ABSTRACT

Dual energy x-ray absorptiometry (DXA) is a popular technique used to quantify physique in athletic populations. Due to biological variation, DXA precision error (PE) may be higher than desired. Adherence to standardised presentation for testing has shown improvement in consecutive-day PE. However, the impact of short-term diet and physical activity standardisation prior to testing has not been explored. This warrants investigation, given the process may reduce variance in total body water and muscle solute, both of which can have high daily flux amongst athletes. Twenty (male n = 10, female n = 10) recreationally active individuals (age: 30.7±7.5 yrs; stature: 176.4±9.1 cm; mass: 74.6±14.3 kg) underwent three DXA scans; two consecutive scans on one day, and a third either the day before or after. In addition to adhering to standardised presentation for testing, subjects recorded all food/fluid intake plus activity undertaken in the 24 hours prior to the first DXA scan and replicated this the following 24 hours. International Society of Clinical Densitometry recommended techniques were used to calculate same-day and consecutive-day PE. There was no significant difference in PE of whole-body fat mass (479 vs. 626 g) and lean mass (634 vs. 734 g) between same-day and consecutive-day assessments. Same-day and consecutive-day PE of whole-body fat mass and lean mass were less than the smallest effect size of interest. Inclusion of 24 hours standardisation of diet and physical activity has the potential to reduce biological error further but this needs to be verified with follow-up investigation.

INTRODUCTION

Physique assessment is commonly undertaken amongst athletic populations to evaluate longitudinal adaptations in response to training and/or dietary interventions. However, adaptations in physique exhibited by highly trained individuals are usually small (Argus et al., 2010; Harley et al., 2011; Lees et al., 2017), requiring a highly precise assessment tool. While precision of multi-component models is high (Withers et al., 1999), resource constraints typically ensures use is restricted to research activities. In practice, dual energy x-ray absorptiometry (DXA) has gained popularity in the assessment of elite athletes for its ability to assess body composition, incorporating measures of whole body, and regional lean mass (LM) and fat mass (FM) (Meyer et al., 2013). However, the ability of DXA to validate small, but potentially important longitudinal changes in body composition may be questioned, especially when precision is quantified via consecutive day assessments, which takes into consideration both technical and biological sources of error.

In an attempt to facilitate the standardisation of DXA data capture, clear recommendations on data acquisition and reporting have been established that account for a range of variables potentially contributing to technical and biological error (Hind et al., 2018). While issues such as subject positioning and clothing worn, plus demarcation of regional composition, are clearly articulated, control of biological error is limited to specifying subjects should present in a rested, overnight fasted state after voiding the bladder. Unfortunately, this likely fails to account for biological variation in estimates of LM that can arise from fluctuations in gastrointestinal content (Kerr et al., 2017; Nana et al., 2012), total body water (TBW) content (Rodriguez-Sanchez & Galloway, 2015; Toomey et al., 2017) and muscle solutes (Bone et al., 2017; Rouillier et al., 2015). This is particularly relevant in athletes who have the potential for larger fluctuations in hydration status and intramuscular solutes such as creatine and glycogen

over a short time frame (Bone et al., 2017). Given this, the impact of standardised diet and physical activity in advance of a DXA scan has been recommended for investigation (Farley et al., 2021; Rose et al., 2021; Rouillier et al., 2015), in the hope of further enhancing precision, when quantified from consecutive day scans.

To our knowledge, no previous investigation has examined the reliability of measurement using standardised dietary intake and physical activity on same-day and consecutive-day DXA precision error (PE). The aim of this investigation was to establish the PE in physique assessment using DXA with best practice protocols in recreationally active individuals whilst standardising dietary intake and physical activity for 24 hours between consecutive day measures. We hypothesised that standardised diet and physical activity would minimise the biological variation in consecutive-day measures so that same-day and consecutive-day PE would be statistically equivalent.

METHODS

Participants

Twenty recreationally active (Tier 1;(McKay et al., 2022)) **Caucasian adults (male n = 10, female n = 10) volunteered to participate in this investigation**. Characteristics of all individuals are presented in Table 1. All subjects were informed of the nature and possible risks of the investigation before giving their written informed consent. The investigation was approved by the Regional Committees for Medical and Health Research Ethics in Norway (2017/2160), in accordance with the declaration of Helsinki.

Experimental Design

An overview of the investigation is presented in Figure 1. In brief, each subject underwent three identical testing sessions over a 24-hour period with each measurement taken by the same technician and conducted soon after waking and at the same time of day under standardised presentation conditions (overnight fasted and well rested). Each testing session commenced with the body mass of subjects measured in minimal clothing followed by a total body DXA scan. The first scan on day 2 (D2S1) was repeated consecutively following re-positioning (D2S2) to quantify technical error (tester and technique error). A third scan was randomly assigned on the day before or after the repeat scans (D3S3), to quantify consecutive-day biological error (between day variance in estimates of body composition).

