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Original research

Food and fluid intake and hydration status does not affect ultrasound measurements of subcutaneous adipose tissue in active adults

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ABSTRACT

Objectives: To investigate the impact of acute food and fluid intake or hydration status on the standardised brightness-mode ultrasound measurement of subcutaneous adipose tissue thickness.
Design: Thirty active adults (female $n = 10$) participated in a randomised cross over study.
Methods: Participants completed three body composition assessment sessions via standardised brightness-mode ultrasound and Dual-energy X-ray absorptiometry. Participants were assessed under standardised presentation during 'food only' and 'food plus water' sessions at baseline and reassessed after their allotted intake. 'Hypohydration plus water' was undertaken in a hypohydrated state at baseline and reassessed after water intake.
Results: The sum of eight subcutaneous adipose tissue thickness was lower when measured after 'food only' or 'food plus water' compared to baseline (−0.1 to −0.9 mm; $p < 0.01$). However, these changes were less than the 95% confidence interval of the technical error of measurement of the investigator. Body mass, dual-energy x-ray absorptiometry total and trunk mass, lean mass and trunk lean mass estimates increased ($p < 0.01$) following 'food only' or 'food plus water', and decreased with hypohydration ($p < 0.01$). Total and regional fat mass estimates were not impacted.
Conclusions: The sum of eight subcutaneous adipose tissue thickness measured via standardised brightness-mode ultrasound was unaffected by acute food and fluid consumption or hydration status changes. Comparatively, these interventions altered dual-energy x-ray absorptiometry body composition estimates, especially that of lean mass components. Standardised brightness-mode ultrasound can therefore be used to monitor changes in fat patterning when standardised client presentation is not practically achievable.

Practical Implications

- Subcutaneous adipose tissue thickness measured by standardised brightness mode ultrasound by a trained practitioner may be a surrogate for fat mass when standardised client presentation for physique assessment is not practically achievable
- Clients should adhere to standardised presentation requirements prior to undertaking dual energy x-ray absorptiometry scans
- Adipose tissue, hence body fat mass appears to be robust against acute manipulations of food and/or fluid intake or hydration status

1. Introduction

Physique assessment is an important aspect of monitoring the health and physical performance of an individual.[1] Nevertheless, the reliability of measurements obtained from most physique assessment methods is subject to both technical and biological variability that can contribute to the overall error of a measurement.[1–3] Technical variability may be associated with the operational skill and experience of the technician, positioning protocols, or intrinsic to the measurement device. On the other hand, biological variability is often attributed to client presentation (e.g., hydration status),[4,5] acute food and fluid intake[2,3] or exercise[6] prior to an assessment. Accordingly, it is recommended that client positioning and presentation (i.e., overnight fasted, well hydrated with limited exercise in the past 12–24 h) be standardised when conducting physique assessments.[1,7,8] However, this means assessment times can be restricted or logistically inconvenient.

Common physique assessment methods such as DXA and skinfolds can be subjected to some of the aforementioned limitations. Accordingly, this has prompted the International Olympic Committee Medical Commission Research Group on Body Composition, Health and Performance in collaboration with the International Association of Sciences in Medicine and Sports (IASMS) to establish a standardised protocol using brightness modulation ultrasound (B-mode US) to measure uncompressed subcutaneous adipose tissue (SAT) thickness as a surrogate estimate of body fat.[9] This method has been shown to be highly accurate over a wide spectrum of physiques,[10,11] and has high intra- and inter-

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measurer reliability when assessing athletes sampled from a broad range of sports.[12]

Following the establishment of a standardised measurement protocol, thereby minimising technical variability, the next logical step therefore is to investigate the impact in which manipulating client presentation may have this on standardised B-mode US method of measuring uncompressed SAT thickness. This is necessary in order for this method to be widely adopted for field assessments.[13] Accordingly, the aims of this study were to assess the impact of 1) acute food and fluid intake i.e., 'food only' (FO) or 'food plus water' (FW) and 2) hypohydrated vs. euhydrated client presentation i.e., 'hypohydration plus water' (HW) on standardised B-mode US measurements of uncompressed SAT thickness in comparison to DXA body composition estimates.

