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Original research

Measures of body composition via Dual-energy X-ray absorptiometry, ultrasound and skinfolds are not impacted by the menstrual cycle in active eumenorrheic females

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ABSTRACT

Objectives: (1) Compare changes in body composition estimates over the menstrual cycle in active females using Dual-energy X-ray absorptiometry, standardised brightness-mode ultrasound and skinfolds (2) Compare the predictability of Dual-energy X-ray absorptiometry fat mass estimate via standardised brightness-mode ultrasound versus skinfolds measurements.
Design: Thirty active females (27 ± 5 y) with regularly occurring menstrual cycles participated in a cross sectional study.
Methods: Participants completed four assessment sessions scheduled according to each individual's menstrual cycle. These sessions took place during their (1) early follicular, (2) mid-to-late follicular, (3) mid-luteal and (4) second early follicular phases. Body composition estimates were acquired using Dual-energy X-ray absorptiometry, subcutaneous adipose tissue thickness was measured at eight sites using standardised brightness-mode ultrasound and skinfolds.
Results: The sum of eight subcutaneous adipose tissue thickness measured using standardised brightness-mode ultrasound and skinfolds were not different between the cycle phases ($p > 0.05$). Body mass and Dual-energy X-ray absorptiometry total mass estimate as well as Dual-energy X-ray absorptiometry estimates of total and regional lean and fat mass were also not different between cycle phases ($p > 0.05$) and any changes were within the 95% confidence intervals of their respective least significant change values.
Conclusions: There were no true and meaningful changes in the sum of eight subcutaneous adipose tissue thickness measured via standardised brightness-mode ultrasound and skinfolds or Dual-energy X-ray absorptiometry total and regional tissue mass estimates across the menstrual cycle in active eumenorrheic females. Body composition may thus be assessed via these methods in this population at any cycle phase with standardised participant presentation.

Practical implications

- Standardised brightness-mode ultrasound is a reliable measure of subcutaneous adipose tissue thickness across the menstrual cycle. The sum of eight subcutaneous adipose tissue thickness measured via standardised brightness-mode ultrasound compared to skinfolds is in closer agreement to Dual-energy X-ray absorptiometry total fat mass estimate.
- Practitioners may conduct body composition assessments via brightness-mode ultrasound, skinfolds or Dual-energy X-ray absorptiometry on active eumenorrheic females at any phase in their menstrual cycle provided standard participant presentation protocols are adhered to.
- Researchers may include this population group as participants and need not account for variations in body composition due to the menstrual cycle in their study design

1. Introduction

Fluctuations in oestrogen, specifically oestradiol, and progesterone concentrations across the menstrual cycle (MC) may alter fat deposition, skin elasticity, thickness and hydration.[1] To date, various studies that determined whether different MC phases may affect body

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composition estimates have produced equivocal results. Some authors have reported body mass,[2–4] lean mass (LM) or fat-free mass,[5,6], fat mass (FM) or body fat % (%BF),[6,7] or skinfolds[2,3] to be relatively stable throughout the MC. However, others have observed LM changes between the early follicular (EF) to mid-late follicular (MF)[7] phase or FM changes between the EF, MF and mid-luteal (ML) phases.[5] Given the uncertainty to which the MC may have an impact on body composition estimates and thus interfere with monitoring such changes in active females, this study therefore aimed to further investigate the variability of body composition estimates across the MC of these individuals using standardised brightness-mode ultrasound (International Association of Sciences in Medicine and Sports [IASMS]) and skinfolds (SF) (International Society for the Advancement of Kinanthropometry [ISAK]) at eight measurement sites, along with Dual-energy X-ray absorptiometry (DXA). The standardised brightness-mode ultrasound (B-mode US) technique has been recently demonstrated to be highly accurate and reliable for measuring subcutaneous adipose tissue (SAT) in athletes of diverse sports and physiques[8,9] and non-athletes of various obesity classes.[10] Nevertheless, the specific impact of the MC on its validity and reliability is yet to be determined.

