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2 **Short Term Precision Error of Body Composition Assessment Methods in**
3 **Resistance Trained Male Athletes**

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21 **Abstract**

22 Athletic populations require high precision body composition assessment to identify true
23 change. Least Significant Change (LSC) determines technical error via same-day consecutive
24 tests but doesn't integrate biological variation, being more relevant for longitudinal
25 monitoring. The aim of this study was to assess biological variation using LSC measures from
26 body composition methods used on athletes, including surface anthropometry (SA), air
27 displacement plethysmography (BOD POD), dual-energy X-ray absorptiometry (DXA) and
28 bioelectrical impedance spectroscopy (BIS). Thirty-two athletic males (age: 31 ± 7 yr; stature:
29 183 ± 7 cm; mass 92 ± 10 kg) underwent three testing sessions over two days using four
30 methods. LSC values were calculated from differences in Day¹Test¹ vs Day¹Test² (same-day
31 precision), as well as Day¹Test¹ vs Day² (consecutive-day precision). There was high
32 agreement between same-day and consecutive-day FM and FFM measurements for all
33 methods. Consecutive-day precision error (PE) in comparison to same-day, was 50% higher
34 for FM estimates from BIS (3607g vs 2331g), 25% higher from BODPOD (1943g vs 1448g)
35 and DXA (1615g vs 1204g), but negligible from SA (442g vs 586g). Consecutive-day PE for
36 FFM was 50% higher from BIS (3966g vs 2276g) and SA (1159g vs 568g), and 25% higher
37 from BODPOD (1894g vs 1450g) and DXA (1967g vs 1461g) than same-day. PE in
38 consecutive-day analysis considers both technical error and biological variation, enhancing
39 identification of small, yet significant changes in body composition of resistance trained
40 male athletes. Given change in physique is likely to be small in this population, the use of
41 DXA, BOD POD or SA is recommended.

42 *Key Words:* Body composition, least significant change, BOD POD, dual energy x-ray
43 absorptiometry, bioelectrical impedance spectroscopy, anthropometry, fat mass, fat free
44 mass.

45 **Introduction**

46 The association between athletic physique traits and competitive sporting success is well
47 established (Meyer et al., 2013; Olds, 2001). In sports requiring high force production,
48 athletes with high levels of muscularity can gain a competitive advantage (Bilsborough,
49 Greenway, Livingston, Cordy, & Coutts, 2016; Gabbett, 2009; Olds, 2001) yet these athletes
50 tend to see only small adaptations or improvements in physique over time (Binkley,
51 Daughters, Weidauer, & Vukovich, 2015; Harley, Hind, & O'Hara, 2011; Lees et al., 2017;
52 Smart, Hopkins, & Gill, 2013). Given this, assessment methods with high precision are
53 required to measure body composition on a regular basis in these athletes. By accurately
54 quantifying changes in physique, more refined training and dietary interventions may be
55 implemented which can positively influence performance outcomes (Slater et al., 2005).

56

57 A variety of body composition assessment methods are available to quantify fat free mass
58 (FFM) and fat mass (FM) (Ackland et al., 2012; Kerr, Slater, & Byrne, 2017). Depending on
59 time and resources, the four most popular methods used on athletic populations are air
60 displacement plethysmography (BODPOD), dual energy x-ray absorptiometry (DXA),
61 bioelectrical impedance spectroscopy (BIS) and surface anthropometry (SA) (Meyer et al.,
62 2013). Despite differences in technology, resources and technical expertise required, they
63 are all susceptible to technical error and biological variation (Ackland et al., 2012; Meyer et
64 al., 2013), significantly affecting precision (Kerr et al., 2017; Kerr, Slater, & Byrne,
65 2018). Technical error is influenced by quality control procedures such as subject clothing
66 (D. A. Fields, Hunter, & Goran, 2000; Vescovi, Zimmerman, Miller, & Fernhall, 2002), and
67 positioning during assessment (Kerr, Slater, Byrne, & Nana, 2016; Lambrinoudaki et al.,
68 1998; Tegenkamp, Clark, Schoeller, & Landry, 2011) level of technical expertise (Hume &