2. Methods

Thirty recreationally active adults (males: $n = 20$, height $= 182.1 \pm 5.6$ cm, weight $= 84.2 \pm 6.2$ kg; females: $n = 10$, height $= 167.6 \pm 4.4$ cm, weight $= 59.9 \pm 6.5$ kg) were recruited to participate in the study. Included participants (1) met the physical activity and exercise guidelines for adult Australians[14] (2) fit within the active scanning area of the DXA bed (198 cm × 66 cm), (3) were not carbohydrate or creatine loading,[15] or (4) were exposed to ≤1 mSv of ionising radiation from medical imaging in the past year (<1 spine x-ray or equivalent). This study was approved by the Human Ethics Research Committee of the University of Western Australia (RA/4/20/4798), and informed written consent was obtained from all participants.

Participants attended an initial familiarisation session, followed by three experimental sessions, each separated by one week. These sessions were assigned via block randomisation such that participants undertook FO or FW first before completing the study with HW or vice versa (Fig. 1). During familiarisation, participants were provided with an information sheet that included instructions on recording dietary intake and physical activity over the 24 h before their first session. These were to be replicated on the day prior to FO or FW. The information sheet also contained instructions on how to passively hypohydrate via fluid restriction in the 24 h prior to HW. Digital scales (WW® Body Weight Digital Scale WW58A, Conair, Australia) calibrated against the laboratory platform scales (August Sauter GmbH, Ebingen, Germany), were loaned out to participants to record their nude body mass (BM) daily for five consecutive days upon waking prior to their first assessment session to determine an average baseline BM. Female participants were asked to confirm their pregnancy status in accordance to local radiation safety regulation requirements. Participants maintained their habitual exercise and dietary routine throughout the duration of the study.

Participants were assessed under standardised presentation at baseline for FO or FW sessions, but in a hypohydrated state for the HW session. Accordingly, participants consumed 500 mL of water upon waking on the day of FO or FW but not HW to standardise fluid intake.[4,16] With HW, participants consumed only ~600–700 mL of fluid in the 24 h prior to testing and only consumed foods that have low water content. This was expected to induce at least a 1% reduction in BM in 24 h.[16] In all sessions, a urine sample was collected and specific gravity was measured using a desktop refractometer (T3-NE, Atago, Tokyo, Japan) on arrival to the laboratory in the morning to ascertain hydration status. Participants were weighed in their underwear on the laboratory platform scales accurate to 0.01 kg after voiding their bladder and height was measured using a wall mounted stadiometer (Novel Products Inc., Illinois, USA) accurate to 0.1 cm. Participants were marked up at eight standardised sites in accordance to the IASMS protocol[9,17] and corresponding B-mode US images (Telemed Echo Blaster 128 EXT-1Z, Telemed UAB, Vilnius, Lithuania) were captured in duplicates via a linear transducer and requisite software (Telemed Echo Wave II v 3.2.0). These were used to evaluate SAT thickness with the Fat Analysis Tool software (v 3.3, Rotosport, Austria; rotosport.at) and a mean sum of eight SAT thicknesses (including embedded structures, S8US) was used for data analysis (Supplementary Fig. 1). Whole body DXA scans were performed with standard thickness mode on a narrowed fan-beam machine (Lunar iDXA Advance, GE Healthcare, Wisconsin, USA) and analysed with GE Encore (v 16.0, GE Healthcare, Wisconsin, USA). The machine was calibrated with phantoms as per the manufacturer's guidelines each morning before measurements were taken. Participants were positioned on the DXA scanning bed according to the Nana et al positioning protocol,[18] standardised with Velcro straps and customised radiolucent foam blocks i.e., a constant distance of 22 cm between the

