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**Reliability and precision of the Nana protocol to assess body composition using dual
energy X-ray absorptiometry**

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26 ABSTRACT

27 The Nana positioning protocol is widely used to position participants to minimise technical
28 error when undertaking body composition scanning and analysis with a Dual Energy X-Ray
29 Absorptiometry (DXA) machine. Once biological and technical errors are accounted for, the
30 only variation in test re-test results is from statistical fluctuation or machine error. Therefore,
31 the aim of this study is to assess the test re-test reliability of the Nana positioning protocol,
32 and establish the smallest real difference percentage (SRD%). A gender balanced group of
33 thirty participants (15 males, 15 females) underwent two scans in succession using the Nana
34 positioning protocol, with repositioning between scans. Percentage change in mean with
35 typical error, Intraclass Correlation Coefficients (ICC) and standard error measurement
36 percentage (SEM%) were used to identify the test re-test reliability and error rate of these
37 protocols. Additionally, SRD% was calculated to assess the point at which clinically
38 important changes occurred in a participant. The reliabilities of the whole body and regional
39 scans were excellent. Percentage change in mean ranged between 0.00% and 0.23%. High
40 reproducibility of the Nana positioning protocol was evident through an ICC ranging between
41 0.966 – 1.000. Additionally, typical error, SEM% and SRD% were all low. Interestingly, fat
42 mass was associated with the largest fluctuations observed to be associated with any of the
43 parameters assessed. When all sources of biological and technical errors have been accounted
44 for, the Nana positioning protocol has excellent test re-test reliability and produces low
45 SEM% and SRD%.

46

47 KEYWORDS

48 DXA; Test Re-test; Smallest Real Difference

49

50

51 INTRODUCTION

52 Dual energy X-ray absorptiometry (DXA) uses a machine originally developed to provide
53 information about bone mineral density, with the additional capability to assess and analyse
54 body composition (BC) while imparting only low levels of radiation (less than a thousandth
55 of the maximum recommended dosage of 5mSv) (ANZBMS, 2005; Bazzocchi et al., 2016;
56 Lewiecki, 2005; Nana et al., 2012). The distinct characteristics of lean mass (LM), fat mass
57 (FM) and bone when scanned with DXA enable clinicians and researchers to gain a greater
58 understanding of both the pathogenic processes involved in a variety of conditions (obesity,
59 diabetes, undernourished individuals, renal, gastrointestinal diseases) and the physiological
60 changes in healthy populations associated with the process of growth and aging (Bazzocchi et
61 al., 2016; Lee et al., 2008; Rothney et al., 2009). Body composition scans are also used
62 extensively in athletic populations to investigate physiological and para-physiological
63 conditions affecting athlete performance (Bazzocchi et al., 2016; Georgeson et al., 2011).

64

65 Dual energy X-ray absorptiometry results for body composition have been found to be
66 reliable in assessments of test retest reliability that have used a wide variety of reliability
67 statistics (Bilsborough et al., 2014; Colyer et al., 2016; Covey et al., 2010; Covey et al., 2008;
68 Kerr et al., 2016; Lohman et al., 2009; Moon et al., 2013; Nana et al., 2012, 2013; Smith-
69 Ryan et al., 2016). However, in order to produce the most reliable results, provisions in
70 methodology are required to minimize the chance occurrence of errors, both biological and
71 technical, that create false or misleading results (Hangartner et al., 2013). The most important
72 provision to minimise technical errors is to use a consistent manner in which participants are
73 positioned. As such, two positioning protocols exist - the National Health and Nutrition
74 Examination Survey (NHANES) Body Composition positioning protocol of the National
75 Centre for Health Statistics, and the Nana positioning protocol designed and described by

76 Alisa Nana (Nana et al., 2012; NHANES, 2013). These two positioning protocols are used to
77 minimise the movement of the participant during scanning, which creates artifacts, and
78 consistently produce higher reliability scores than DXA scanning without a repeatable
79 positioning protocol (Bilsborough et al., 2014; Colyer et al., 2016; Covey et al., 2010; Covey
80 et al., 2008; Kerr et al., 2016; Lohman et al., 2009; Moon et al., 2013; Nana et al., 2012,
81 2013; Smith-Ryan et al., 2016).