Subject Presentation

Instructions were provided to subjects in advance of testing days to encourage adherence to standardised presentation for all three of the tests (D2S1, D2S2 and D3S3) as per current best practice guidance (Hind et al., 2018). Subjects were required to present overnight fasted (including nil fluid intake), bladder voided and well rested (no prior physical activity) on the mornings before D2S1 and D3S3. They were asked to wear minimal fitted clothing (i.e. underwear) with metal objects and jewellery removed, plus clothing checked for metal zips or studs. Additionally, prior to both days of scanning, participants were instructed to remain well hydrated, consume their normal diet and partake in recreational activity as usual. Participants were also instructed to document all food and fluid intake, plus physical activity undertaken in the first 24 hours via a food and activity diary and replicate this in the second 24 hours (i.e., Day 2), in order to minimise biological variation over the testing period. Participants were advised to select commonly available foods so that they could replicate intake as closely as possible on the day following. Food and fluid intake were analyzed on

both days using Foodworks (version 10.0, Xyris Software, Sydney, Australia). Activity diaries were visually inspected to confirm physical activity compliance

Dual Energy X-ray Absorptiometry

All DXA scans were undertaken in the total body mode on a narrow fan beam DXA scanner scanner (Lunar iDXA, GE Healthcare, Madison, WI) with analysis performed using GE enCORE v.13.60 software (GE Healthcare) with the NHANES reference database. The DXA was calibrated with phantoms as per the manufacturer's guidelines each day before measurements were taken. All scans were conducted by the same radiation health licensed technician using the standard thickness mode as determined by the auto scan feature in the software and all safety protocols as per the institution's radiation safety protection plan were adhered to. The scans were performed according to a protocol developed that emphasises a consistent positioning of subjects on the DXA scanning bed as previously described i.e. Nana protocol (Kerr et al., 2016). Two Velcro straps were used to minimise any subject movement during the scan as well as provide a consistent body position for subsequent scans. One strap was secured around the ankles above the foot positioning pad and the other strap was secured around the trunk at the level of the mid forearms to assist in maintaining the hands in the mid prone position while secured within the hand positioning pads. All scans were analysed automatically by the DXA software, but all regions of interest were reconfirmed before being included in the subsequent statistical analysis.

Statistical Analysis

Statistical analyses were conducted using R version 3.6.1 (R Core Team, 2019). Same-day and consecutive-day PE was established by calculating the root-mean-square standard deviation (RMS-SD) and within-subject coefficient of variation (CV) between repeated

measures following guidelines of the International Society for Clinical Densitometry (ISCD) (Lewiecki et al., 2016). The 95% confidence intervals of precision error estimates were calculated using the chi-square distribution (Leslie & Moayyeri, 2006). Coefficients of determinant were also calculated to determine test-retest correlation of repeated measures for same-day and consecutive-day assessments. Systematic and proportional bias between repeated trials were inspected using Bland-Altman plots with 95% limits of agreement (Giavarina, 2015). Paired sample t-tests were conducted to test for differences between repeated measures and identify systematic bias. An alpha value of 0.05 was used to indicate statistical significance and the mean bias reported with 95% confidence intervals.

Traditional null-hypothesis testing and equivalence testing was used to test the hypothesis that consecutive-day PE would be statistically equivalent to same-day PE with standardised diet and exercise. Paired sample t-tests on within-subject CV estimates were used to determine if there was a difference between same-day and consecutive-day PE. The 'TOSTER' package (D. Lakens, 2017) was used to determine whether any increase in consecutive-day PE compared to same-day PE was less than the smallest effect size of interest (Daniël Lakens et al., 2018). The upper equivalence bound was set at 1%, which corresponds to acceptable inter-rater reliability for anthropometric assessment (Carsley et al., 2019). Further, it was anticipated that an increase in error of 1% due to biological variation would result in acceptable error as recommended by the International Society of Clinical Densitometry (2% and 3% for lean mass and fat mass, respectively) and based on the same-day PE of best practice protocols reported in previous research (Hind et al., 2018; Kerr et al., 2017).

RESULTS

There were no differences in dietary intake between days, including total energy (9606±2311 kJ vs. 9616±2304 kJ, p = 0.77), carbohydrate (283±82 g vs. 283±81 g, p = 0.89), fat (73±14 g vs. 73±15 g, p = 0.67), protein (124±34 g vs. 124±35 g, p = 0.64), fluid (1986±317 ml vs. 2001±326 ml, p = 0.26) or sodium intake (1881±283 mg vs. 1890±326 mg, p = 0.52) . Similarly, training diaries confirmed compliance with replication of physical activity each day.