The standardised B-mode US method is also relatively new and foreign compared to DXA and ISAK SF, which are two of the most commonly utilised body composition assessment methods.[11] Collecting SF data is relatively cheap and convenient, making it a highly accessible and popular field assessment tool among practitioners. Nevertheless, inherent in the technique is a measure of a compressed double layer of SAT and skin.[8] Comparatively, by placing an US probe on the epidermis with a thick layer of gel (3 to 5 mm), a clear image of the SAT sandwiched between the skin and muscle fascia can be captured, avoiding skinfold measurement errors associated with dual skin layer inclusion, skin elasticity and SAT compression.[8,12] As opposed to SF and US which only samples the SAT, DXA is able to ascertain estimates of absolute total and regional body composition and hence viewed as a laboratory reference method.[12,13] Consequently, a secondary aim of this study was to examine how well B-mode US predicts and agrees with DXA total FM estimates via compared with skinfolds. Results of this study will therefore provide further clarity on the impact the MC may have on body composition assessment methods as well as determine the credibility and worthiness of standardised B-mode US as a plausible field assessment tool.

2. Methods

Thirty females (age = 27 ± 5 y, height = 162.9 ± 31.8 cm, mass = 61.20 ± 7.66 kg, MC length = 29 ± 3 d) were recruited to volunteer in this study via convenience sampling and provided written informed consent during the familiarisation session. Inclusion crietria included: normal body mass index (BMI: 18.5–24.9 $kg \cdot m^{-2}$), eumenorrheic, not using oral contraceptives or hormonal implants for ≥6 months at the time of recruitment and meeting Australia's Physical Activity & Sedentary Behaviour Guidelines for Adults.[14] The study protocol was approved by the Human Ethics Research Committee of the University of Western Australia (RA/4/20/5369).

Participants attended five separate sessions: a familiarisation session followed by four assessment sessions over 4–6 weeks, scheduled according to each individual's MC. Assessment sessions occurred during the EF (EF1, menses), MF, and ML phases, and at the start of the participant's new cycle (EF2). Body composition assessments were conducted under standardised participant presentation[15] using standardised B-mode US, skinfolds and DXA at each of these sessions and a finger-prick blood sample (0.5 ml) was taken during the MF and ML phase assessments for subsequent hormonal analysis to verify the MC phase of the participant.

Information on the participant's MC history and associated signs and symptoms as well as physical activities was collected electronically to ascertain their participation eligibility prior to their familiarisation session. During the familiarisation session, participants installed the smartphone app Clue (v5.15 onwards, BioWink GmBH, Adalbertstraße, Berlin, Germany) to monitor their MC for at least one complete cycle and throughout the study. Participants were provided a thermometer (Surgipack Flexitip Ovulation Digital Thermometer, Vega Technologies Inc., Dong Guan, China) and urinary ovulation test strips (Nantong Egens Biotechnology, Nantong, China) to measure their oral basal body temperature (BBT) and urinary luteinising hormone surge as an indicator of ovulation respectively. Additionally, participants were instructed to record, standardise and replicate their food intake and

Table 1
(a) Mean difference (± Standard Error) of Dual-energy X-ray absorptiometry total mass, total and regional lean and fat mass estimates and body mass between different menstrual cycle phases (b) Mean difference (± Standard Error) of sum of eight subcutaneous adipose thickness measurements via standardised brightness-mode ultrasound and sum of eight skinfolds between different menstrual cycle phases.