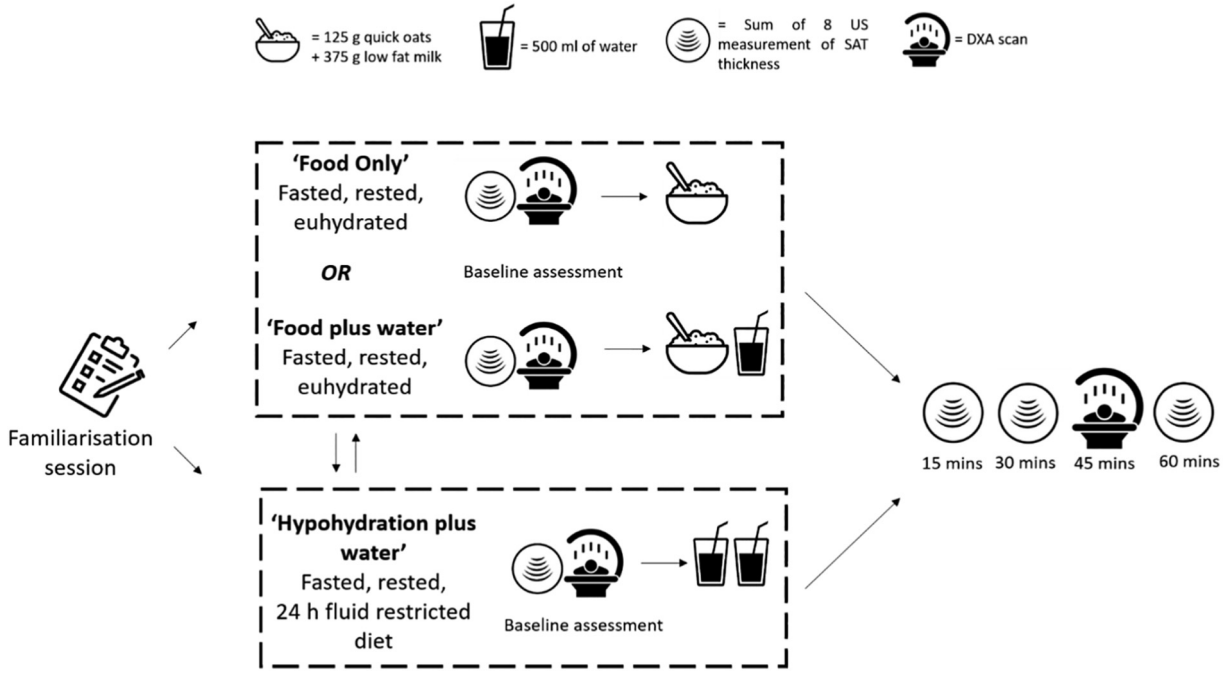

Fig. 1. Schematic of experimental protocol. Participants were block randomised to undertake either 'food only' or 'food plus water' first in random order before undertaking 'hypohydration plus water' or undertake 'hypohydration plus water' first before undertaking 'food only' or 'food plus water' in random order.

feet and 5 cm between the palms and trunk. Regions of interest automatically demarcated by the software in the scans were subsequently confirmed by the investigator.

With FO, participants consumed 125 g of quick oats and 375 g of low fat milk within 15 min following baseline measurements and an additional 500 mL of water was consumed with FW. With HW, participants consumed 1 L of water within 15 min following baseline measurements. Participants were reassessed via standardised B-mode US at 15, 30 and 60 min and via DXA at 45 min after their allotted intake. The same IASMS certified technician took all measurements and analysed the data.

Linear mixed effects models were implemented via the *nlme* R package (v 4.05 RStudio Inc., Massachusetts, USA)[19] to analyse the impact of the interventions on physique assessment results. The analyses were not stratified by sex since no such effects were observed in previous studies.[5,20,21] The modelling included the interventions and the time points at which the assessments were conducted as fixed effects and individual participants as random effects. The inclusion of random effects allows the additional within-participant biological variability between interventions to be accounted for. An interaction term between the fixed effects was included to determine if changes due to time were dependent on intervention. Correlations between measurements and time points were compared pairwise between interventions using Pearson and Filon's Z statistic.[22] 95% confidence intervals (95% CI) of the mean difference between pre- and post-interventions were used to assess statistical significance and clinical meaningfulness of the mean difference. The precision error of S8US is represented as the percentage coefficient of variation (%CV) and true change in S8US is assessed via the technical error of measurement (TEM-95% CI) of the investigator.[23] All statistical significance was set at $p < 0.05$.