82

83 Upon reviewing (critical appraisal and level of evidence) studies of reliability of the DXA
84 results yielded using each of these protocols, we found there was a high level of evidence and
85 very high reliability for the Nana positioning protocol even though all studies using the Nana
86 protocol involved Alisa Nana the founder of the protocol. The NHANES protocol had a
87 moderate level of evidence but suggested very high reliability.

88

89 Additionally, in previous studies investigating the reliability of DXA measurements of body
90 composition there has been inconsistent use of statistical procedures. To date, no study has
91 included the calculation of smallest real difference (SRD) or smallest real difference
92 percentage (SRD%), which constitute the benchmark statistical analysis used to determined
93 whether a real change beyond measurement error has occurred at the defined confidence level
94 (Beckerman et al., 2001; Chen et al., 2009). Previously authors have reported typical error
95 (usually expressed as a coefficient of variation percentage (CV%)), or smallest worthwhile
96 effect (SWE). There are inconsistencies in the definition of the SWE statistic, as most authors
97 propose that the SWE can only be determined by consultation with participants who received
98 the intervention, and not by researchers or clinicians using statistical analysis in isolation,
99 however some authors have calculated it based simply on dividing the between-subject
100 standard deviation by one third (Ferreira et al., 2013; Ferreira et al., 2012; Herbert, 2000;

101 Kerr et al., 2016; Nana et al., 2012, 2013).

102

103 On this basis, technical error was the primary focus of this study. Specifically, the aim of this
104 study was to determine the test re-test reliability of DXA results obtained using the Nana
105 positioning protocol to assess total body and regional BC.

106

107 **METHODS**

108 **Study overview**

109 In order to assess the reliability of the Nana positioning protocol, each participant was
110 scanned twice using the DXA machine by a trained scanner, in a single session at Bond
111 University, Gold Coast, Australia. Scanning was undertaken in accordance with the Nana
112 positioning protocol (feet and hands positioned in radio-opaque pads). Each subject was
113 repositioned between scans, with the total session running for approximately fifteen minutes
114 per participant.

115

116 **Participants**

117 Prior to commencing the research, this study was granted ethics approval by Bond University
118 Human Research Ethics Committee (RO0000015221). Each subject was informed of all risks
119 and the testing procedure, with the informed consent process taking place prior to scans
120 proceeding. A gender balanced group of fifteen males and females (n=30) was enlisted from
121 Bond University on the Gold Coast, Australia, and from the wider public of the Gold Coast
122 community. Participants were included based on the inclusion criteria that participants
123 wholly physically fitted within the scanning area (197cm x 60.5cm) so as to avoid minimise
124 the confounding variable of segmental scans (i.e. participant too tall or too wide for scanning
125 area).

126 The participant demographics (mean \pm SD) were females (n=15), age = 31.3 \pm 11.9 years,
127 height = 164.7 \pm 8.9cm, mass = 62.4 \pm 9.7 kg; and males (n=15), age = 27.8 \pm 7.2 years,
128 height = 178.7 \pm 7.3 cm, mass = 78.9 \pm 8.8 kg. The number of subjects recruited was based
129 on recommendations regarding sample sizes published in previous reliability studies (Lexell
130 et al., 2005). A Stadiometer (Harpenden, Holtain Limited, Crymych, UK) and scales
131 (WM202, Wedderburn, Bilinga, Australia) were utilised to undertake an anthropometric
132 analysis of height and mass of each subject prior to BC scanning on the DXA machine.

133

134 **Standardised Baseline Conditions**

135 The subject reported for their morning scan having fasted overnight; refrained from exercise;
136 and with their bladders voided. Male subjects wore minimal attire i.e. underwear, whereas
137 female subjects wore either two-piece underwear or bathers, as they wished. Furthermore, all
138 subjects were required to remove any metal from their bodies and clothes.

139

140 **DXA instrument and operation**

141 The Lunar Prodigy DXA machine (GE Healthcare, Madison, WI) was calibrated every day
142 according to the manufacture's guidelines, using a phantom. A single Australia and New
143 Zealand Bone and Mineral Society (ANZBMS) densitometry qualified scanner performed
144 each BC scan using the narrow angle fan beam DXA machine, and thereafter used the GE
145 enCORE 2016 software (GE Healthcare) to analyse the data (Figure 1).