(a)	Same-day	Menstrual cycle phase						
	EF1R – EF1	MF – EF1	ML – EF1	ML - MF	TEM	%TEM	TEM-68% CI	TEM-95% CI
Σ of 8 US SAT thickness (mm)	-	-0.5 ± 0.6	0.5 ± 0.7	1.0 ± 0.7	0.7	0.9	1.0	2.0
Σ of 8 Skinfold thickness (mm)	-	-1.4 ± 0.9	-0.6 ± 1.0	0.8 ± 1.0	1.7	2.0	2.4	4.8
(b)							LSC-95% CI	
					RMS-SD	% CV	RMS-SD	% CV
Body Mass (g)	-	-279 ± 158	23 ± 169	302 ± 81				
Arms Lean (g)	34 ± 37	-70 ±45	-34 ± 48	37 ± 51	143	3.1	395	8.6
Arms Fat (g)	37 ± 19	-13 ± 19	-2 ± 21	11 ± 22	76	4.2	211	11.6
Legs Lean (g)	-30 ± 46	-47 ± 75	-83 ± 80	36 ± 86	175	1.2	486	3.3
Legs Fat (g)	-14 ± 28	-91 ± 47	-80 ± 50	11 ± 54	107	1.5	297	4.1
Trunk Lean (g)	105 ± 68	-88 ± 129	161 ± 138	249 ± 148	268	1.3	743	3.6
Trunk Fat (g)	27 ± 43	14 ± 70	33 ± 75	19 ± 80	166	2.4	460	6.7
Total Lean (g)	-23 ± 57	-196 ± 167	69 ± 179	265 ± 192	216	0.5	599	1.4
Total Fat (g)	19 ± 51	-86 ± 99	-46 ± 106	39 ± 113	194	1.2	538	3.3
Total Mass (g)	-1 ± 15	-287 ± 161	6 ± 173	293 ± 185	58	0.1	160	0.3

EF1R = Early follicular 1 (repeat), MF = mid-late follicular, ML = mid-luteal, RMS-SD = root-mean-square standard deviation, %CV = percent coefficient of variation, LSC = least significant change, CI = confidence interval, SAT = Subcutaneous adipose tissue, TEM = Technical error of measurement. A one way analysis of variance (ANOVA) of the S8US and S8SF at EF1 was performed to calculate the respective technical error of measurement (TEM) of the technician where TEM = $\sqrt{(MSe)}$, $\sqrt{(MSe)}$ = mean square error and the 95% CI for true change = $2 \times \sqrt{2} \times$TEM.