3. Results

The %CV and TEM-95% CI for S8US was determined to be 1.8% and 2.1 mm respectively. Although there was no main effect of intervention ($p = 0.449$), there was a main effect of time ($p < 0.001$) and a significant interaction between intervention and time ($p = 0.049$) on S8US measurements (interactions presented in Supplementary Table 1). Accordingly, hypohydration did not affect S8US ($p = 0.52$). However, S8US decreased ($p < 0.05$) at 15 (−0.6 mm), 30 (−0.8 mm) and 60 min (−0.8 mm) following FO, at 30 (−0.5 mm) and 60 min (−0.6 mm) following FW and at 15 (−0.9 mm) and 30 min (−0.7 mm) and 60 min (−0.5 mm) following HW from pre-values (Table 1). Nevertheless, all changes to S8US observed with time were less than the TEM-95% CI to qualify for any meaningful changes.

There was a significant interaction between time and intervention for BM ($p < 0.01$) in addition to DXA estimates of total mass ($p < 0.01$), total LM ($p < 0.01$), trunk mass ($p < 0.01$) and trunk LM ($p = 0.01$) (interactions presented in Supplementary Table 2). These regional estimates were lower than respective baseline values at pre-FO and pre-FW with hypohydration ($p < 0.05$) (Table 2), i.e., there was a main effect of intervention, but their differential increase ($p < 0.05$) following FO and FW was similar to the intake mass. Additionally, leg and arm LM decreased with FW ($p < 0.05$). Hypohydration also resulted in decreased BM (1.02 kg; −1.3%) from the participants' average baseline BM. Consuming water following hypohydration returned BM (99.9%) to participants' average baseline values but was reflected as an increase in DXA total mass along with total LM and trunk mass and trunk LM estimates. Additional comparisons following interventions across assessment time points and their interactions are contained in Tables 1, 2, Supplementary Tables 1 and 2. No other changes in DXA body composition estimates were observed.

4. Discussion

Our study is the first to quantify the impact of FO, FW or HW on S8US in active individuals. Whilst we observed the S8US to be lower post-intervention compared to pre-intervention, these reductions were much lower than the TEM-95% CI (ranging from −0.1 to −0.9 vs 2.1 mm) and thus not qualified to be meaningful changes. Importantly, our study found S8US to be unaffected by hydration status since there were no differences in S8US across interventions at each time point (Supplementary Table 1). Similarly, Wagner and Cotter[21] recently reported neither hypohydration (ranging from −0.9 to −2.7% BM) nor hyperhydration (ranging from 0.7 to 1.5% BM) impacted SAT thickness (11 sites, including 8 IASMS standard sites) measured via A-mode (amplitude modulation) US. It should be noted however that the accuracy and reliability of the 2.5 MHz A-mode US system used in this study is an order of magnitude below the fine scale of the standardised 10 MHz B-mode US system employed in our study. Because the A-mode system is cruder, it has limited tissue border detection and cannot distinguish between skin and adipose tissue. Furthermore, moving the transducer along the measurement site to determine SAT thickness with A-mode US, increases the risk of uncontrolled pressure variation on the skin and resultant SAT compression. Despite the disadvantages of using A-mode over B-mode US however, our results together with that of Wagner and Cotter[21] concur that measurements derived from US appear resistant to hydration status. This may be attributed to US being a direct measure of SAT thickness rather than indirect estimate of FM, as well as SAT possessing a low water content (7.4% water, 92.6% lipids).[24]

