (d) subscapula, (e) abdomen, (f) anterior suprailiac and (g) thigh. The constant error presented as solid line. The 95% limits of agreement presented as dotted lines. All values are presented in millimeters.
Results from the present study coincide with results from the cadaver analysis (Wagner et al. 2019). In both studies the mean difference between devices was <0.7 mm, and the correlation coefficients exceeded 0.80 at most sites. It was noted in the cadaver study that the weakest relationship between ultrasound devices occurred at the suprailiac and abdomen. Although the correlation coefficients exceeded 0.80 at both sites in the present study, the variability between devices was greatest at the abdomen, as evidenced by the large 95% limits of agreement at that site (Fig. 3e). At least a portion of this error can likely be attributed to the difficulty of interpreting the B-mode scan at that site as much as an errant measurement by the A-mode ultrasound. In a study to validate specialized software to measure subcutaneous fat thickness when using B-mode ultrasound, Müller et al. (2013) commented that the iliac crest and abdominal areas accounted for the majority of images that could not be interpreted correctly because of the difficulty identifying the underlying structures in these areas. They reported that the evaluations of their 3 examiners differed substantially in 10 of 19 athletes at the abdomen because of the anatomic complexity at this measurement site. Subsequently, this research group recommended alternative measurement sites and were able to improve the intertester reliability to a standard error of the estimate = 0.55 mm for the sum of 8 sites when measuring lean athletes with an 18 MHz B-mode ultrasound and proprietary software (Müller et al. 2016). Even with this improved measurement strategy, the greatest variability among the 8 sites measured was at the lower abdomen. Thus, the finding in the present study that the greatest variability between devices occurred at the abdomen is not surprising.

In addition to greater variability at the trunk, there also appeared to be heteroscedasticity at the abdomen and suprailiac sites (Figs. 3e and 3f). In contrast, no heteroscedasticity was apparent at the limbs. This dichotomy between trunk and limb errors is understandable. Again, the difficulty of interpreting images from the trunk relative to the limbs likely contributed to this result. In addition, the largest fat thicknesses were measured at the abdomen. Thus, it is logical that the site producing the largest values would be at most risk for a heteroscedasticity error.

This study was limited such that all the participants were lean to average in body fatness and young to middle-aged adults. The fattest participant had a %BF of 32.4%. This value is at the upper end of acceptable for females by some experts (Lohman et al. 1997). The oldest participant was 57 y of age. Assuming that ultrasound is an alternative to the skinfold method (Ackland and Müller 2018), we selected a sample that spanned the range of the acceptable limits for the skinfold method. The skinfold method is not recommended for obese clients with large folds or for older clients with loose connective tissue (Heyward and Wagner 2004). Consequently, the results of the present study are limited to nonobese, nonelderly adults. Little is known about the accuracy of the BodyMetrix BX2000 on an obese population. Smith-Ryan et al. (2014) reported that the device was reliable but underpredicted the %BF of 47 overweight and obese participants. However, this was a study of total %BF and evaluated the accuracy of the predictive algorithm rather than fat thickness at individual sites. Thus, this is an area of research that is still lacking. Likewise, there is a dearth of research regarding the validity of the ultrasound method on an older population.

Although only one researcher interpreted the B-mode ultrasound scan, multiple technicians took both the A-mode and B-mode ultrasound measurements. Research documents excellent interrater reliability specific to the BX2000 device (ICC = 0.987, 95% confidence interval of 0.976-0.993) (Wagner et al. 2016). Nevertheless, inter-rater reliability was not assessed in the present study, and multiple examiners is another potential study limitation. This could have contributed to some of the sex-specific variability in the data with consistently higher ICCs for men compared with women at each measurement site (Table), as male technicians measured male participants and female technicians measured female participants. Whether the ultrasound method, and more specifically the BX2000, has greater validity in males than in females requires more research.