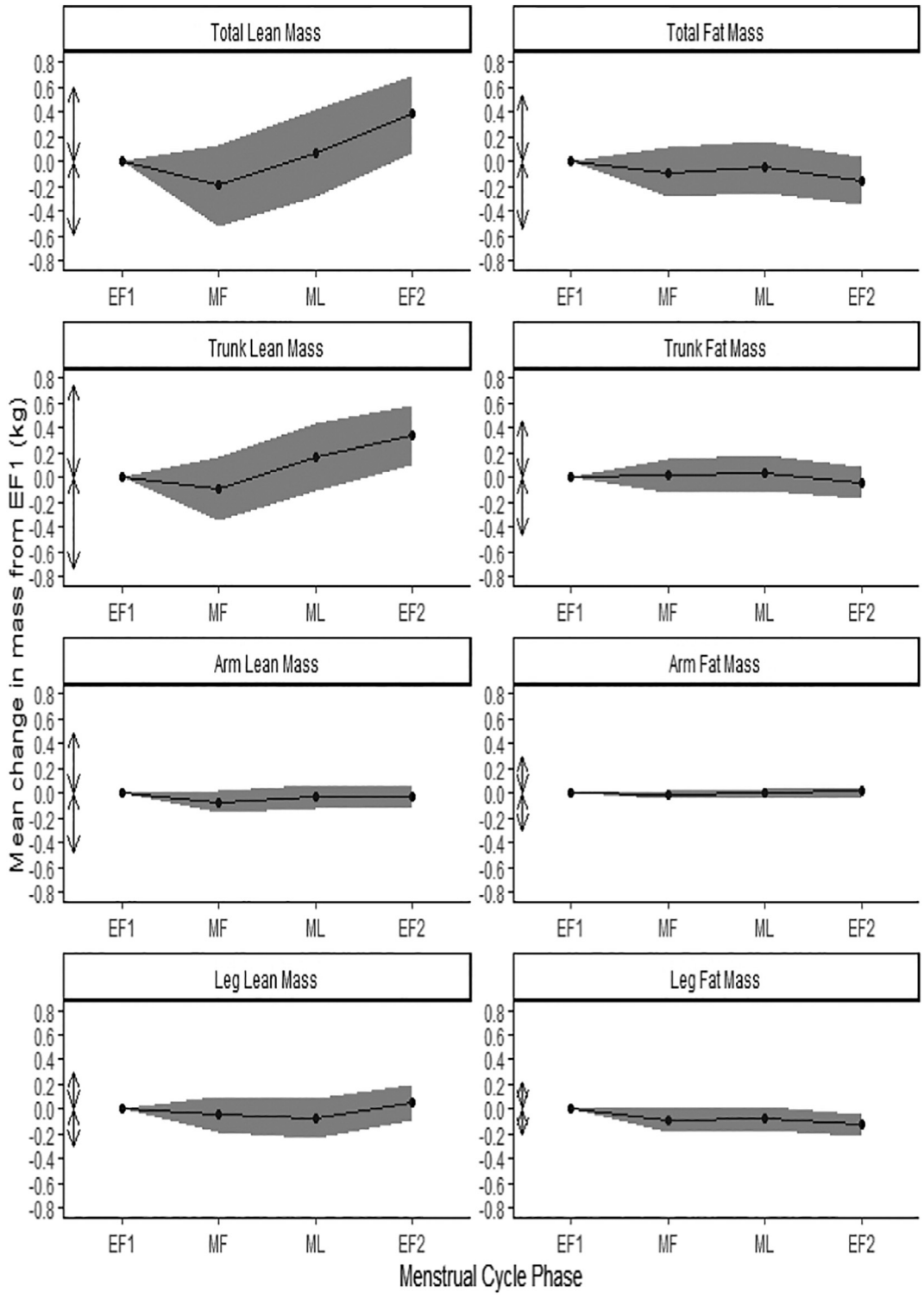
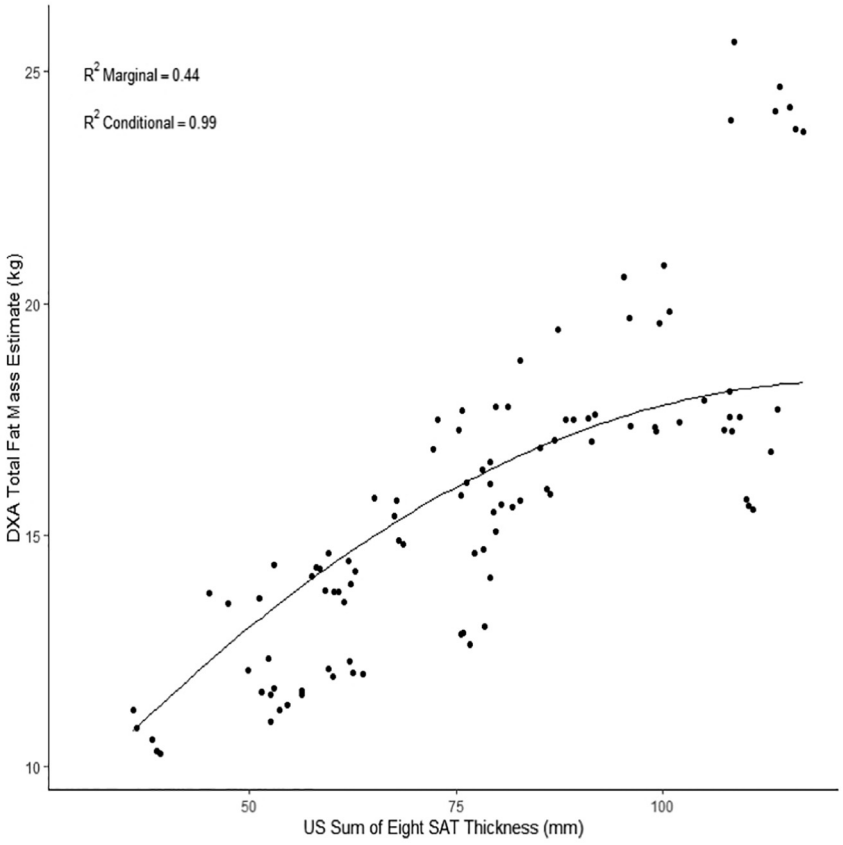


Fig. 1. Estimated change in means of lean and fat mass from the early follicular phase (EF1, menses) at each subsequent phase of the menstrual cycle (technical error and biological variation). MF = mid-late follicular phase, ML = mid-luteal phase ML, EF2 = early follicular phase 2 (new cycle). Grey areas indicate 95% CI from mean change. Arrows indicate least significant change (LSC-95% CI) as a measure of true and meaningful change. All estimates were computed with linear mixed effects model to adjust for the within-individual dependent data.

