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Ultrasound Measurements of Subcutaneous Fat Thickness Are Robust Against Hydration Changes

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Ultrasound is an appealing tool to assess body composition, combining the portability of a field method with the accuracy of a laboratory method. However, unlike other body composition methods, the effect of hydration status on validity is unknown. This study evaluated the impact of acute hydration changes on ultrasound measurements of subcutaneous fat thickness and estimates of body fat percentage. In a crossover design, 11 adults (27.1 ± 10.5 years) completed dehydration and hyperhydration trials to alter body mass by approximately ±2%. Dehydration was achieved via humid heat (40 °C, 60% relative humidity) with exercise, whereas hyperhydration was via ingestion of lightly salted water. Ultrasound measurements were taken at 11 body sites before and after each treatment. Participants lost 1.56 ± 0.58 kg (−2.0 ± 0.6%) during the dehydration trial and gained 0.90 ± 0.21 kg (1.2 ± 0.2%) during the hyperhydration trial even after urination. The sum of fat thicknesses as measured by ultrasound differed by <0.90 mm across trials ($p = .588$), and ultrasound estimates of body fat percentage differed by <0.5% body fat. Ultrasound measures of subcutaneous adipose tissue were unaffected by acute changes in hydration status by extents beyond which are rare and overtly self-correcting, suggesting that this method provides reliable and robust body composition results even when subjects are not euhydrated.

Keywords: body composition, dehydration, hyperhydration

Body composition is a health-related component of physical fitness, and as such, the American College of Sports Medicine recommends measuring body fat percentage (%BF) as part of a comprehensive health-fitness evaluation (American College of Sports Medicine, 2018). In addition, body composition assessments can be used to evaluate nutrition and exercise interventions, estimate healthy competitive body weights for athletes, and monitor body changes related to growth, aging, and certain diseases or treatments (Risoul-Salas et al., 2020). Consequently, accurate and reliable measurements of body composition are important in various sport, fitness, health, and medical settings.

Numerous methods and techniques exist for measuring human body composition. Common laboratory methods include dual-energy X-ray absorptiometry (DXA), hydrodensitometry, and air displacement plethysmography (e.g., BOD POD). These measurement devices are large and expensive and typically require travel to a research setting for measurement. In contrast, field methods are small, portable, and less costly. Field methods include tape measures and anthropometers, skinfold calipers, and bioelectrical impedance analysis (BIA). Accuracy of estimating %BF is 2–3% for laboratory methods and 3–4% for field methods (Lohman et al., 2020).

Ultrasound spans the laboratory and field method categories of body composition assessment. High-frequency (12–18 MHz) B-mode (brightness modulation) ultrasound combined with specialized software is capable of measuring subcutaneous fat thickness to within 0.2 mm (Müller et al., 2016), placing it in laboratory-category accuracy (Lohman et al., 2020). Yet, some A-mode (amplitude modulation) ultrasound transducers will fit within a shirt pocket and can plug into a laptop, giving ultrasound the portability of field methods. Although ultrasound has been used to measure fat thickness for over 50 years, interest in this method is resurgent due to improved technology (Wagner, 2013), and a working group under the auspices of the International Olympic Committee concluded that B-mode ultrasound has potential to replace BIA and skinfolds for measuring athletes' body fat (Müller et al., 2013).

An underlying assumption and recommendation for nearly all laboratory and field methods of body composition is that the individual is euhydrated during measurement (Slater et al., 2018). However, it is not always practical or realistic to have clients adhere to strict hydration guidelines, particularly if measuring in field settings. Furthermore, most technicians do not take the time or have the resources to assess hydration status before assessing body composition. In a survey of professionals responsible for taking body composition measurements of national and international athletes, only 36% reported assessing hydration status before measurement (Meyer et al., 2013). Hydration status presumably receives less, if any, thought when measurements are made for scholastic athletes or clients in fitness centers. Measuring hydration status is also more fraught than it may appear (e.g., it entails both water and solute contents and concentrations); no single variable provides a definitive measure, and all indices have problems of sensitivity, specificity, and/or practicality. Thus, it is important to identify methods for measuring body composition that are robust against potential violations of assumed euhydration.