146

147 **Nana body composition positioning protocol**

148 During each scan, the Nana positioning protocol requires the subject's feet to be placed on a
149 transparent styrofoam block, which is custom-made to keep a consistent distance of 15cm
150 between the feet; together with a strap around the ankles to keep movement minimal, and

151 reduce artifacts (Nana et al., 2012). The subject is also placed centrally and in a supine
152 position, with custom-made foam and plastic paddles used to position the subject's hands in a
153 mid-prone position with a consistent gap of 3cm between the inside of the hands and the
154 trunk; again, the hand paddles reduced the risk of any movements (Figure 1) (Nana et al.,
155 2012).

156

157 **Statistical Analysis**

158 This study used a range of statistical approaches to collect, analyse and present data. IBM
159 SPSS 24 and custom-made spreadsheets from the Sportsscience website (www.sportsci.org)
160 aided with determining percentage change in mean, confidence intervals (CI), typical error as
161 CV%, standard error of measurement percentage ($SEM\% = ((\sqrt{\text{mean square error from ANOVA}}/\text{mean}) \times 100\%)$), and smallest real difference percentage ($SRD\% = ((1.96 \times SEM \times \sqrt{2})/\text{mean}) \times 100\%$) (Lexell et al., 2005). Intraclass Coerrelation Coefficients, type 3,1, were
164 calculated as the primary measure of level of agreement between paired results, using IBM
165 SPSS 24 (Trevethan, 2016). Bland Altman plots were also generated and means and standard
166 deviations were established for anthropometric data.

167

168 **RESULTS**

169 All the collated results from the Nana positioning protocol test re-test reliability analysis are
170 presented in Table 1. When assessing the BC on two different occasions with repositioning
171 of the participant between scans, the reliabilities of the whole body (Tissue, FM, LM and
172 BMC) and all regional (arms, legs and trunk) scan results were very high. Additionally, these
173 results are also demonstrated in the Bland Altman plots (Figure 2), displaying close precision
174 in all areas.

175

176 Percentage change in mean of the Nana positioning protocol ranged between -0.23% and
177 0.23%. Arms were the regional area with the smallest variance in the parameters (Table 1),
178 with results ranging from -0.02% to 0.02%. The trunk had the largest variance, with results
179 ranging from -0.23% to 0.12%.

180

181 The typical error, expressed as CV%, when using the Nana positioning protocol ranged
182 between 0.01% and 0.75%. The arms showed the smallest typical error (Table 1), ranging
183 between 0.01% and 0.11%; whereas for other body areas the typical error ranged from 0.03%
184 to 0.75%, with the whole body LM producing the largest value of 0.75%.

185

186 High reproducibility of the Nana positioning protocol is evident in the ICC, ranging between
187 0.966 and 1.000. FM consistently presented the lowest ICC for whole body and regional
188 scans except for trunk BMC, which produced the lowest ICC of 0.966 (95% CI 0.931-0.984).
189 Whole body tissue produced the highest ICC of 1.000 (95% CI 1.000 – 1.000).

190

191 The SEM% reflected the results of the ICC, with FM results consistently showing the highest
192 SEM%. Tissue mass of the whole body produced the lowest SEM% scores (Table 1).

193

194 Smallest real difference percentages (SRD%) also followed the pattern of ICC and SEM%,
195 with FM consistently displaying the highest results, ranging between 5.9% and 11.1%.
196 Tissue, LM and BMC illustrated an overall low SRD% score throughout, except for the
197 regional trunk of BMC, which indicated a high SRD% of 9%.

198

199 **DISCUSSION**

200 The aim of this study was to provide an unbiased assessment of the reliability of the Nana
201 positioning protocol and establish the SRD% of the Nana positioning protocol.

202

203 The Nana positioning protocol produced excellent test re-test reliability results when the
204 parameters of tissue mass, FM, LM and BMC were assessed in the total body, and in the
205 regions of the arms, legs and trunk. These results confirm the findings of previous research
206 indicating that the Nana positioning protocol is a reliable positioning protocol when using a
207 DXA machine to assess body composition (Kerr et al., 2016; Nana et al., 2012, 2013).