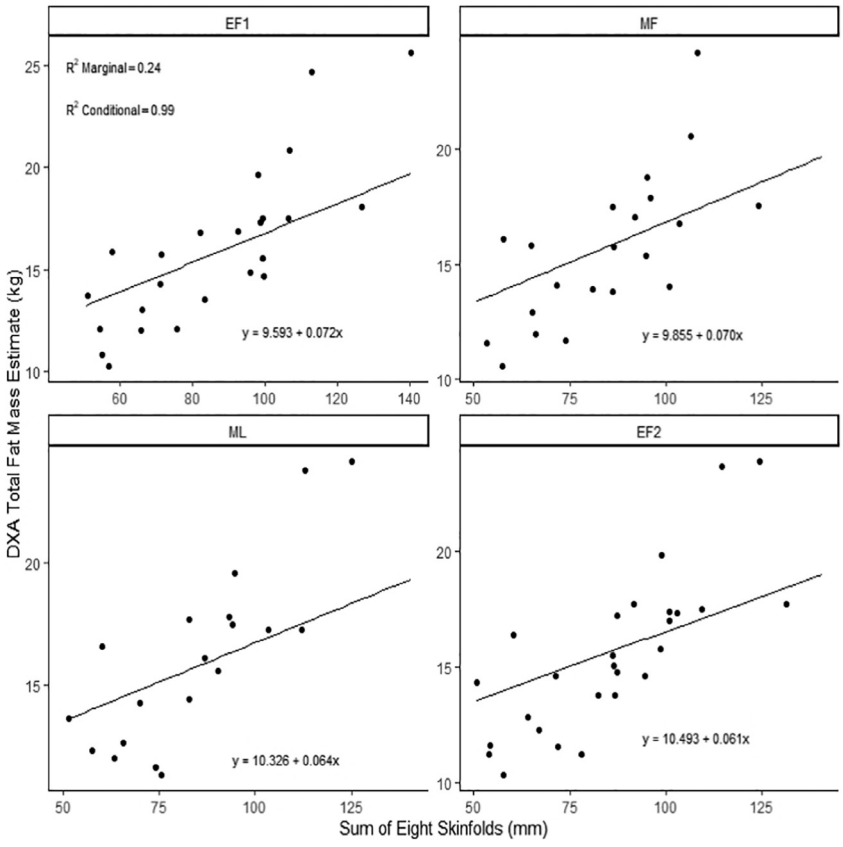
exercise on the day prior to each assessment session. They were also reminded to maintain their dietary and exercise habits throughout the study. Participants were requested to notify the investigators at (1) the onset of menses and (2) when they observed a sustained elevation in BBT plus a positive result on their urinary ovulation test strip. If a positive test was not registered by 7 days after the app predicted ovulation date, the predicted date was then taken as the acutal ovulation date. Subsequent body composition assessment sessions were scheduled according to the predicted cycle phases of the participant's MC after self-monitoring one complete cycle.

Participants were instructed to adhere to standardised presentation protocols[13] prior to each assessment day and consume an additional 500 ml of fluid to standardise hydration in the morning of the assessment.[16] Upon arrival to the laboratory each assessment morning,

(a)


R² Marginal = 0.44
R² Conditional = 0.99
DXA Total Fat Mass Estimate (kg)
US Sum of Eight SAT Thickness (mm)

(b)


EF1
MF
ML
EF2
R² Marginal = 0.24
R² Conditional = 0.99
y = 9.593 + 0.072x
y = 9.855 + 0.070x
y = 10.326 + 0.064x
y = 10.493 + 0.061x
DXA Total Fat Mass Estimate (kg)
Sum of Eight Skinfolds (mm)