69 Marfell-Jones, 2008; Ruiz, Colley, & Hamilton, 1971) and equipment calibration (Marfell-
70 Jones, Stewart, & de Ridder, 2012). Biological variation may result from food and fluid
71 ingestion or exercise prior to assessment and appears to influence most body composition
72 methods albeit to different degrees (Bone et al., 2016; Kerr et al., 2017). Other biological
73 variables known to impact on estimates of body composition include body temperature and
74 skin moisture (Fields, Higgins, & Hunter, 2004), gastrointestinal contents (Bone et al., 2016)
75 and muscle solutes (Rouillier, David-Riel, Brazeau, St-Pierre, & Karelis, 2015).

76

77 While quantification of precision error (PE) is frequently done by calculating differences in
78 same-day repeat assessments of body composition (Hangartner, Warner, Braillon,
79 Jankowski, & Shepherd, 2013; Hind et al., 2018) using least significant change (LSC) values,
80 this fails to account for biological variability in the absence of controls, and can be evident
81 during longitudinal monitoring (Meyer et al., 2013). Given this, we have advocated for
82 identifying the LSC for same-day (technical error) and consecutive-day (biological variation)
83 precision in estimates of FM and FFM, finding a more accurate interpretation of true and
84 meaningful change (Zemski et al., 2019). Currently, the precision of BOD POD, BIS and SA
85 using LSC values for same-day and consecutive-day analysis has not been explored.
86 Therefore, aims of this study were to 1) to establish the same-day technical error of the four
87 methods and 2) determine the consecutive-day PE of the methods using LSC values to
88 determine the threshold of meaningful change in resistance trained male athletes.

89

90 **Methods**

91 *Subjects*

92

93 Thirty-two Caucasian volunteers participated in this study and met the inclusion criteria
94 which included being male gender, at least two or more years resistance training
95 experience, and with a BMI of ≥ 25 . Subjects were excluded from the study if they were >190
96 cm tall due to the limitation of the active scanning area of the DXA bed. Characteristics of all
97 individuals are presented in Table 1. All subjects were informed of the nature and possible
98 risks of the investigation before giving their written informed consent. This study was
99 conducted according to the guidelines laid down in the Declaration of Helsinki and all
100 procedures involving subjects were approved by the Human Research Ethics Committee of
101 the University of the Sunshine Coast (Ethics Approval No. S/12/450).

102

103 *Experimental design*

104

105 Each subject underwent three testing sessions during a 24-h window over a two day period
106 (Fig. 1) with each measurement taken by the same technician. The sessions commenced
107 with body mass and stretch stature measured in minimal clothing, a total body DXA scan
108 immediately followed by a BIS estimation of total body water (TBW), a BOD POD test and an
109 assessment of subcutaneous FM via the skinfold technique, in that sequence. Each subject
110 undertook tests 1 (D1T1) and 2 (D1T2) on day 1 under standardised conditions (early
111 morning, overnight fasted, well rested and bladder voided). D1T2 was undertaken
112 immediately after D1T1 and test 3 (D2) was undertaken the following morning, 24 hours
113 after D1T1. Comparison of these testing sessions allowed the calculation of typical error of
114 measurement (TEM), random consecutive-day biological variability, and the difference in
115 estimates of body composition data.

116

117 *Subject Presentation*

118

119 Guidance was provided on both days to encourage adherence to standardised presentation
120 for all three of the tests (D1T1, D1T2 and D2). Subjects were required to present overnight
121 fasted, bladder voided and well rested (no prior physical activity) on the mornings before
122 D1T1 and D2. They were asked to wear minimal fitted clothing with metal objects and
123 jewellery removed, plus clothing checked for metal zips or studs. Hydration status was
124 assessed by a mid-stream sample of urine provided by the subjects early on both mornings
125 before testing (D1T1 and D2). The specific gravity of the urine sample was measured using a
126 digital refractometer (UG-Alpha; Atago Corporation). All subjects voided their bladder
127 before tests.