208

209 In this study, when percentage change in mean was used to assess reliability, the Nana
210 positioning protocol produced similar results as previous studies, which have used this
211 statistic (Kerr et al., 2016; Nana et al., 2012, 2013). The actual figure of change in this
212 study's result was consistently lower in comparison to those from previous studies that
213 utilised the Nana positioning protocol (Kerr et al., 2016; Nana et al., 2012, 2013). This may
214 be possibly due to the strict methodology followed and that the machine used was relatively
215 new. The results fluctuated among studies as to which parameter (tissue, FM, LM or BMC)
216 produced the smallest change in mean from zero. Only the parameters of tissue mass when
217 assessed on the whole body, together with BMC when assessed in the legs, produced results
218 that were similar across all the studies. Consequently, these produced the smallest change in
219 mean scores from zero in all studies.

220

221 When using percentage change in mean, it is required to present the typical error, this has
222 usually been presented as a percentage of typical error otherwise known as a CV% (Hopkins,
223 2000; Hopkins et al., 2009). The CV% results of this study typically were smaller values
224 when compared to other studies (Kerr et al., 2016; Nana et al., 2012, 2013), and this is likely

225 due to the provisions in methodology used to reduce effects of biological and technical error.
226 Once again differences occurred in regards to which parameter produced the smallest
227 percentage typical error. It was found that only BMC in the legs produced the same results
228 across all studies.

229

230 This study is the only study so far to include ICC results for all parameters in whole body and
231 regional body areas. The ICC results of this study ranged between 0.966 and 1.000,
232 demonstrating very high reliability (Munro et al., 2005). Other studies, have presented ICC
233 ranging between 0.4 and 0.99 (Nana et al., 2012, 2013). These results varied significantly as
234 they have not reported ICC for individual variables but instead have reported overall figures.

235

236 To our knowledge, this is the first time that SRD% has been used when assessing use of
237 DXA to measure BC. In this study, the SRD% was calculated to fall between 0.6% and 5.9%
238 (whole body) and 2.3% and 11.1% (regional), thus providing an indication of the point at
239 which real change occurs. Using SRD% produced results that were similar to the other
240 studies that have used SWE, in that FM produced the largest figure that may be attributed to
241 statistical error or fluctuation before a real change can be confidently assessed. As such
242 SRD% should be calculated on each individual machine if longitudinal analysis of BC is
243 being undertaken.

244

245 As the most fluctuation of SRD% scores occurred in the trunk and arm regions, the authors
246 postulate this may be due to automatic region of interest lines were applied automatically and
247 adipose tissue may have encroached over the region of interest line into another region, i.e.
248 the arm fat may have been assessed in both the arm and trunk in one scan but may have been
249 only in the arm region on the next scan. To address this possible issue, future research should

250 be undertaken with ROI adjusted and standardized between patients to ensure that the region
251 of interest line follows the defined anatomical landmarks and tissue does not encroach into
252 other regions.

253

254 In summary, once biological and technical errors have been justified, the Nana positioning
255 protocols produced very high test re-test reliability, and therefore can be the trusted choice
256 for clinicians assessing an individual's BC. Additionally, we urge future clinicians and
257 researchers using the Nana positioning protocol to establish the SRD%. This calculation will
258 enable a scanner to determine the figure at which a change in results can confidently be
259 attributed to a true change of the participant between test re-test, and not due to statistical
260 fluctuation or error.

261

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267 interpretation and manuscript preparation were undertaken by FS and CP. All authors
268 approved the final version of the paper.