The influence that hydration has on most methods of body composition assessment is already known (Kerr et al., 2017; Rodriguez-Sanchez & Galloway, 2015). Significant measurement errors in estimating fat-free mass from skinfolds, BIA, DXA, and the BOD POD have occurred under both acute hypohydration (Rodriguez-Sanchez & Galloway, 2015) and hyperhydration

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(Kerr et al., 2017). However, it is still unknown what effect hydration changes have on ultrasound measures of body composition. Thus, the purpose of this study was to determine the extent to which acute dehydration and hyperhydration affect subcutaneous fat thickness measurements obtained by A-mode ultrasound. Given that fat is essentially anhydrous, we hypothesized that hydration changes would have no significant effect on ultrasound-measured fat thickness.

Methods

Subjects

Adults aged 18–65 years were recruited by word of mouth in an Exercise Science Department and via an advertisement posted at the University of Otago's fitness center. Exclusion criteria included a self-reported inability to be confined in a hot environment or to drink a large volume of lightly salted water, pregnancy, currently using an NSAID, or prior history of hyponatremia. The University of Otago Human Ethics Committee approved the study. Participants received an information sheet and consent form to review when they expressed interest in the study, and they provided written informed consent upon initial arrival in the laboratory.

Sample size was estimated with G*Power (version 3.0.10; Heinrich Heine Universität Düsseldorf, Düsseldorf, Germany; Faul et al., 2007). Repeated-measures analysis of variance with one group performing four tests (two baseline, one dehydration, and one hyperhydration) assuming an effect size of 0.25, alpha of .05, power of 0.95, and correlation among repeated measures of 0.9 yielded a sample size of 9. Previously published test–retest reliability data of the ultrasound device used in this study justify the assumed correlation among repeated measures of 0.9 as a conservative estimate (Loenneke et al., 2014; Smith-Ryan et al., 2014; Wagner et al., 2016).

Procedures

Preliminary measures. Participants came to the laboratory on 2 consecutive days. They were instructed to follow a presession euhydration protocol of drinking 10 ml/kg water approximately 2 hr before arriving at the lab, maintain their typical daily routine, and refrain from exhaustive exercise prior to each trial. Upon arrival at the lab, participants emptied their bladder and provided a urine sample. Urine specific gravity (USG) was measured with a refractometer (Uricon-N, Atago, Tokyo, Japan) to verify euhydration status. A USG of <1.020 was the criterion for euhydration (American College of Sports Medicine, 2016). Height was measured to the nearest 0.1 cm with a custom-made, wall-mounted stadiometer. Body mass was measured to the nearest 0.02 kg with a digital scale (DI/10; Wedderburn Scales Ltd., Dunedin, New Zealand). Male participants wore shorts only, whereas female participants wore shorts and a sports bra for all measurements.

Ultrasound. Ultrasound measurements were taken with the BodyMetrix BX2000 (IntelaMetrix, Inc., Livermore, CA) operating in A-mode. Illustrations are available elsewhere (e.g., Wagner, 2013). The BX2000 is a portable, fixed-frequency device that operates at 2.5 MHz at an average sound speed of 1,450 m/s. The BX2000 transmitter and receiver are each 5 mm × 10 mm, and the sampling rate exceeds 10 Hz (L. Da Silva, personal communication, September 15, 2020).

Prior to taking measurements, 11 anatomical sites (Table 1) were marked on the right side of the body with a surgical pen (1450XL skin marker; Viscot Medical, East Hanover, NJ). Identification, marking, and body positioning for measurement of eight of these sites were consistent with International Olympic Committee standardized ultrasound sites described by Müller et al. (2016) and illustrated therein. The additional three sites were specific to the Jackson and Pollock three-site skinfold locations (Jackson & Pollock, 1978; Jackson et al., 1980). Participants were standing for these three measurements per the manufacturer's guidelines. The Body View Pro software (IntelaMetrix, Inc., Livermore, CA) that accompanies the BX2000 modifies popular skinfold formulas into proprietary ultrasound formulas unavailable to the public. This modification of the Jackson and Pollock three-site formula was used to provide an estimate of %BF.