The same-day PE of whole body and regional body composition measures are presented in Table 2, showing an absence of heteroscedasticity. There were no differences between repeated same-day measures of body composition ($p \geq 0.1$), except for a systematic decrease in whole body lean mass (Δ = -157 [290] g, P = 0.03, Figure 2), which was well below the corresponding lean mass least significant change (LSC). Coefficients of determination showed there were almost perfect test-retest correlations between same-day measures of whole-body bone mineral content, fat mass and lean mass (Figure 2). There were also almost perfect test-retest correlations between same-day measures of regional bone mineral content (R2 = 0.994 – 0.999), fat mass (R2 = 0.995 – 0.998) and lean mass (R2 = 0.992 – 0.998).

Table 3 presents the consecutive-day PE of whole-body and regional body composition measures. Assessments of absolute and relative PE were higher for consecutive-day than same-day measures, except for some regional fat mass measures. There were no differences between repeated consecutive-day measures of whole-body or regional body composition ($p \geq 0.12$). Figure 3 presents the test-retest correlations and Bland-Altman plots showing the agreement and absence of heteroscedasticity between whole-body measures on consecutive days. There were almost perfect correlations between consecutive-day measures of whole-body and regional bone mineral content (R2 = 0.993 – 0.998), fat mass (R2 = 0.995 – 0.999)

and lean mass (R2 = 0.989 – 0.999).

Figure 4 presents the outcome of inferiority tests establishing whether any difference between same-day and consecutive-day PE is equal to or less than the smallest effect size of interest (i.e., $\Delta \geq 1\%$). There was no significant increase in PE of whole-body fat mass ($\Delta = 0.3\%$ [-0.2 – 0.9%], $p = 0.22$) and lean mass ($\Delta = 0.05\%$ [-0.16 – 0.26%], $p = 0.62$) between same-day and consecutive-day assessments. Additionally, the difference between same-day and consecutive-day PE of whole-body fat mass ($t = -2.6$, $p = 0.009$) and lean mass ($t = -9.7$, $p < 0.001$) was less than the smallest effect size of interest. There was a significant difference in PE between same-day and consecutive-day assessments for whole-body bone mineral content ($\Delta = 0.2\%$ [0.04 – 0.35%], $p = 0.015$), however the increase in PE was less than the smallest effect size of interest ($t = -11$, $p < 0.001$). These results show that consecutive-day PE for whole-body fat mass, lean mass and bone mineral content is statistically equivalent to same-day PE.

There were similar results for most regional body composition measures. The same-day and consecutive-day PE of regional arm, leg and trunk lean mass ($\Delta = 0.04 – 0.5\%$, $t = -5.2 – -2.9$, $p \leq 0.005$) and bone mineral content ($\Delta = 0.12 – 0.33\%$, $t = -7.2 – -3.6$, $p \leq 0.001$) were equivalent or less than the smallest effect size of interest (i.e., $\Delta \geq 1\%$). The equivalence of same-day and consecutive-day PE for regional fat mass measures were inconclusive, meaning there was inadequate statistical power to determine whether there was a difference between repeated measures or if repeated measures were equivalent, except for arms fat mass ($\Delta = -0.4\%$, $t = -2.4$, $p = 0.013$). There were no significant differences between same-day or consecutive day PE for leg ($\Delta = 0.3\%$ [-0.7 – 1.2%], $p = 0.07$) and trunk fat mass ($\Delta = 0.97\%$ [-0.6 – 2.5%], $p = 0.21$). However, the hypothesis that the difference in PE of leg ($t = -1.6$, p

= 0.07) and trunk fat mass was equal to or greater than the smallest effect size of interest could not be rejected (t = 0.05, p = 0.48).

DISCUSSION

This study investigated the impact of short term (24 hour) diet and physical activity standardisation, in addition to adherence to current best practice, on PE of DXA derived estimates of total body, as well as regional FM and FFM. Diet and physical activity standardisation prior to DXA appears to reduce biological error, as inferred from the fact no differences in PE were observed when assessed on consecutive days compared to the same day, but this needs to be verified with follow up investigation.