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397 TABLES

398 **Table 1. Nana Positioning Protocol Test Retest Reliability**

		% Δ in mean	Typical error as CV%	ICC	CI (95%)	SEM%	SRD%	
whole body	Tissue	0.03	0.14	1.000	1.000 - 1.000	0.2	0.6	
	Fat	0.23	0.36	0.996	0.990 – 0.998	2.1	5.9	
	Lean	-0.03	0.75	0.996	0.991 - 0.998	1.5	4.1	
	BMC	0.02	0.03	0.997	0.993 – 0.999	1.1	3.1	
Regional	arms	Tissue	0.00	0.10	0.998	0.996 – 0.999	1.1	3.0
		Fat	-0.02	0.08	0.986	0.972– 0.994	3.8	10.6
		Lean	0.02	0.11	0.997	0.995 – 0.999	1.7	4.7
		BMC	0.00	0.01	0.996	0.992 - 0.998	1.6*	4.5*
	legs	Tissue	0.07	0.29	0.996	0.991 – 0.998	1.2	3.3
		Fat	0.10	0.20	0.992	0.982 – 0.996	3.0	8.3
		Lean	-0.03	0.29	0.995	0.989 – 0.998	1.7	4.6
		BMC	0.00	0.01	0.996	0.998 – 0.999	0.8*	2.3*
	trunk	Tissue	-0.10	0.32	0.997	0.994 – 0.999	1.0	2.8
		Fat	0.12	0.33	0.990	0.979 – 0.995	4.0	11.1
		Lean	-0.23	0.45	0.991	0.981 – 0.996	2.0	5.5
		BMC	0.01	0.03	0.966	0.931 – 0.984	3.3*	9.1*

399 *assessed in milligrams

400 % Δ in Mean – percentage change in mean, CV- confidence variance, ICC – intraclass
 401 correlation coefficient, CI – confidence interval, SEM% - percentage standard error of
 402 measurement, SRD% - percentage smallest real difference

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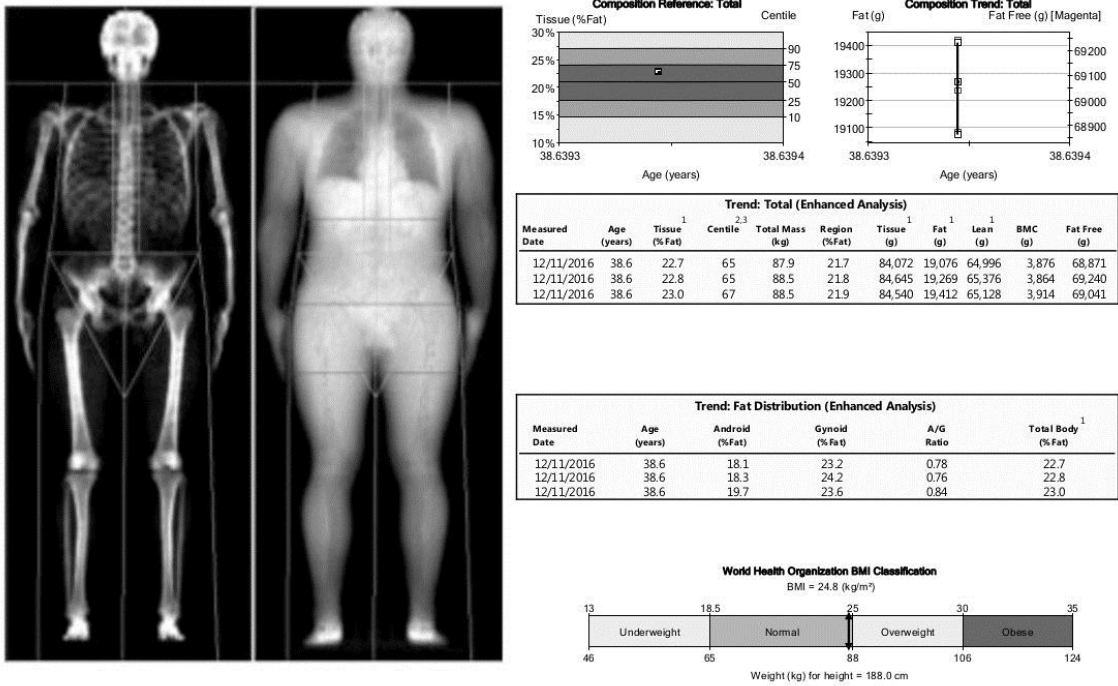
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415 FIGURES



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417 **Figure 1.** Nana positioning protocol analysis