Our study observed no changes to the participants' total or regional FM DXA estimates following all three interventions (Table 2 and Supplementary Table 2), corroborating the results of previous studies on the effects of acute food and fluid intake[3,20] or exercise induced hypohydration.[5] However, Tinsley et al[25] observed decreases in total and trunk FM when active adults ($n = 48$) were assigned to ingest a high carbohydrate (9 $g \cdot kg^{-1}$) or very low carbohydrate meal (1–1.5 $g \cdot kg^{-1}$) prior to undertaking DXA assessments (Hologic Discovery W; Hologic APEX v 3.3). Conversely, Nana et al[2] observed small but meaningful increases in trunk FM when active adults ($n = 31$) underwent DXA Scans (Lunar Prodigy; GE Encore v 12.2) after consuming meals of variable quantities (200–2000 mL). The disparate observations may be due to different calibration algorithms used by different manufacturers to resolve lipid and lean fractions from soft tissue hydration.[1] Accordingly, it is recommended to not only standardise client presentation but also the assessment machine and evaluation software if serial body composition assessments are conducted for longitudinal monitoring.[8]

Our study observed FO or FW to increase total and trunk LM DXA estimates, which corroborate findings from previous studies investigating the effects of acute food and fluid intake on DXA body composition estimates.[2,3,25] For example, Kerr et al[3] observed that a 500 g meal plus 1 L of water elicited a moderately substantial increase in LM in

Table 1
Sum of eight subcutaneous adipose tissue thickness (including embedded fibrous structures) measured via standardised B-mode ultrasound (S8US, mm). ΔMean = change in mean from pre-intervention values. Standard error (SE) is reported as results are based on modelling from raw values. CI = confidence interval. * = $p < 0.05$, different from pre-intervention.

Condition	Pre-intervention	15 min post		30 min post		60 min post	
	Mean ± SE	Mean ± SE	ΔMean ± SE (95% CI)	Mean ± SE	ΔMean ± SE (95% CI)	Mean ± SE	ΔMean ± SE (95% CI)
'Food only'	54.0 ± 4.8	53.4 ± 4.8	−0.6 ± 0.2 (−1.0, −0.3)*	53.2 ± 4.8	−0.8 ± 0.2 (−1.1, −0.4)*	53.2 ± 4.8	−0.8 ± 0.2 (−1.1, −0.4)*
'Food plus water'	54.0 ± 4.9	53.8 ± 4.9	−0.1 ± 0.2 (−0.5, 0.2)	53.5 ± 4.9	−0.5 ± 0.2 (−0.9, −0.2)*	53.4 ± 4.9	−0.6 ± 0.2 (−1.0, −0.2)*
'Hypohydration plus water'	54.5 ± 4.7	53.6 ± 4.7	−0.9 ± 0.2 (−1.2, −0.5)*	53.8 ± 4.7	−0.7 ± 0.2 (−1.0, −0.3)*	53.9 ± 4.7	−0.5 ± 0.2 (−0.9, −1.9)*

Table 2
Total and regional dual X-ray absorptiometry (DXA) body composition estimates. ΔMean = change in mean from pre-intervention values. Standard error (SE) is reported as results are based on modelling from raw values. CI = confidence interval. * = $p < 0.05$, different from pre-invention; ‡ = $p < 0.05$, different from pre-'Food only' and pre-'Food plus water'; § = $p < 0.05$, different from pre-'Food plus water'.