128

129 *Dual-energy X-ray absorptiometry*

130 All DXA scans were undertaken in the total body mode on a pencil beam DXA scanner (Lunar
131 DPX; GE Healthcare) with analysis performed using GE enCORE version 13.60 software (GE
132 Healthcare) with the combined Geelong/Lunar reference database. CV for the laboratory
133 being 0.1, 2.2, 0.6 and 1.0% for BM, FM, lean mass and BMC respectively. The DXA was
134 calibrated with phantoms as per the manufacturer's guidelines each day before
135 measurements were taken. All scans were conducted by the same Queensland Radiation
136 Health licensed technician using the standard thickness mode as determined by the auto
137 scan feature in the software and all safety protocols as per the Institution's Radiation Safety
138 Protection Plan were adhered to. The scans were performed according to a protocol
139 developed that emphasises a consistent positioning of subjects on the DXA scanning bed
140 (Alisa Nana, Gary J Slater, Will G Hopkins, & Louise M Burke, 2012a) as previously described

141 (Alisa Nana, Gary J. Slater, Will G. Hopkins, & Louise M. Burke, 2012b). In addition, two
142 Velcro straps were used to minimise any subject movement during the scan as well as
143 provide a consistent body position for subsequent scans. One strap was secured around the
144 ankles above the foot positioning pad and the other strap was secured around the trunk at
145 the level of the mid forearms (Kerr et al., 2016). All scans were analysed automatically by
146 the DXA software, but all regions of interest were reconfirmed before being included in the
147 subsequent statistical analysis.

148

149 *Bioelectrical impedance spectroscopy*

150

151 Immediately after each DXA scan, whilst the subjects were still positioned on the DXA
152 scanning bed, body composition derived from TBW obtained values, was measured using
153 the SFB7 BIS device (ImpediMed, Brisbane, Australia). Subject positioning was standardised
154 (Kyle et al., 2004) to ensure supine positioning on the non-conductive foam mattress
155 without contact to the metal side supports of the DXA scanner for a minimum of 15 min
156 before BIS measurements (Ward, Isenring, Dyer, Kagawa, & Essex, 2015). The BIS was
157 calibrated as per the manufacturer's instructions with each participant's stature, body mass,
158 age and sex programmed into the unit. Sites of attachment for the electrodes (ImpediMed)
159 were first shaved and cleaned with alcohol wipes before the dual-tab electrodes were
160 attached as follows: one electrode was attached centrally on the top side of the wrist in
161 alignment with the ulnar head and 5 cm lower on the dorsal surface of the hand. The
162 second electrode was attached centrally on the dorsal surface of the ankle between the
163 lateral and medial malleoli and 5 cm lower on the dorsal surface of the foot which is in
164 accordance with previous guidelines (Ava Kerr, Slater, Byrne, & Chaseling, 2015). The SFB7

165 measures impedance using 256 frequencies between 4 and 1024 kHz to estimate TBW
166 based on a Cole-Cole plot (Cornish, Ward, Thomas, Jebb, & Elia, 1996). Three measurements
167 were taken consecutively and the median of these used in subsequent analysis. The TBW
168 value, as per the Pace et al model (Pace & Rathbun, 1945), was used to estimate body
169 composition of FFM and FM by simple subtraction from body mass.

170

171 *Air displacement plethysmography*

172

173 Immediately after BIS measurement, assessment of body density was undertaken using the
174 BOD POD (Life Measurement Instruments) following the recommended procedures of the
175 manufacturer (Dempster & Aitkens, 1995) utilising a validated, predicted thoracic lung
176 volume (VTG) estimation (McCrorry, Molé, Gomez, Dewey, & Bernauer, 1998). Subjects wore
177 Lycra clothing and a silicone swim cap, with all metal objects removed before measurement.
178 Body density was calculated by the BOD POD's software system (COSMED version 5.3.2) as
179 follows:

180

$$181 \quad D \text{ (density) } = \frac{\text{Mass (scale)}}{\text{Volume (BOD POD)}}$$

182 An estimate of FM and FFM was obtained to calculate %BF as defined by the Siri equation
183 (Siri, 1961), as follows:

$$184 \quad \%BF = (497.1 / \text{body density}) - 451.9.$$

185

186 *Surface anthropometry*

187

188 Immediately after completion of the BOD POD assessment, duplicate skinfold

189 measurements were taken according the International Society of the Advancement of
190 Kinanthropometry (ISAK) technique by the same technician certified by ISAK as previously
191 described (Norton et al., 1996).