It has been argued that PE is best quantified using consecutive-day data, given this takes into consideration both technical and biological error (Zemski et al., 2019). However, when this is undertaken using current best practice guidance for data capture (Hind et al., 2018), PE has previously been elevated sufficiently to a level that it may draw into question the validity of DXA for tracking longitudinal changes in physique traits amongst athletic populations (Farley et al., 2021; Zemski et al., 2019). However, unlike prior research by our group (Farley et al., 2021; Zemski et al., 2019), there was no difference in PE for BMC, FM and LM when calculated via same-day vs consecutive-day assessments in the current investigation. Whole body LM LSC was three times smaller in the current investigation compared to Zemski et al (734 g vs. 2083 g), while whole body FM LSC was reduced by half (626 g vs. 1261 g) (Zemski et al., 2019). This observation was also observed for regional FM and LM including trunk, arms, and legs, with similar improvements in PE observed when contrasted against the data of Farley et al. (Farley et al., 2021), using a similar study protocol

but without pre-test diet and physical activity standardisation. The implementation of diet and physical activity standardisation prior to DXA scans undertaken on consecutive days is proposed to be primarily responsible for the marked reduction in LSC estimates when compared to prior data from our group.

Prior studies in which hydration status and muscle solute content have acutely been manipulated suggest variance in these biological variables influence DXA derived body composition estimates. For example, adherence to a high carbohydrate diet for 3 days resulted in a significant increase in DXA derived LM, presumably because of an associated increase in muscle glycogen (Rouillier et al., 2015), as has been proposed elsewhere (Toomey et al., 2017). However, adherence to a similarly high carbohydrate diet for one day had no influence on estimates of LM (Tinsley et al., 2017), perhaps because the duration of enhanced carbohydrate ingestion was insufficient to substantially influence glycogen storage. To date, only one investigation has systematically explored the influence of change in muscle glycogen on DXA derived body composition estimates. Bone et al. confirmed diet and exercise manipulations to both increase and decrease muscle glycogen (as confirmed via muscle biopsies), resulted in significant elevations and reductions in LM, respectively, and reflected associated changes in TBW (Bone et al., 2017). A similar outcome was observed with creatine supplementation, because of the elevation in TBW in response to creatine loading. Perhaps not surprisingly, manipulation of carbohydrate and creatine status had no influence of estimates of FM and bone mass, given their low water content.

Given the influence of TBW change on DXA derived estimates of LM, it is not surprising activities that result in hydration status variance also have a similar impact on DXA derived

estimates of body composition (Rodriguez-Sanchez & Galloway, 2015; Toomey et al., 2017). Thus, while variance in muscle solute content and TBW clearly increase biological error, their standardisation likely moderates error, as has been postulated in this investigation. The time frame of diet and physical activity standardisation necessary to 'normalise' muscle solute and TBW to a point where it mitigates biological error of DXA scans remains to be confirmed. While a 24-hour period appeared to be adequate in this investigation, it could be argued a longer period may be necessary amongst athletic populations given the potential for larger fluctuations in hydration status and intramuscular solutes over a short time frame (Bone et al., 2017).

The authors acknowledge that there are limitations of the study design that may have affected findings. Firstly, the sample size of participants was slightly below that recommended by the ISCD for calculating LSC. Secondly, the findings are largely compared to those of Zemski et al who used a Hologic Discovery A DXA machine (Zemski et al., 2019), as compared to the present investigation which used a GE Lunar iDXA. It might be speculated that comparing the results of DXA machines from different manufacturers could give reason as to why such markedly different PE was found between the consecutive-day scans. However, it must be acknowledged that the results of same-day scans (technical error only) were similar, along with mean participant age, stature and mass. Thus, the large difference seen in consecutive-day scanning data is thus hypothesised to be attributable to reduced biological variation. This warrants follow up investigation, in which diet and physical activity standardisation is directly contrasted against PE generated from the same individuals when standardisation is not enforced. We would also advocate in future studies for provision of food to participants so as to enhance dietary compliance relative to self-reported intake (Jeacocke & Burke, 2010). Furthermore, activity monitors should be integrated into future studies so as to better

confirm the impact of physical activity guidance on both incidental and structured physical activity, in place of the subjective nature of a training diary. Given assessments were undertaken across ~48 hour period, consideration was not given to phase of menstrual cycle amongst women volunteers, given this does not influence DXA estimates of body composition (Ong et al., 2022), or any effect is trivial (Thompson et al., 2021).

Currently, the easiest and most practical way to minimise the biological ‘‘noise’’ associated with undertaking a DXA scan is to have a standardised scanning protocol with fasted and rested subjects (Hind et al., 2018). Any potential further improvement in PE from standardised diet and physical activity prior to testing may be important for athletic populations as the ability to track small changes may facilitate more refined interventions for diet and training, and thus enhancing athletic performance. Feasibility of implementing such standardisations among athletic populations requires consideration for its practicality.