participants were marked up IASMS protocol [9,17] and US images were captured using B-mode US (Telemed Echo Blaster 128 EXT-1Z, REV:C, Milan, Italy) with a linear transducer (Telemed Linear Transducer HL9.0/40/128Z-4, Milan, Italy) and requisite software (Telemed Echo Wave II v3.2.0, Milan, Italy) in duplicates. The Fat Analysis Tool software (v3.3, Rotosport, Graz, Austria) was used to evaluate SAT thicknesses from the US images and a sum of eight SAT thicknesses (including embedded fibrous structures, S8US) was used for data analysis. One same advanced level IASMS accredited technician performed all the US measurements. When determined accurately, S8US can detect changes in SAT mass with an accuracy of about 0.2 kg[8]. Participants were also marked up as per ISAK protocol[18] and skinfold thickness measurements (British Indicators, Hertfordshire, UK) were conducted in duplicates at each site by one same ISAK Level 1 anthropometrist. A third measure was repeated if the first two measures differed by > 5% and the subsequent mean or median thickness value was recorded. A sum of eight skinfolds (S8SF) was used for data analysis. Body mass (BM) was measured on digital platform scales (August Sauter GmbH, Ebingen, Germany) to the nearest 0.01 kg and height was measured using a wall-mounted stadiometer to the nearest 0.1 cm (Novel Products Inc., Illinois, USA).

For all DXA scans, participants were aligned on the scanning bed according to the Nana et al. positioning protocol[13] and standardised with velcro straps and customised radiolucent foam blocks i.e. a constant distance of 22 cm between the feet and 5 cm between the palms and trunk. Scans were performed were performed in standard thickness mode (GE Lunar iDXA, GE Medical Systems, Wisconsin, USA) and anaylsed using GE enCORE v16.0 by one trained technician. Regions of interest in the scans automatically demarcated by the software were subsequently confirmed by the investigator. Dual-energy X-ray absorptiometry scans at EF1 were performed in duplicate to determine precision error and least significant change with 95% confidence (LSC-95% CI).[19]

A fingertip capillary blood sample (0.5 ml) was collected (MiniCollect® tubes, Z Serum Separator, Greiner Bio-One GmbH, Kremsmünster, Austria) at assessments scheduled during each participant's MF and ML phase. Samples were centrifuged at 4000*g* for 10 min at 4 °C and frozen at −80 °C for subsequent analysis of serum progesterone concentration via enzyme-linked immunosorbent assay (Human Progesterone ELISA Kit ab108670, Abcam, Melbourne, Australia). All samples were analysed in duplicates using the same kit to avoid inter-assay variability. The intra- and inter-assay coefficients of variation reported by the manufacturer were 4.0 and 9.3%, respectively. Serum progesterone concentrations $< 3\ ng \cdot ml^{-1}$ and $\geq 5\ ng \cdot ml^{-1}$ verified assessments were indeed undertaken during MF and ML phases respectively.

Comparison between serum progesterone concentrations collected at the MF and ML phases were made using a paired samples *t*-test. Linear mixed effects models were estimated to evaluate the mean differences in S8US, S8SF and DXA tissue mass estimates between the phases of the MC. Results of modelled data are reported as mean ± standard error (SE). The fixed and random effects in the model were the MC phases (four levels) and the participants respectively. Precision of DXA estimates are reported as the root-mean-square SD (RMS-SD) and percentage coefficient of variation (%CV) with LSC-95% CI calculated from these values.[19] Ordinary least square regression analysis using linear mixed effects models was also used to assess the agreement between S8US and S8SF vs. DXA total FM estimation. All linear mixed models were computed using R (v4.1 RStudio Inc., Massachusetts, USA) with the *nlme* and *emmeans* packages and statistical significance was set at $p < 0.05$.

3. Results

Serum progesterone concentrations confirmed the MC phases with a 25 fold increase in progesterone ($p < 0.01$) in the ML compared with the MF phase. Body composition data from five participants acquired during the MF phase and seven participants during the ML phase were excluded from subsequent data analysis due to their progesterone concentrations falling outside the criterion values during the phase of their assessment.