	'Food only'			'Food plus water'			'Hypohydration plus water'		
	Pre-intervention	45 min post		Pre-intervention	45 min post		Pre-intervention	45 min post	
	Mean ± SE	Mean ± SE	ΔMean ± SE (95% CI)	Mean ± SE	Mean ± SE	ΔMean ± SE (95% CI)	Mean ± SE	Mean ± SE	ΔMean ± SE (95% CI)
	(kg)	(kg)	(g)	(kg)	(kg)	(g)	(kg)	(kg)	(g)
Body mass	75.95 ± 2.47	76.38 ± 2.47	440 ± 10 (420, 460)*	76.20 ± 2.45	77.15 ± 2.45	950 ± 10 (930, 970)*	75.10 ± 2.41‡	76.08 ± 2.41	980 ± 10 (960, 1000)*
Total mass	76.30 ± 2.50	76.74 ± 2.50	430 ± 30 (370, 500)*	76.53 ± 2.48	77.49 ± 2.48	960 ± 30 (900, 1020)*	75.42 ± 2.45‡	76.42 ± 2.45	1000 ± 30 (940, 1060)*
Trunk mass	34.77 ± 1.18	35.42 ± 1.18	650 ± 130 (400, 900)*	34.90 ± 1.19	36.24 ± 1.19	1220 ± 130 (970, 1470)*	34.24 ± 1.15‡	35.17 ± 1.15	930 ± 130 (680, 1190)*
Leg mass	26.51 ± 0.79	26.39 ± 0.79	−120 ± 90 (−300, 60)	26.58 ± 0.78	26.40 ± 0.78	−170 ± 90 (−360, 10)	26.28 ± 0.78§	26.34 ± 0.78	60 ± 90 (−120, 240)
Arm mass	10.09 ± 0.52	10.03 ± 0.52	−64 ± 73 (−210, 80)	10.16 ± 0.52	10.03 ± 0.52	−130 ± 70 (−270, 20)	10.03 ± 0.52	10.00 ± 0.52	−30 ± 70 (−180, 110)
Total lean mass	58.91 ± 2.39	59.24 ± 2.39	426 ± 67 (290, 560)*	59.12 ± 2.36	59.97 ± 2.36	840 ± 70 (710, 980)*	58.06 ± 2.37‡	58.98 ± 2.37	920 ± 70 (780, 1050)*
Trunk lean mass	27.63 ± 1.08	28.29 ± 1.08	653 ± 116 (420, 880)*	27.82 ± 1.07	28.96 ± 1.07	1140 ± 120 (910, 1370)*	27.18 ± 1.07‡	28.07 ± 1.07	890 ± 120 (660, 1170)*
Leg lean mass	19.68 ± 0.80	19.56 ± 0.80	−130 ± 80 (−290, 30)	19.77 ± 0.78	19.58 ± 0.78	−190 ± 80 (−350, −30)*	19.49 ± 0.79§	19.51 ± 0.79	20 ± 80 (−150, 180)
Arm lean mass	8.00 ± 0.50	7.93 ± 0.50	−71 ± 61 (−190, 50)	8.07 ± 0.50	7.93 ± 0.50	−140 ± 60 (−260, −20)*	7.94 ± 0.50§	7.91 ± 0.50	−30 ± 60 (−150, 90)
Total fat mass	14.45 ± 0.65	14.47 ± 0.65	15 ± 57 (−100, 130)	14.38 ± 0.65	14.50 ± 0.50	120 ± 60 g (0, 230)	14.33 ± 0.63	14.41 ± 0.63	80 ± 60 (−40, 190)
Trunk fat mass	6.23 ± 0.35	6.24 ± 0.35	6 ± 55 (−100, 110)	6.17 ± 0.35	6.26 ± 0.35	80 ± 60 g (−20, 190)	6.15 ± 0.33	6.19 ± 0.33	40 ± 60 (−60, 150)
Legs fat mass	5.68 ± 0.28	5.69 ± 0.28	10 ± 30 (−50, 80)	5.67 ± 0.28	5.68 ± 0.28	20 ± 30 g (−40, 80)	5.66 ± 0.28	5.69 ± 0.28	30 ± 30 (−40, 90)
Arms fat mass	1.65 ± 0.08	1.65 ± 0.08	0 ± 20 (−40, 40)	1.65 ± 0.8	1.65 ± 0.08	0 ± 20 g (−40, 40)	1.64 ± 0.08	1.65 ± 0.08	10 ± 20 (−30, 50)

males ($n = 16$, 18–47 y) but only a trivial increase when a 500 g meal was consumed. This led the authors to conclude that DXA may reliably estimate LM if acute food and fluid intake is kept to <500 g. Nevertheless, this study cohort consisted of larger resistance-trained males (91.5 ± 10.1 kg) that may not apply to participants that are of a smaller physique.[3] Indeed, 500 g of food was sufficient to increase LM estimates of our participants (76.1 ± 13.7 kg), which consisted of recreationally active males and females of mixed physiques. Our study also demonstrated FW to decrease leg and arm LM, which was similarly reported by Nana et al.[2] Furthermore, we observed that whilst FO and FW differentially increased BM, DXA total mass and total LM that was similar to the intake mass (Supplementary Table 1), increases in trunk mass and trunk LM exceeded that of the intake mass on both occasions (Table 2). This result was not replicated following water consumption only following hypohydration. A redistribution of blood from the periphery to the trunk region resulting in gastrointestinal hyperaemia with food consumption is a likely cause,[26] as evidenced by a decrease in leg and arm LM following FW.