The procedures for taking ultrasound measurements using the BX2000 have been described previously (Wagner et al., 2016). Briefly, this included placing ultrasound gel liberally on the measurement site and the transducer head so that the ultrasound glided easily over the skin. Care was taken to not apply pressure while taking the measurement, as skin compression affects thickness measurement (Toomey et al., 2011). Spot measurements using the BX2000 involved moving the transducer head slightly above and below (0.5–1 cm) the marked site several times. Each measurement was repeated several times for reliability averaging following the computer software prompts. The same technician, with 8 years of experience with the BX2000, performed all of the ultrasound scans.

Dehydration and hyperhydration. For their initial trial, participants were randomly assigned to dehydration or hyperhydration with the opposite treatment applied during the subsequent trial. Dehydration took place in an environmental chamber with the temperature and relative humidity held constant at 40 °C and 60%, respectively. Participants had access to a cycle ergometer and treadmill, and exercise was ad libitum. A scale was available for participants to self-monitor changes in mass. Participants remained in the chamber until approximately 2% of body mass was lost or 2 hr elapsed, whichever occurred first.

The hyperhydration protocol required participants to drink 2% body mass of lightly salted water (30 mmol/L NaCl; 1.76 g NaCl/L). They were encouraged to finish this volume within 30 min. Once the volume was consumed, they waited an additional 30 min before beginning the posttreatment measurements.

Posttreatment measurements. Participants emptied their bladder, and body mass was measured. The change in mass (Δmass) between the baseline and this posttreatment measurement confirmed and quantified dehydration and hyperhydration. Ultrasound measurements were repeated following the same standards described for baseline testing.

Statistical Analyses

Data were checked for normality by visual inspection of plots and the Shapiro–Wilk test. Reliability across the four trials was assessed via intraclass correlation coefficient, standard error of measurement (*SEM*), and minimal difference according to Weir (2005). Mean differences in the variables of interest (body mass, individual-site fat thicknesses, Σfat thicknesses, and %BF) between the two baseline measurements and the dehydration and hyperhydration posttreatments were evaluated using repeated-measures analysis of variance. If the assumption of sphericity was violated, the Greenhouse–Geisser correction was used. When the *F* score was significant, Bonferroni pairwise comparisons were made. In addition, since the amount of change between baseline and posttreatment was variable across participants, plots were made to evaluate the change in scores (e.g., Δmass plotted against ΔΣfat

Table 1 Ultrasound Measurement Sites

Site name	Anatomical location
Müller et al. (2016) IOC standardized sites	
1. Upper abdomen	0.02 × ht superior and lateral to umbilicus
2. Lower abdomen	0.02 × ht inferior and lateral to umbilicus
3. Erector spinae	0.14 × ht superior to supporting surface and 0.02 × ht lateral to spinous process
4. Distal triceps	0.05 × ht superior to supporting surface
5. Brachioradialis	0.02 × ht distally from biceps brachii tendon
6. Lateral thigh	At level of gluteal fold
7. Front thigh	0.14 × ht proximally from patella
8. Medial calf	0.18 × ht superior to supporting surface
Jackson and Pollock (1978)—three sites (males)	
9. Chest	Midway between anterior axilla and nipple
10. Abdomen	2-cm right of umbilicus
11. Thigh	Anterior; midway between inguinal crease and superior border of patella
Jackson et al. (1980)—three sites (women)	
9. Triceps	Midway between acromion process and olecranon process
10. Suprailiac	2-cm above iliac crest at anterior axillary line
11. Thigh	Anterior; midway between inguinal crease and superior border of patella

Note. ht = height (in centimeters); IOC = International Olympic Committee.