In conclusion, the standardisation of diet and physical activity modelled in this study for DXA scanning of body composition changes has the potential to enhance PE for whole body LM, FM and BMC in a recreationally active population. More research on the effects of standardised diet and physical activity on biological PE is warranted before we can advocate for the integration of pre-test diet and physical activity standardisation into current best practice methods.

Authorship: The authors' responsibilities were as follows; GS, GP, IG and JLA helped in the study concept and design; GS helped in the acquisition of data; GS and LH assisted in analysis and interpretation of data; AF helped in drafting the manuscript; GS, GP, IG, JLA, LH and AF assisted in the critical revision of the manuscript for important intellectual content; LH helped in statistical analysis; and GS in study supervision. GS had full access to all the data in the investigation and takes responsibility for the integrity and the accuracy of the data analysis.

Conflict of interest: The results of this investigation are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The authors have no financial or personal conflicts of interest to declare.

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References

Argus, C. K., Gill, N., Keogh, J., Hopkins, W. G., & Beaven, C. M. (2010). Effects of a short-term pre-season training programme on the body composition and anaerobic performance of professional rugby union players. *Journal of Sports Sciences, 28*(6), 679-686.

Bone, J. L., Ross, M. L., Tomcik, K. A., Jeacocke, N. A., Hopkins, W. G., & Burke, L. M. (2017). Manipulation of Muscle Creatine and Glycogen Changes Dual X-ray Absorptiometry Estimates of Body Composition. *Medicine and Science in Sports and Exercise, 49*(5), 1029-1035.

Carsley, S., Parkin, P. C., Tu, K., Pullenayegum, E., Persaud, N., Maguire, J. L., & Birken, C. S. (2019). Reliability of routinely collected anthropometric measurements in primary care. *BMC Med Res Methodol, 19*(1), 84.

Farley, A., Slater, G. J., & Hind, K. (2021). Short-Term Precision Error of Body Composition Assessment Methods in Resistance-Trained Male Athletes. *International Journal of Sport Nutrition and Exercise Metabolism, 31*(1), 55-65.

Giavarina, D. (2015). Understanding Bland Altman analysis. *Biochem Med (Zagreb), 25*(2), 141-151.

Harley, J. A., Hind, K., & O'Hara J, P. (2011). Three-compartment body composition changes in elite rugby league players during a super league season, measured by dual-

299 energy X-ray absorptiometry. *Journal of Strength and Conditioning Research, 25*(4),
300 1024-1029.
301 Hind, K., Slater, G., Oldroyd, B., Lees, M., Thurlow, S., Barlow, M., & Shepherd, J. (2018).
302 Interpretation of Dual-Energy X-Ray Absorptiometry-Derived Body Composition
303 Change in Athletes: A Review and Recommendations for Best Practice. *Journal of*
304 *Clinical Densitometry, 21*(3), 429-443.
305 Jeacocke, N. A., & Burke, L. M. (2010). Methods to standardize dietary intake before
306 performance testing. *International Journal of Sport Nutrition and Exercise*
307 *Metabolism, 20*(2), 87-103.
308 Kerr, Slater, G. J., & Byrne, N. (2017). Impact of food and fluid intake on technical and
309 biological measurement error in body composition assessment methods in athletes.
310 *British Journal of Nutrition, 117*(4), 591-601.
311 Kerr, Slater, G. J., Byrne, N., & Nana, A. (2016). Reliability of 2 Different Positioning
312 Protocols for Dual-Energy X-ray Absorptiometry Measurement of Body Composition
313 in Healthy Adults. *Journal of Clinical Densitometry, 19*(3), 282-289.
314 Lakens, D. (2017). Equivalence Tests: A Practical Primer for t Tests, Correlations, and Meta-
315 Analyses. *Soc Psychol Personal Sci, 8*(4), 355-362.
316 Lakens, D., Scheel, A. M., & Isager, P. M. (2018). Equivalence Testing for Psychological
317 Research: A Tutorial. *Advances in Methods and Practices in Psychological Science,*
318 *1*(2), 259-269.
319 Lees, M. J., Oldroyd, B., Jones, B., Brightmore, A., O'Hara, J. P., Barlow, M. J., . . . Hind, K.
320 (2017). Three-Compartment Body Composition Changes in Professional Rugby
321 Union Players Over One Competitive Season: A Team and Individualized Approach.
322 *Journal of Clinical Densitometry, 20*(1), 50-57.
323 Leslie, W. D., & Moayyeri, A. (2006). Minimum sample size requirements for bone density
324 precision assessment produce inconsistency in clinical monitoring. *Osteoporosis*
325 *International, 17*(11), 1673-1680.
326 Lewiecki, E. M., Binkley, N., Morgan, S. L., Shuhart, C. R., Camargos, B. M., Carey, J. J., . .
327 . Leslie, W. D. (2016). Best Practices for Dual-Energy X-ray Absorptiometry
328 Measurement and Reporting: International Society for Clinical Densitometry
329 Guidance. *Journal of Clinical Densitometry, 19*(2), 127-140.
330 McKay, A. K. A., Stellingwerff, T., Smith, E. S., Martin, D. T., Mujika, I., Goosey-Tolfrey,
331 V. L., . . . Burke, L. M. (2022). Defining Training and Performance Caliber: A
332 Participant Classification Framework. *International Journal of Sports Physiology and*
333 *Performance, 17*(2), 317-331.
334 Meyer, N. L., Sundgot-Borgen, J., Lohman, T. G., Ackland, T. R., Stewart, A. D., Maughan,
335 R. J., . . . Muller, W. (2013). Body composition for health and performance: a survey
336 of body composition assessment practice carried out by the Ad Hoc Research
337 Working Group on Body Composition, Health and Performance under the auspices of
338 the IOC Medical Commission. *British Journal of Sports Medicine, 47*(16), 1044-
339 1053.
340 Nana, A., Slater, G. J., Hopkins, W. G., & Burke, L. M. (2012). Effects of daily activities on
341 dual-energy X-ray absorptiometry measurements of body composition in active
342 people. *Medicine and Science in Sports and Exercise, 44*(1), 180-189.
343 Ong, J. N., Ducker, K. J., Furzer, B. J., Dymock, M., & Landers, G. J. (2022). Measures of
344 body composition via Dual-energy X-ray absorptiometry, ultrasound and skinfolds are
345 not impacted by the menstrual cycle in active eumenorrheic females. *Journal of*
346 *Science and Medicine in Sport, 25*(2), 115-121.