Hypohydration followed by water intake reduced and increased our participants' total and trunk LM respectively. This corroborates previous findings that fluid loss negatively impacts LM DXA estimates.[4,5,20] For example, Going et al[20] observed a 2.7% reduction in total LM in participants ($n = 17$) following 24 h without fluid intake. A staggered rehydration protocol allowed their participants to regain >99.7% of their LM. Similarly, when our participants consumed water (1 L) following hypohydration, total and trunk LM returned to ~99.9% of baseline euhydrated values. This was however reflected as a gain in the DXA estimates of these values pre-water consumption. Consuming water following hypohydration also returned BM and DXA total and trunk mass estimates to baseline (>99.8%), verifying participants successfully achieved the intended decrease in BM via our fluid restriction protocol. Additionally, it was observed that total and trunk mass, total and trunk LM estimates assessed at hypohydrated baseline were lower than baseline values assessed at FO and FW but were not different following water consumption (Table 2). This further confirms that participants were euhydrated at baseline for FO and FW. A small discrepancy (0.45%, within 1% agreement[27]) was observed between BM and total mass DXA estimate. This is due to the fact that DXA determines total mass via the summation of bone mineral, fat and fat-free soft tissue estimates, assuming water and lipid constancy in tissue composition across skin, adipose, muscle and bone tissue.[1,28] This assumption seldom holds true, especially following hypohydration, where regional variation in water loss occurs and acute water ingestion, where water still remains largely in the gut (Table 2).

One of the limitations of this study was that we did not conduct baseline duplicate DXA scans to determine precision error from which the meaningfulness of a change can be evaluated,[29] given ethical concerns surrounding the number of scans in such a short period of time. However, Buehring et al[30] reported their observed least significant change (LSC) 95% CI of LM of student athletes (30 males [20.6 y] and 30 females [19.9 y]) based on Lunar iDXA to be 575 g and 381 g for males and females respectively suggesting that food and/or fluid consumption ≥500 g may affect the precision of DXA LM estimates.[3,31] Other limitations include: a) differences in activities of participants prior to each assessment day were not fully controlled and b) food and fibre intake of participants prior to the assessment day were not fully controlled but rather relied on participants to maintain their habitual diet and replicate as much as possible prior to each session. It has been suggested that high fibre and low residue diets and gas may also introduce error in DXA estimates.[7]

In conclusion, our study demonstrated that standardised B-mode US measurement of SAT is robust against acute intake of FO, FW, or hydration status. Additionally, this research further verifies that acute food and food and water consumption, and hydration status will induce errors in DXA estimates of body composition, especially LM, and advocates the need for standardised client presentation prior to DXA scans.

Therefore, should changes in fat patterning be of main interest, B-mode US could be used in place of DXA to minimise exposure to ionising radiation. Should resources permit, it may be advantageous for practitioners to incorporate both DXA and US into their arsenal of assessment tools, given the advantage of DXA over US to quantify total and regional absolute tissue mass.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jsams.2022.03.016.

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Declaration of Interest Statement

None.

The authors declare that all personal data arising from this investigation remains confidential, and that no authors have any conflicts of interest to declare.

Confirmation of Ethical Compliance

Approval for this work to commence was granted by the Human Ethics Research Committee of the University of Western Australia (RA/4/20/4798) and was conducted in accordance to the National Statement on Ethical Conduct in Human Research (2007) - Updated 2018, under the National Health and Medical Research Council Act 1992.

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