Table 2 Mean ± *SD* of the Sample (*N* = 11) From the Two Baseline and Two Posttreatment Assessments

Variable	Baseline dehydration	Postdehydration	Percentage of change from baseline	Baseline hyperhydration	Posthyperhydration	Percentage of change from baseline
Body mass (kg)	78.0 ± 20.4	76.5 ± 20.2*	–2.0 ± 0.6	78.0 ± 20.6	78.9 ± 20.8*	1.2 ± 0.2
Upper abdomen fat (mm)	9.2 ± 3.7	9.4 ± 4.1	2.4 ± 16.5	9.1 ± 3.5	9.0 ± 3.5	–1.3 ± 4.9
Lower abdomen fat (mm)	10.4 ± 4.0	10.6 ± 3.9	3.1 ± 5.9	10.5 ± 3.8	10.4 ± 3.6	0.4 ± 8.2
Erector spinae fat (mm)	7.8 ± 3.8	7.9 ± 3.8	1.2 ± 3.8	7.7 ± 3.6	7.9 ± 3.7	4.2 ± 3.3
Distal triceps fat (mm)	6.8 ± 3.0	6.9 ± 3.1	0.2 ± 6.1	6.9 ± 3.0	6.9 ± 2.9	0.7 ± 6.9
Brachioradialis fat (mm)	5.7 ± 4.7	5.4 ± 4.8	–2.4 ± 22.6	5.9 ± 5.3	5.9 ± 4.7	7.7 ± 18.0
Lateral thigh fat (mm)	7.9 ± 3.9	8.2 ± 3.7	5.3 ± 18.4	8.2 ± 3.9	8.0 ± 3.6	–1.3 ± 8.5
Front thigh fat (mm)	7.9 ± 3.5	7.8 ± 3.6	–1.7 ± 4.7	7.6 ± 3.3	7.8 ± 3.4	1.7 ± 6.3
Medial calf fat (mm)	5.0 ± 1.5	5.2 ± 1.5	7.8 ± 6.4	4.9 ± 1.5	4.9 ± 1.3	3.3 ± 18.4
Ultrasound Σfat thickness[a] (mm)	60.7 ± 24.6	61.6 ± 24.8	1.4 ± 2.9	60.7 ± 24.8	60.9 ± 23.2	1.0 ± 3.1
Ultrasound %BF[b] (%)	16.6 ± 8.4	17.0 ± 8.5	3.1 ± 2.5	16.7 ± 8.3	16.7 ± 8.2	0.4 ± 2.3

Note. %BF = body fat percentage; IOC = International Olympic Committee.
[a]Σfat thickness is from the eight IOC sites (Müller et al., 2016). [b]%BF is estimated from the Jackson and Pollock three-site measurements (Jackson & Pollock, 1978; Jackson et al., 1980).
*$p < .001$ from baseline.

thicknesses). A significant correlation was indicative of bias. Statistical significance was accepted as $p < .05$. All statistical analyses were performed with SPSS (version 25.0; IBM Corp., Armonk, NY).

Results

Eleven recreationally active adults (eight males and three females), ranging in age (19–54 years; 27.1 ± 10.5 years), height (156.5–