Rodriguez-Sanchez, N., & Galloway, S. D. (2015). Errors in dual energy x-ray absorptiometry estimation of body composition induced by hypohydration. *International Journal of Sport Nutrition and Exercise Metabolism, 25*(1), 60-68.

Rose, G. L., Farley, M. J., Slater, G. J., Ward, L. C., Skinner, T. L., Keating, S. E., & Schaumberg, M. A. (2021). How body composition techniques measure up for reliability across the age-span. *American Journal of Clinical Nutrition, 114*(1), 281-294.

Rouillier, M. A., David-Riel, S., Brazeau, A. S., St-Pierre, D. H., & Karelis, A. D. (2015). Effect of an Acute High Carbohydrate Diet on Body Composition Using DXA in Young Men. *Annals of Nutrition and Metabolism, 66*(4), 233-236.

Thompson, B. M., Hillebrandt, H. L., Sculley, D. V., Barba-Moreno, L., & Janse de Jonge, X. A. K. (2021). The acute effect of the menstrual cycle and oral contraceptive cycle on measures of body composition. *European Journal of Applied Physiology, 121*(11), 3051-3059.

Tinsley, G. M., Morales, F. E., Forsse, J. S., & Grandjean, P. W. (2017). Impact of Acute Dietary Manipulations on DXA and BIA Body Composition Estimates. *Medicine and Science in Sports and Exercise, 49*(4), 823-832.

Toomey, C. M., McCormack, W. G., & Jakeman, P. (2017). The effect of hydration status on the measurement of lean tissue mass by dual-energy X-ray absorptiometry. *European Journal of Applied Physiology, 117*(3), 567-574.

Withers, R. T., Laforgia, J., & Heymsfield, S. B. (1999). Critical appraisal of the estimation of body composition via two-, three-, and four-compartment models. *American Journal of Human Biology, 11*(2), 175-185.

Zemski, A. J., Hind, K., Keating, S. E., Broad, E. M., Marsh, D. J., & Slater, G. J. (2019). Same-Day Vs Consecutive-Day Precision Error of Dual-Energy X-Ray Absorptiometry for Interpreting Body Composition Change in Resistance-Trained Athletes. *Journal of Clinical Densitometry, 22*(1), 104-114.

Table 1. Characteristics and physique measures in the participant cohort. Data presented are the average of same-day and consecutive-day repeated measures.