192

193 The sum of eight skinfolds was determined following measurements of the triceps, biceps,
194 sub scapulae, iliac crest, supra-spinale, abdominal, quadriceps and calf skinfold using a
195 calibrated skinfold caliper (Harpenden; Baty International). Due to the similar procedure,
196 equipment and population used, the 4C validated Evans equation of three skinfolds (triceps,
197 abdominal and thigh) was utilised to calculate %BF as (Evans, Rowe, Misic, Prior, &
198 Arngrímsson, 2005):

199

$$200 \quad \%BF = 8.997 + 0.24658 \times (3SKF) - 6.343 \times (gender) - 1.998 \times (race),$$

201 Gender coded as 0 = female, 1 = male, and race coded as 0 = white, 1 = black.

202

203 Stretch stature was measured with a stadiometer (Harpenden; Holtain Limited) to the
204 nearest 0.1 cm. Body mass was measured on a calibrated scale to the nearest 0.01 kg (SECA
205 GMBH).

206

207 *Statistical analysis*

208

209 Data analysis was performed using Microsoft Excel (Microsoft, Redmond, WA). Descriptive
210 data are reported as the mean \pm standard deviation (SD). The precision is reported as the
211 root mean-square SD (RMS_ SD) and percentage coefficient of variation (%CV). The resulting
212 LSC with 95% confidence intervals (LSC_ 95% CI) were calculated following the ISCD protocol
213 (Hangartner et al., 2013). The %CV was derived from the equation $\%CV = (SD / \text{mean}) * 100$.
214 Coefficients of determination (R^2) were calculated for measurements to establish the
215 relationship between same-day and consecutive-day measures. Paired t tests were utilized
216 to test for differences based on same-day versus consecutive-day test results and precision
217 for all techniques. Statistical significance was set at 0.05. Bland Altman plots were created
218 to compare individual same-day and consecutive-day precision for all techniques.

219

220 **Results**

221 Descriptive statistics for the participants in this study are given in Table 1. The mean
222 differences between same-day (technical error) and consecutive-day (technical error and
223 biological variation) for FM and FFM in all methods are shown in Table 2. Differences
224 between same-day and consecutive-day testing demonstrating the LSC values for all
225 methods are given in Figure 2.

226

227 Table 3 shows the PE for each method of testing, represented as the %CV, with the RMS-SD,
228 LSC and %LSC. Strong agreement was found for all methods for same-day, and for
229 consecutive-day FM regression analysis (SA $R^2 = 1.00 - 1.00$, BOD POD $R^2 = 0.99 - 0.99$, DXA
230 $R^2 = 1.00 - 0.99$, BIS $R^2 = 0.98 - 0.96$) as shown in Figures 3 and 4. Regression analysis
231 undertaken for same-day and consecutive-day FFM for all methods revealed strong
232 relationships (SA $R^2 = 1.00 - 1.00$, BOD POD $R^2 = 0.99 - 0.99$, DXA $R^2 = 0.99 - 0.99$, BIS $R^2 =$

233 0.99 – 0.96) as shown in Figures 5 and 6.

234

235 Bland Altman analysis revealed SA had the smallest level of bias between same-day and
236 consecutive-day precision for FM (83 g) and FFM (178 g) with very low limits of agreement
237 (FM: -7 g to 173 g; FFM: -185 g to 172g). DXA and BOD POD had low levels of bias between
238 same-day and consecutive-day precision for FM (DXA: 226 g; BOD POD: 318 g) and FFM
239 (DXA: 309 g; BODPOD: 321 g) with low limits of agreement for DXA (FM: -365 g to 87 g; FFM:
240 -59 g to 558 g) and for BOD POD (FM: -275 g to 361 g; FFM: -251 g to 390 g). The largest
241 level of bias between same-day and consecutive-day precision came from BIS for FM (524 g)
242 and FFM (580 g) with wider limits of agreement (FM: -108 to 939 g; FFM: -930 g to 230 g) as
243 shown in Figures 7 and 8.