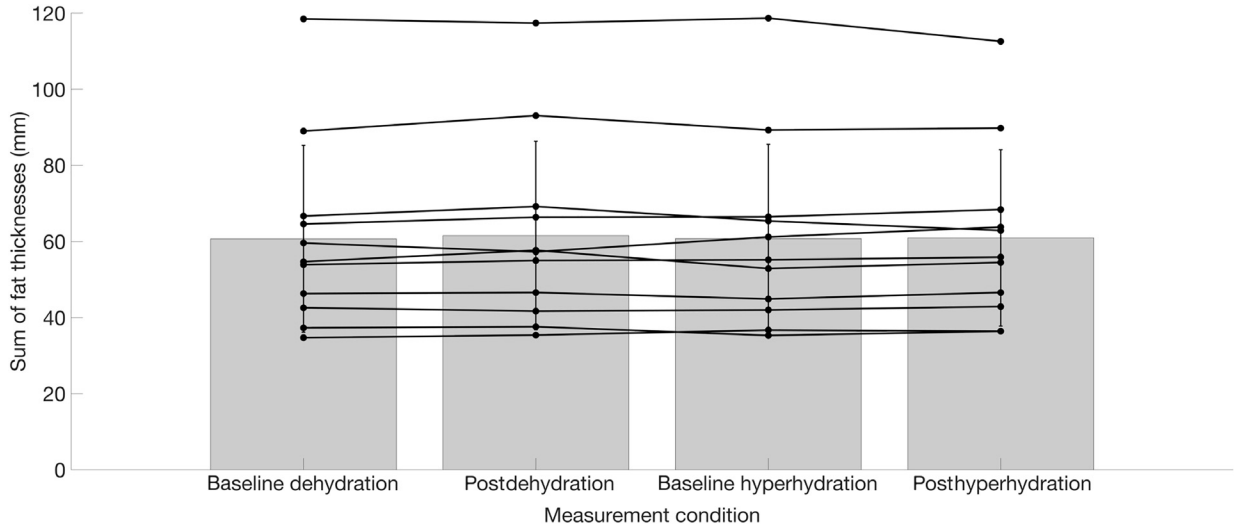

Figure 1 — Mean ± *SD* and individual data for the sum of fat thicknesses across four measurement conditions. Bars indicate means, error bars represent *SD*, and plots represent individual data.

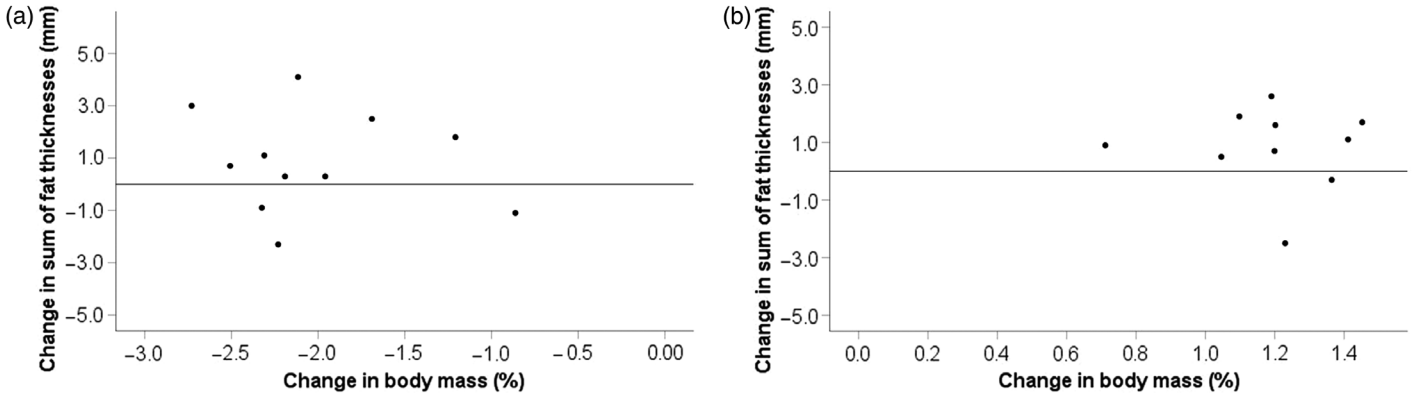

Figure 2 — Percentage change in body mass plotted against the change in the sum of fat thicknesses from the (a) dehydration trial and the (b) hyperhydration trial.