The mean change ± SE values of S8US and S8SF between each phase of the MC and the respective technical error of measurement (TEM) of the technician are described in Table 1a. Changes in S8US (p = 0.51 – 0.88) and S8SF (p = 0.44 – 0.93) were within the TEM-95% CI of the technician and not different across the MC. Body mass and DXA total mass estimates were not different between the MC phases ($p > 0.05$). A breakdown of the mean changes in total and regional DXA LM and FM estimates at each phase of the MC are further described in Table 1b, along with the precision error for each region, represented as the %CV, with the RMS-SD and LSC-95% CI. Mean changes in these estimates were within their respective LSC-95% CIs and not different between the phases. Mean changes in total and regional DXA LM and FM estimates at different MC phases, together with changes during the new cycle (EF2) with respect to EF1 are further illustrated in Fig. 1.

A quadratic relationship that was MC phase independent was found between S8US and DXA total FM estimates across the MC. A marginal $R^2 = 0.44$ indicated S8US alone accounted for 44% of the variability in DXA total FM estimates and this increased to 99% (conditional $R^2 = 0.99$) when individual variability of the MC was included. Additionally, as S8US increased, DXA total FM estimates were predicted to increase at a decreasing rate (Fig. 2a). Comparatively, a linear relationship dependent on the MC phase (i.e. the linear relationship is different for each phase) was found between S8SF and DXA total FM estimates. A marginal $R^2 = 0.24$ indicated S8SF alone accounted for 24% of the variability in DXA total FM estimates and this increased to 99% (conditional $R^2 = 0.99$) when individual variability of the MC was included (Fig. 2b).

4. Discussion

Our study did not observe any changes to the participant's mean S8US and S8SF across the MC. Changes in total and regional DXA LM or FM estimates between different MC phases were also not significant and less than their respective LSC-95% values and hence not considered true and meaningful. A secondary finding was that a quadratic relationship, where the agreement was independent of the MC phase, was found between S8US and DXA total FM estimates. In contrast, a linear relationship where the agreement was dependent on the phase of the MC was found between S8SF and DXA total FM estimates. These results have implications for the use and interpretation of DXA, S8US and S8SF to monitor body composition in physically active eumenorrheic females.

Using B-mode US, Perin, et al.[20] previously reported the SAT thicknesses of both the abdomen and thigh of ten participants to vary between a maximum during the EF and a nadir during ML phase, with an amplitude of 1.3 mm and 1.1 mm for the abdomen and thigh respectively. However, neither variations reached significance, which the authors attributed to the small sample of participants. In contrast, our study found the SAT thicknesses of both the upper abdomen and front thigh to vary between a maximum during the ML and a nadir during

Fig. 2. Linear mixed effects models were employed to model the relationship between measurements of total fat mass and subcutaneous adipose tissue and skinfolds whilst adjusted for the within-individual dependent data. Polynomial relationships and interactions between measurements and menstrual cycle phases were tested. (a) Quadratic relationship between S8US and DXA total fat mass estimate that is independent of menstrual cycle phase. (b) Linear relationship between S8SF and DXA estimates of total fat mass that varies with different menstrual cycle phases. S8US = sum of eight ultrasound measures of subcutaneous adipose tissue, S8SF = sum of eight skinfolds, DXA = Dual-energy X-ray absorptiometry.

EF phase with a much smaller amplitude of 0.2 mm and 0.1 mm respectively. Nevertheless, neither SAT variations in these sites ($p = 0.24$, 0.83) nor S8US between the MC phases reached significance ($p = 0.58$). Accordingly, our study is the first to employ the IASMS protocol to assess the possible impact of the MC on changes in S8US.

Oestrogen has been documented to promote increases in skin thickness,[21] so it is plausible that fluctuations across the MC may impact skinfold assessments . Despite not measuring changes in skin thickness independently from SAT thickness in our study, this did not affect our overall measure of S8US (which includes skin thickness) or S8SF. Indeed, mean differences in S8SF between the MC phases were not significant in our participants ($p = 0.49$) and were within the TEM-95% CI of our investigator. Accordingly, no true change in S8SF occurred across the MC, which corroborates the results of previous investigations.[2,3] This further suggests that practitioners may measure the S8US or S8SF of their female participants at any phase in their MC.