244

245 **Discussion**

246 To our knowledge this is the first study exploring both technical error and the short-term
247 biological variation within a 24-hour period, using four independent methods of body
248 composition assessment. The body composition PE was greater when quantified from
249 consecutive-day compared to same-day results on a resistance trained athletic male cohort.
250 This was evident across all body composition assessment techniques. Consecutive-day PE
251 was 25% higher for DXA and BOD POD (FM and FFM) estimations and nearly 50% higher for
252 BIS (FM and FFM) and SA (FFM) than same-day PE. It must be noted that same-day and
253 consecutive-day PE in SA (FFM) was lower than all other methods. In contrast the SA FM PE
254 for same-day and consecutive day analysis was lower for consecutive-day but only by 8%
255 and was not significantly different ($p < 0.5$). This shows that biological variation affects
256 measurement precision even within very short time frames (24 hours), at least when using

257 BIS, DXA and BOD POD methodology. Therefore, the use of consecutive-day PE is advocated
258 as longitudinal monitoring of physique will always include both technical error and
259 biological variation.

260

261 Excellent SA same-day precision was found for estimations of FM (CV 1.0%) and FFM (CV
262 0.2%) as well as for consecutive-day testing with FM (CV 1.0%) and FFM (CV 0.3%)
263 respectively. Raw measurements from SA (mm) have been shown to be robust and
264 unaffected by biological variation caused by prior food and fluid ingestion or exercise (Kerr
265 et al., 2017) yet this study included body mass to obtain estimates of FM and FFM using the
266 Evans equation (Evans et al., 2005). It would be expected then, that consecutive-day PE
267 would be larger given that body mass is acutely influenced by hydration status,
268 gastrointestinal tract contents and muscle glycogen stores (Rouillier et al., 2015). Due to
269 adopting previous recommendations of subject presentation including overnight fasting,
270 post bladder and bowel evacuation with body measurements taken early in the morning in
271 minimal clothing, the biological impact on precision was expected to be minimal (Kerr et al.,
272 2017; Nana, Slater, Stewart, & Burke, 2015).

273

274 DXA is prone to biological variance due to changes in hydration, significantly affecting FFM
275 estimates (Kerr et al., 2017). This is particularly noticeable in large muscular males with high
276 levels of FFM (Barlow et al., 2015; J. C. Bilsborough et al., 2014). Previous literature and
277 manufacturing guidance suggest that a standardized testing protocol be adopted to
278 minimise technical error and biological variation (Kerr et al., 2016; Nana et al., 2012a). This
279 is in agreement with the literature finding a CV of 0.5 and 1.5% respectively (De Lorenzo,
280 Andreoli, & Candeloro, 1997; Nana et al., 2012a) and more recently, results from Zemski et

281 al with a consecutive-day FM CV of 2.9% and lean mass CV of 1.1% (Zemski et al., 2019).
282 Despite obtaining excellent precision from utilising a standardized presentation protocol
283 those authors found biological variance (consecutive-day) to be higher than technical error
284 (same-day) most probably due to short-term changes in hydration (Nana et al., 2012a),
285 sleep hygiene (Vitale, Owens, Hopkins, & Malhotra, 2019) and intramuscular solute levels
286 (Bone et al., 2016). These findings would support the results from this study with a FM and
287 FFM CV of 2.4 and 0.5% respectively. While current best practice guidance was followed,
288 this may not account for variance in muscle solute content which is known to influence
289 reliability. The impact of standardised training and diet on consecutive-day precision
290 warrants investigation.