188.8 cm; 176.2 ± 11.5 cm), mass (49.2–120.3 kg; 78.0 ± 20.6 kg), and body mass index (20.3–33.5 kg/m^2; 24.7 ± 3.9 kg/m^2) completed the study. Measured data from postdehydration, posthyperhydration, and both baselines are in Table 2. All participants began each trial with USG < 1.020. The difference in USG was not significant between trials ($\Delta = 0.0035$, 95% CI [–0.0074, 0.0005], $p = .081$), suggesting similar euhydration at the start of each session. Body mass was similar at the start of each trial ($\Delta = 0.03$ kg, 95% CI [–0.60, 0.66] kg, $p = 1.000$) but decreased significantly postdehydration ($\Delta = –2.0\%$ or –1.56 kg, 95% CI [–2.13, –0.99] kg, $p < .001$) and increased significantly posthyperhydration ($\Delta = 1.2\%$ or 0.90 kg, 95% CI [0.68, 1.11] kg, $p < .001$). Six of the 11 subjects stayed in the heat chamber for the full 2 hr, whereas the others achieved the desired mass loss in less time. The average time to dehydrate 2% was 99 ± 28 min. The average amount of water consumed during the hyperhydration trial was 1,560 ± 413 ml.

Reliability for the sum of fat thicknesses across the four trials was intraclass correlation coefficient = .994, 95% CI [0.986, 0.998], *SEM* = 1.89 mm, and minimal difference = 5.24 mm. The sum of fat thickness from the eight International Olympic Committee measurement sites (Müller et al., 2016) did not differ among the four trials ($p = .588$) with the greatest difference between trials being 0.86 mm (Figure 1). None of the fat thicknesses at the individual measurement sites was significantly different across trials ($p > .05$). Similarly, estimated %BF derived from the three Jackson and Pollock sites (Jackson & Pollock, 1978; Jackson et al., 1980) was not significantly different across trials ($p = .070$) with the largest change being an increase of 0.49% BF postdehydration compared with baseline. Finally, when the percentage of Δmass was plotted against the ΔΣfat thicknesses for each individual, the correlation was small and not significant for both the dehydration trial ($r = –.143$, $p = .674$; Figure 2a) and hyperhydration trial ($r = .210$, $p = .536$; Figure 2b). These random differences indicate no bias or pattern of change in ultrasound measurements with magnitude of hydration change.

Discussion

The primary finding from this study was that ultrasound measures of subcutaneous fat thickness are not meaningfully affected by changes

in hydration status. Dehydration of 2% body mass was chosen for this study because this magnitude of dehydration has been shown to compromise cognitive and athletic performance (American College of Sports Medicine, 2016) and larger fluid deficits would be atypical by virtue of corrective behavior, especially when resting. Similarly, subjects were overhydrated by drinking a volume (1.56 ± 0.41 L) equivalent to 2% of their body mass to create a clear state of hyperhydration (+1.2%). Participants were pushed to achieve these levels of hypohydration and hyperhydration. As ultrasound measurements are unaffected by such changes in body mass, this method should be even less prone to error with smaller hydration variations that are more likely when athletes or clients present themselves for a body composition assessment. In contrast, other body composition methods are significantly affected by hydration changes of similar magnitude. For example, DXA underestimates lean tissue mass by 1.3–1.7 kg following exercise-induced dehydration of approximately 2% body mass (Rodriguez-Sanchez & Galloway, 2015; Toomey et al., 2017), and the BOD POD overestimates %BF with increasing amounts of water consumption ≥1 L (Heiss et al., 2009; Kerr et al., 2017; Vukovich & Peeters, 2003). These overestimates range from 1.3% with 1 L of water to 2.3% with 2 L (Vukovich & Peeters, 2003). By comparison, the difference of <0.5% BF across the four ultrasound tests in the present study was not significant and well below the reported minimal difference of 1.8% BF for detecting a meaningful change with this method (Wagner et al., 2016).