Women's energy intake can increase by 380–2100 $kJ \cdot day^{-1}$ during the luteal compared to the follicular phase due to changes in ovarian hormones levels,[22] that may result in weight gain just prior to menses, or during late luteal phase. Indeed, Johnson, et al.[23] reported a significant increase of 686 kJ from the MF to ML phase in 26 females (32 ± 4 y). Nevertheless, the authors did not observe any changes in BM or %BF over the MC. Congruent with other studies,[2,4,6] our study also did not observe any difference in BM between the MC phases. Notably, Byrd, Thomas[4] observed no changes in BM, density or %BF of participants in a study that extended across two MCs. No changes in BM or % BF were also observed in studies that employed DXA to compare body composition across the MC phases. For example, DXA estimates of BM, BF% and LM in fifty-one females (18–45 y) were reported not to vary across the MC phases in a study by Champion, et al.[24] Our study is the first however to compare and report no absolute mean changes in total and regional DXA FM and LM estimates across different MC phases. Body mass and composition stability in active females across the MC may be due to an increased resting[25] or sleeping[26] metabolic rate coupled with greater post-exercise energy expenditure and fat utilisation[27] despite increased energy intake and unchanged exercise energy expenditure in the ML phase.[23]

Skinfolds and US both quantify the SAT as a surrogate estimate of total body fat, which make up about 80–90% of anatomically detectable fat mass.[8] In our study, we found S8US better than S8SF (marginal $R^2 = 0.44$ compared to $R^2 = 0.24$) to agree with and predict DXA total FM estimates (which includes visceral adipose tissue that accounts for the remaining 10–20%). Given that a S8US measurement error of 1.4 mm can translate into a SAT mass error of up to 0.2 kg[8], this is quite a promising result for S8US since the ability of S8US to accurately predict DXA total FM estimates is further compounded by daily biological variations that contribute to the precision error in DXA total FM estimates. Indeed, Zemski, et al.[28] demonstrated that the consecutive-day precision error can be almost twice as large for DXA total FM estimates in comparison to same-day precision error. Additionally, accounting for the fact that individual variation (~300%) in SAT compressibility and skin thickness can further diminish the accuracy of S8SF,[8] S8US may compartively provide a superior estimation of body fat.

Acknowledging that fluctuating levels of oestrogen and progesterone may affect fluid regulation and distribution,[29] one of the limitations of our study was we did not determine changes in total body water, which may have impacted body composition estimates. However, total body water has been demonstrated to be relatively stable between the MC phases via deuterium dilution.[30,31] Another limitation of this study was that we were unable to include all the body composition estimates for all of the 30 participants at each of the intended phases of their cycle due to either participant availability, inaccuracies in cycle phase prediction or technical error during hormonal analysis. We were however able to collect body composition data from all 30 participants in duplicates at baseline (EF1), which enabled us to calculate the precision error and LSC-95% CIs of DXA body composition estimates as recommended,[19] to verify true changes between MC phases. Should the monitoring of longitudinal changes in body composition be required however, calculating precision error and LSC-95% CIs values from analysis of consecutive-day rather than same-day DXA scans may be even more advantageous since both technical error and biological variation will be accounted for when interpreting accuracy and meaning.[28]

5. Conclusion

To our knowledge, this is the first controlled study to investigate the reliability of using standardised B-mode US as a novel measure of S8US variation across the MC in active eumenorrheic females. We also examined impact of the MC on DXA estimates of total and regional body composition measurements and S8SF in this population and compared the level of predictability of S8US and S8SF to DXA in estimating total fat mass. Our results showed that S8US is a reliable measure of SAT thickness across the MC and is a better predictor of DXA total FM estimates compared to S8SF. Our results also suggest that the MC does not contribute to additional biological variability to DXA body composition estimates, provided standardised participant presentation protocols are adhered to prior to assessment. However, it must be noted that these results may only apply to physically active eumenorrheic females of normal BMI between the ages of 18 to 36 and may be invalid for females that experience menstrual disturbances or changes (amenorrhoea, oligomenorrhea, perimenopausal), or are sedentary with BMI > 24.9 $kg \cdot m^{-2}$. Therefore should resources permit, future studies could include oligomenorrheic females as well as having a participant sample of diverse physical activity levels, race/ethnicities, and body mass and composition profiles assessed over multiple cycles to provide better insights into intra- and inter-individual variation in body composition across the MC.

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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/4/20/5369) 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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