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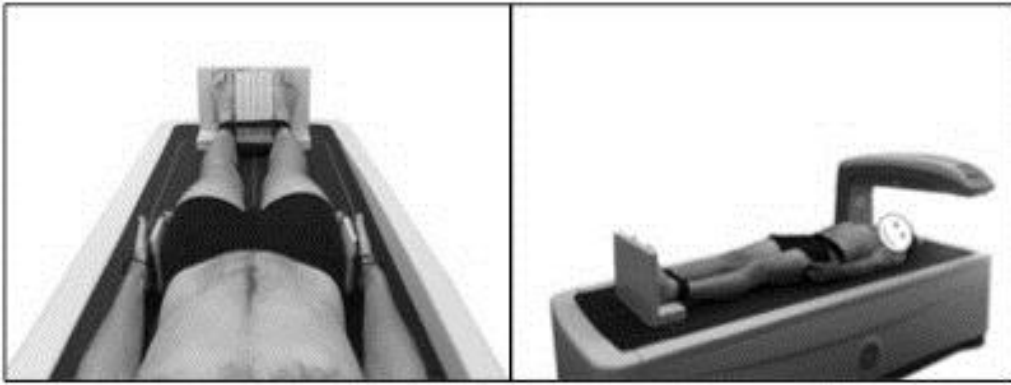
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431 **Figure 2.** Nana positioning protocol

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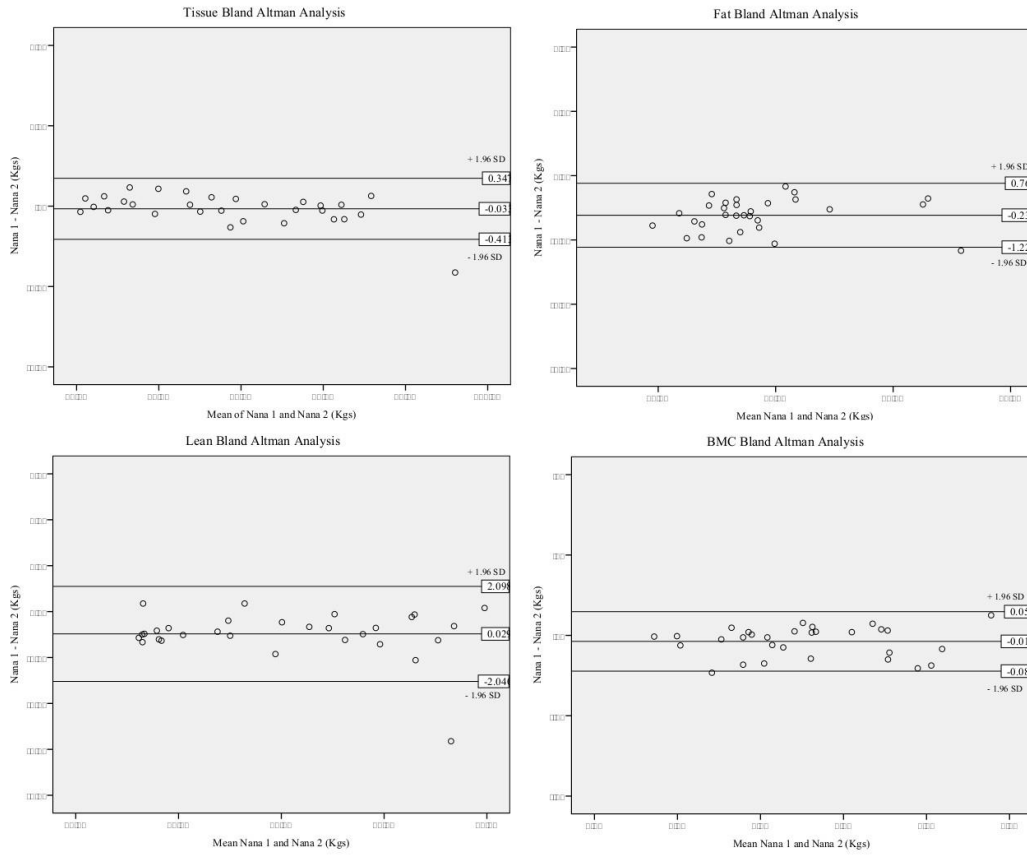
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Figure 3. Bland Altman Plots for whole body Nana versus Nana positioning