The intent of the present study was to induce a hydration change of ±2% of body mass. In reality, the percentage change in body mass following the dehydration protocol ranged from –0.9% to –2.7% (Figure 2a), and the percentage increase that was retained after drinking a volume equivalent to 2% of mass ranged from 0.7% to 1.5% (Figure 2b). Regardless of the magnitude of hydration change, the impact on the ultrasound fat thickness measurements was consistent; there was not an increasing error with increasing change in hydration status (Figure 2a and 2b). This finding is different from what has been observed with DXA and the BOD POD. Kerr et al. (2017) noted that a 500 g meal had minimal influence on DXA and BOD POD results but that an additional 1 L of water caused significant changes in the estimation of fat-free mass for these methods. Similarly, Vukovich and Peeters (2003) reported no significant difference in the estimate of %BF from the BOD POD with hydration changes <1 L but significant and increasing errors with overhydration >1 L. These studies suggest that DXA and the BOD POD are reliable if hydration changes are small but not reliable if changes exceed 1 L.

It is logical that hydration status would have little impact on ultrasound measures of subcutaneous fat thickness because adipocytes are nearly anhydrous and the water content of adipose tissue is considerably less than that of skeletal muscle. The water content of subcutaneous adipose tissue of adults is approximately 10–19% (Baker, 1969; Thomas, 1962), varying with age (Baker, 1969) and adiposity (Thomas, 1962). In contrast, nearly 80% of skeletal muscle is water (Mitchell et al., 1945). Thus, due to the small water content of adipose tissue and the relatively small contribution of subcutaneous adipose tissue to the total body mass, it is not surprising that variations in hydration status had no impact on measures of subcutaneous fat thickness.

This premise that hydration status has minimal influence on measures of subcutaneous fat thickness is also evident from skinfold studies. Nickerson et al. (2020) recently reported no bias for skinfold measures when the fat-free mass hydration levels of their participants varied from 65% to 74%. Similarly, the difference in the sum of eight skinfold measurements before and after exercise-induced hypohydration of 2% body mass was only –1.4 mm (Rodriguez-Sanchez & Galloway, 2015). Also, skinfolds were more reliable than BIA, DXA, or the BOD POD when measurements were taken after daily activities or a meal and drink rather than a euhydrated state (Kerr et al., 2017). However, hydration changes could alter the elasticity and compressibility of skinfolds (Ward et al., 1999). Ultrasound has an advantage over the skinfold method in this regard because ultrasound can provide a direct measure of subcutaneous fat thickness without tissue compression (Müller et al., 2013).

This study used a low resolution (2.5 MHz), A-mode ultrasound. Some experts recommend using only high-resolution, B-mode ultrasound to measure subcutaneous fat thickness because fibrous structures embedded within the fat layer could confound the identification of the fat–muscle interface when using A-mode ultrasound (Ackland & Müller, 2018). Yet, laboratory-grade B-mode ultrasounds are considerably more expensive and require extensive training compared with the field-method A-mode devices (Wagner et al., 2020). Considering the lower cost and ease of use, A-mode ultrasound is more likely to be used by fitness professionals and coaches in field settings where hydration status might not be as well controlled as in the laboratory; thus, it was selected for this study rather than B-mode ultrasound. Nevertheless, given that the basic principle of using sound waves reflected from underlying tissue back to the transducer to determine the fat–muscle interface is the same for both A-mode and B-mode ultrasound (Wagner, 2013), the finding that acute hydration changes do not affect A-mode ultrasound measures of subcutaneous fat thickness may apply to B-mode ultrasound as well.

In conclusion, negligible and statistically nonsignificant differences were found between euhydration, hypohydration, and hyperhydration for the sum of ultrasound-measured fat thicknesses from eight anatomical sites and for estimates of %BF. This finding indicates that ultrasound is robust and unaffected by typical variations in hydration status. Ideally, individuals should be in a euhydrated state when presenting themselves for a body composition test, and technicians should verify this with a USG measurement (Meyer et al., 2013; Slater et al., 2018). However, this research offers the technician and researcher some confidence that ultrasound provides reliable measures of subcutaneous fat thickness even in situations when the individual might not be euhydrated.

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