<table>
<thead>
<tr>
<th>Site</th>
<th>Mean difference* (mm)</th>
<th>Intraclass correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chest</td>
<td>-0.27</td>
<td>.947</td>
</tr>
<tr>
<td>Male</td>
<td>-0.71</td>
<td>.987</td>
</tr>
<tr>
<td>Female</td>
<td>0.17</td>
<td>.896</td>
</tr>
<tr>
<td>Subscapula</td>
<td>0.01</td>
<td>.879</td>
</tr>
<tr>
<td>Male</td>
<td>-0.47</td>
<td>.946</td>
</tr>
<tr>
<td>Female</td>
<td>0.49</td>
<td>.817</td>
</tr>
<tr>
<td>Midaxilla</td>
<td>0.42</td>
<td>.763</td>
</tr>
<tr>
<td>Male</td>
<td>-0.28</td>
<td>.968</td>
</tr>
<tr>
<td>Female</td>
<td>1.12</td>
<td>.618</td>
</tr>
<tr>
<td>Triceps</td>
<td>-0.59</td>
<td>.968</td>
</tr>
</tbody>
</table>
Limitations exist with the BodyMetrix device. The fat thickness measurement provided by the software includes the epidermis and dermis. Although skin thickness varies throughout the body, the thickness in the sites measured was likely 1.5–2.0 mm (Bergman et al. 2018). This could be meaningful when measuring very lean individuals. In addition, fibrous structures embedded in the subcutaneous adipose tissue could possibly confound the A-mode measurement. For this reason, some investigators recommend that only high-resolution B-mode ultrasound with proprietary software that can detect embedded structures be used to measure subcutaneous fat thickness (Ackland and Müller 2018). Higher ultrasound frequencies (e.g., 12–18 MHz) allow for clearer images at the expense of limited tissue depth (Wagner 2013). However, because subcutaneous fat is near the body surface, the depth that the ultrasound signal can penetrate is of less importance, particularly when measuring the subcutaneous fat of lean individuals.

Despite these limitations, the low-resolution, A-mode BodyMetrix BX2000 produced measurements of subcutaneous fat thickness at various sites that were similar to a high-resolution B-mode ultrasound, with mean differences between devices <0.7 mm, correlation coefficients >0.80 and evenly distributed residual scores. There was greater variability between devices at trunk sites, specifically the abdomen, than at limb sites. High-resolution B-mode ultrasound with proprietary software capable of excluding embedded structures (Müller et al. 2013, 2016) is becoming the gold standard for the measurement of subcutaneous fat thickness and is the recommended method when possible (Ackland and Müller 2018). However, high-resolution B-mode ultrasound units typically exceed US$30,000. Proprietary software and a 2-d training course are recommended (Ackland and Müller 2018) at an additional US$4,000 and US$1,100, respectively, and this does not include the cost and time associated with travel to the training site. This is cost prohibitive for many independent clinicians who want to assess body composition in field settings or in health and wellness or fitness facilities. Data from the present study, particularly when combined with the recent cadaver analysis study (Wagner et al. 2019), suggest that low-resolution A-mode ultrasound provides measures of subcutaneous fat thickness that are similar to high-resolution B-mode results. This might be an acceptable and feasible alternative to those who do not have access to the more costly B-mode devices.

The comparison between A-mode and B-mode ultrasound is somewhat analogous to the comparison between single-frequency bioelectrical impedance analysis (BIA) and multifrequency BIA. For a simple estimation of total body water of a healthy adult, the lower-cost single-frequency BIA provides an estimation similar to the more costly multifrequency BIA (Cornish et al. 1996). However, the multifrequency BIA provides additional information regarding intracellular versus extracellular water. Similarly, if the goal is a simple measurement of subcutaneous fat thickness, the inexpensive A-mode ultrasound will provide a measurement comparable with a more costly B-mode ultrasound. If it is important to identify the thickness of the dermis or embedded structures within the subcutaneous fat layer, then a B-mode ultrasound is needed.

Finally, it is important to note that the present study evaluated the validity of the BodyMetrix BX2000 for measuring subcutaneous fat thickness at individual measurement sites and not the proprietary algorithms used to estimate total %BF. Verifying that a measurement device or method can accurately measure what was intended (subcutaneous fat thickness in the case of A-mode ultrasound) validates the method, not necessarily the predictions that follow. For example, hydrostatic weighing is a known valid method for measuring body density, but considerable error can occur when predicting %BF from body density (Heyward and Wagner 2004). Results from many investigators are equivocal regarding the validity of the total %BF estimates from the preprogrammed equations in the BodyMetrix device (Utter and Hager 2008; Johnson et al. 2012, 2014, 2017; Loenneke et al. 2014; Smith-Ryan et al. 2014; Ripka et al. 2016; Wagner et al. 2016; Baranauskas et al. 2017; Schoenfeld et al. 2017). Thus, we recommend this device for measuring subcutaneous fat thickness at individual sites, but the total %BF estimates may or may not be valid.

**Acknowledgments**
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Conflict of Interest disclosure

The authors have no conflict of interest to disclose.

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**Queries and Answers**

**Query:** Please confirm that givennames and surnames have been identified correctly.

**Answer:** Yes

**Query:** Please indicate the significance of the bold values in the table.

**Answer:** Bold values are for the total sample (male and female combined)

**Query:** The author names have been tagged as given names and surnames (surnames are highlighted in teal color). Please confirm if they have been identified correctly.

**Answer:** These are correct.