291

292 Close comparisons between DXA and BODPOD were identified in this study with strong
293 agreement found in same-day and consecutive-day FM PE (BOD POD $R^2 = 0.99 - 0.99$, DXA
294 $R^2 = 1.00 - 0.99$) and FFM (BOD POD $R^2 = 0.99 - 0.99$, DXA $R^2 = 0.99 - 0.99$). In support,
295 previous research using DXA and BODPOD technology shows consistent results with this
296 study, with only small or trivial PE in FM and FFM estimates from consecutive-day testing
297 conducted under standardised presentation conditions (Kerr et al., 2017). Despite BOD POD
298 estimates of FM and FFM being subject to biological variation if unrestricted subject
299 presentation occurs (food and fluid intake plus physical activity), BOD POD precision in this
300 study showed that very high resolution can be obtained if these variables are controlled for
301 (FM CV 2.8%, FFM CV 0.6%). A limitation of this study is that the DXA scanner used to
302 estimate body composition (GE Lunar DPX Pro) has been superseded by newer models with
303 enhanced precision. PE from the DPX estimations has been found to be twice as high as the
304 GE Lunar Prodigy in athletes (J. C. Bilsborough et al., 2014) whereas the iDXA model

305 resolution has improved bone edge detection thus allowing superior algorithms for body
306 composition estimation (Toombs, Ducher, Shepherd, & De Souza, 2012).

307

308 Factors that impact TBW such as prior food and fluid intake, physical activity or medical
309 conditions make BIS vulnerable to imprecision (Kyle et al., 2004). Additionally, variance in
310 fluid and electrolyte content will affect TBW (Saunders, Blevins, & Broeder, 1998) and
311 confound any change in physique traits inferred from BIS (O'brien, Young, & Sawka, 2002).
312 Given normal daily fluctuations in TBW, it is unsurprising that the change between same-day
313 and consecutive-day precision using BIS derived estimates of FM and FFM showed nearly a
314 50% increase in PE for both FM (3607 vs 2331 g) and FFM (3966 g vs 2276 g) estimates. BIS
315 also had the highest CV % of all methods for both same-day FM and FFM (5.2% and 0.6%)
316 and consecutive-day values (9.4% and 1.1%) respectively. This suggests that despite
317 implementing a rigorous athlete presentation protocol prior to testing, a lower tolerance
318 level for precision still occurs. Given this, the ability of BIS to accurately track small changes
319 in physique among athletic populations is questionable.

320

321 **Conclusion**

322 In conclusion, consecutive-day PE was larger than same-day for FM and FFM estimates
323 obtained from DXA, BOD POD, and BIS (except for SA FM which was marginally lower) in a
324 cohort of muscular resistance trained male athletes. This is despite PE limits for FM and FFM
325 estimates being within acceptable precision thresholds, at least for DXA. Clearly all methods
326 are subject to some imprecision due to daily biological fluctuations, especially BIS which
327 calculates physique traits from a TBW estimation. Given that both technical error and
328 biological variation contribute to precision, we recommend the use of LSC values calculated

329 from consecutive-day analysis when interpreting longitudinal change for true changes in
330 physique. Application of DXA, BOD POD or SA should be advocated over BIS for athletic
331 populations where only small changes are observed over time.

332

333 Practical Implications

334 Adopting LSC values from consecutive-day analysis likely provide a more appropriate
335 benchmark to assess meaningful change in body composition of athletic populations
336 longitudinally.

337

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340 acquisition of data; AK and GJS: analysis and interpretation of data; AK: draft of manuscript;
341 AK, GJS and KH: critical revision of the manuscript for important intellectual content; AK:
342 statistical analysis; and GJS: study supervision. AK had full access to all the data in the study
343 and takes responsibility for the integrity and the accuracy of the data analysis. The results of
344 this study are presented clearly, honestly, and without fabrication, falsification, or
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347

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502 **Figure 1** Study design of three testing sessions conducted over 24 hours.

503

504 **Figure 2** Least significant change for DXA, BODPOD, BIS and SA for same-day

505 and consecutive-day measures.

506

507 **Figure 3** Regression analysis between measures of fat mass (FM) for same-day precision.

508

509 **Figure 4** Regression analysis between measures of fat mass (FM) for consecutive-day

510 precision.

511

512 **Figure 5** Regression analysis between measures of fat free mass (FFM) for same-day

513 precision.

514

515 **Figure 6** Regression analysis between measures of fat free mass (FFM) for consecutive-day

516 precision.

517

518 **Figure 7** Bland Altman plots for differences in same-day vs consecutive-day measures for fat

519 mass (FM).

520

521 **Figure 8** Bland Altman plots for differences in same-day vs consecutive-day measures for fat
522 free mass (FFM).