Variable	Cohort (n = 20) Mean (SD)	Males (n = 10) Mean (SD)	Females (n = 10) Mean (SD)
Age (yrs)	30.7 (7.5)	31.7 (6.1)	29.8 (8.9)
Stature (cm)	176.4 (9.1)	182.2 (6.2)	170.6 (7.8)
Body mass (kg)	74.6 (14.3)	86.1 (8.8)	63.0 (7.6)
WB BMC (g)	2997 (549)	3444 (238)	2550 (370)
WB FM (g)	15968 (7198)	18241 (8896)	13696 (4332)
WB LM (g)	56178 (11115)	65277 (6118)	47080 (6277)
A BMC (g)	409 (101)	500 (48)	319 (35)
A FM (g)	1556 (651)	1706 (811)	1407 (431)
A LM (g)	6791 (2105)	8619 (1091)	4963 (859)
L BMC (g)	1145 (206)	1316 (66)	974 (142)
L FM (g)	5846 (2063)	5384 (2007)	6308 (2117)
L LM (g)	18957 (3488)	21853 (1568)	16061 (2144)
T BMC (g)	910 (187)	1040 (127)	780 (142)
T FM (g)	7745 (5081)	10180 (6097)	5309 (2036)
T LM (g)	27375 (5245)	31241 (4025)	23508 (2944)

WB: whole-body. A: arms. L: legs. T: trunk. BMC: bone mineral content. FM: fat mass. LM: lean mass.

Table 2. Same-day precision error of physique assessment using dual-energy X-ray absorptiometry in recreationally active males and females.

Variable	Day 2 Scan 1 Mean (SD)	Day 2 Scan 2 Mean (SD)	Absolute change Mean (SD), [95% CI]	Percent change Mean (SD), [95% CI]	P value	RMS-SD, [95% CI]	LSC	CV (%), [95% CI]	LSC (%)
WB BMC (g)	2998 (552)	3001 (549)	3 (15) [-4, 10]	0.1 (0.6) [-0.2, 0.4]	0.38	10 [8, 14]	28	0.4 [0.3, 0.6]	1.1
WB FM (g)	15912 (7189)	15968 (7264)	56 (245) [-59, 171]	0.2 (1.9) [-0.7, 1.1]	0.32	173 [132, 253]	479	1.3 [1, 1.9]	3.6
WB LM (g)	56253 (11125)	56096 (11165)	-157 (290) [-293, -21]	-0.3 (0.5) [-0.5, -0.1]	0.03	229 [174, 334]	634	0.4 [0.3, 0.6]	1.1
A BMC (g)	410 (102)	410 (101)	0 (4) [-2, 2]	0.1 (1) [-0.4, 0.6]	0.75	3 [2, 4]	8	0.7 [0.5, 1]	1.9
A FM (g)	1558 (650)	1554 (671)	-4 (56) [-30, 22]	-0.7 (4.1) [-2.6, 1.2]	0.78	39 [30, 57]	108	2.9 [2.2, 4.2]	8
A LM (g)	6776 (2124)	6795 (2103)	20 (112) [-32, 72]	0.4 (1.6) [-0.3, 1.1]	0.44	78 [59, 114]	216	1.1 [0.8, 1.6]	3
L BMC (g)	1147 (207)	1145 (206)	-2 (11) [-7, 3]	-0.2 (0.9) [-0.6, 0.2]	0.39	7 [5, 10]	19	0.6 [0.5, 0.9]	1.7
L FM (g)	5830 (2040)	5833 (2058)	3 (160) [-72, 78]	0 (3.8) [-1.8, 1.8]	0.93	110 [84, 161]	305	2.6 [2, 3.8]	7.2
L LM (g)	19016 (3539)	18896 (3510)	-120 (312) [-266, 26]	-0.6 (1.7) [-1.4, 0.2]	0.10	231 [176, 337]	640	1.2 [0.9, 1.8]	3.3
T BMC (g)	910 (189)	913 (190)	3 (14) [-4, 10]	0.3 (1.5) [-0.4, 1]	0.37	10 [8, 15]	28	1.1 [0.8, 1.6]	3
T FM (g)	7702 (5072)	7761 (5131)	59 (182) [-26, 144]	0.6 (2.8) [-0.7, 1.9]	0.16	132 [100, 193]	366	2 [1.5, 2.9]	5.5
T LM (g)	27405 (5167)	27356 (5297)	-49 (283) [-181, 83]	-0.3 (1) [-0.8, 0.2]	0.45	198 [151, 289]	548	0.7 [0.5, 1]	1.9

SD: standard deviation. RMS-SD: root-mean-square standard deviation. LSC: least signficant change. CV: coefficient of varation. WB: whole-body. A: arms. L: legs. T: trunk. BMC: bone mineral content. FM: fat mass. LM: lean mass. P Value: Alpha values were derived using paired sample t-tests between repeated measures on the same day.

Table 3. Consecutive-day precision error of physique assessment using dual-energy X-ray absorptiometry in recreationally active males and females.

Variable	Day 2 Scan 1 Mean (SD)	Day 3 Scan 3 Mean (SD)	Absolute change Mean (SD)	Percent change Mean (SD)	P value	RMS-SD, [95% CI]	LSC	CV (%), [95% CI]	LSC (%)
WB BMC (g)	2998 (552)	2992 (548)	-7 (23)	-0.2 (0.8)	0.22	17	47	0.6	1.7

			[-18, 4]	[-0.6, 0.2]		[13, 25]		[0.5, 0.9]	
WB FM (g)	15912 (7189)	16024 (7147)	112 (306) [-31, 255]	1 (2.3) [-0.1, 2.1]	0.12	226 [172, 330]	626	1.7 [1.3, 2.5]	4.7
WB LM (g)	56253 (11125)	56186 (11061)	-67 (379) [-244, 110]	-0.1 (0.8) [-0.5, 0.3]	0.44	265 [202, 387]	734	0.5 [0.4, 0.7]	1.4
A BMC (g)	410 (102)	408 (102)	-1 (6) [-4, 2]	-0.3 (1.4) [-1, 0.4]	0.41	4 [3, 6]	11	1 [0.8, 1.5]	2.8
A FM (g)	1558 (650)	1557 (633)	-1 (52) [-25, 23]	0.3 (4) [-1.6, 2.2]	0.92	36 [27, 53]	100	2.6 [2, 3.8]	7.2
A LM (g)	6776 (2124)	6802 (2090)	26 (104) [-23, 75]	0.6 (1.6) [-0.1, 1.3]	0.27	74 [56, 108]	205	1.2 [0.9, 1.8]	3.3
L BMC (g)	1147 (207)	1144 (204)	-3 (12) [-9, 3]	-0.2 (1.1) [-0.7, 0.3]	0.30	9 [7, 13]	25	0.8 [0.6, 1.2]	2.2
L FM (g)	5830 (2040)	5875 (2098)	44 (188) [-44, 132]	0.6 (3.5) [-1, 2.2]	0.31	134 [102, 196]	371	2.4 [1.8, 3.5]	6.6
L LM (g)	19016 (3539)	18959 (3433)	-56 (384) [-236, 124]	-0.2 (1.9) [-1.1, 0.7]	0.52	268 [204, 391]	742	1.4 [1.1, 2]	3.9
T BMC (g)	910 (189)	907 (184)	-3 (14) [-10, 4]	-0.2 (1.9) [-1.1, 0.7]	0.39	10 [8, 15]	28	1.3 [1, 1.9]	3.6
T FM (g)	7702 (5072)	7770 (5044)	68 (226) [-38, 174]	2.1 (6.1) [-0.8, 5]	0.19	163 [124, 238]	452	4.1 [3.1, 6]	11.4
T LM (g)	27405 (5167)	27363 (5290)	-42 (478) [-266, 182]	-0.2 (1.8) [-1, 0.6]	0.70	331 [252, 483]	917	1.3 [1, 1.9]	3.6

SD: standard deviation. RMS-SD: root-mean-square standard deviation. LSD: least signficant change. CV: coefficient of varation. WB: whole-body. A: arms. L: legs. T: trunk. BMC: bone mineral content. FM: fat mass. LM: lean mass. P Value: Alpha values were derived using paired sample t-tests between repeated measures between days.

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Figure 1. An overview of the investigation, incorporating assessment of body composition on three occasions over a 24 hour period, including two scans on oneday, plus another scan randomly assigned on the day before or after the repeat scans.

Figure 2. Test-retest correlation and Bland-Altman plots showing agreement and mean bias in same-day measures of whole-body fat mass (a and b), whole-body bone mineral content (c and d) and whole-body lean mass (e and f).

Figure 3. Test-retest correlation and Bland-Altman plots showing agreement and mean bias in consecutive-day measures of whole-body fat mass (a and b), whole-body bone mineral content (c and d) and whole-body lean mass (e and f).

Figure 4. Inferiority test examining equivalence of same-day and consecutive-day precision error of (a) whole-body fat mass, (b) whole-body bone mineral content, and (c) whole-body lean mass. Data are the group mean difference (±95% confidence intervals) in same-day and consecutive-day precision error (within-subject coefficient of variation). When the 95% confidence intervals are to the right of the vertical line indicating no difference (solid line) then biological error accounts for a significant increase in precision error in consecutive-day testing compared to the technical error established with same-day testing (e.g. figure 4b). When the 95% confidence intervals are completely below the upper equivalence bound (vertical dashed line) then consecutive-day precision error can be considered equivalent to same-day precision error based on the smallest effect size of interest (i.e. $\Delta \geq 1\%$).

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