Reliability and Precision of the Nana Protocol to Assess Body Composition Using Dual Energy X-ray Absorptiometry

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ABSTRACT

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

KEYWORDS

DXA; Test Re-test; Smallest Real Difference

INTRODUCTION

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

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

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

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

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

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

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

METHODS

Study overview

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

Participants

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

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

Standardised Baseline Conditions

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

DXA instrument and operation

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

Nana body composition positioning protocol

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

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

Statistical Analysis

This study used a range of statistical approaches to collect, analyse and present data. IBM SPSS 24 and custom-made spreadsheets from the Sportsscience website (www.sportsci.org) aided with determining percentage change in mean, confidence intervals (CI), typical error as CV%, standard error of measurement percentage (SEM%=((√mean square error from ANOVA)/mean) x100%), and smallest real difference percentage (SRD% = ((1.96 x SEM x √2)/mean) x100%) (Lexell et al., 2005). Intraclass Coerrelation Coefficients, type 3,1, were calculated as the primary measure of level of agreement between paired results, using IBM SPSS 24 (Trevethan, 2016). Bland Altman plots were also generated and means and standard deviations were established for anthropometric data.

RESULTS

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

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

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

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

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

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

DISCUSSION

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

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

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

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

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

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

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

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

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

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

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TABLES

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*

*assessed in milligrams

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

FIGURES

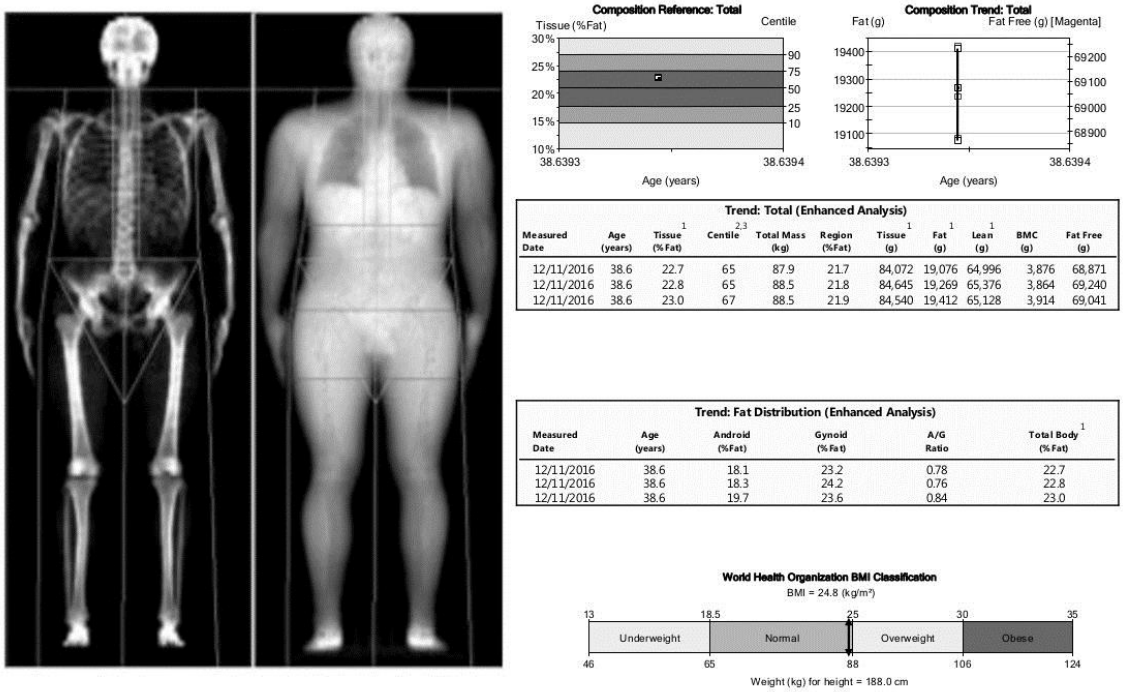

Figure 1. Nana positioning protocol analysis

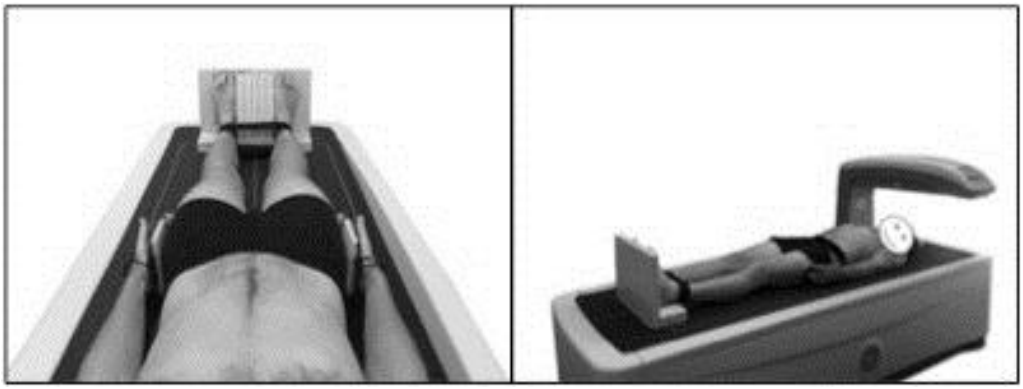

Figure 2. Nana positioning protocol

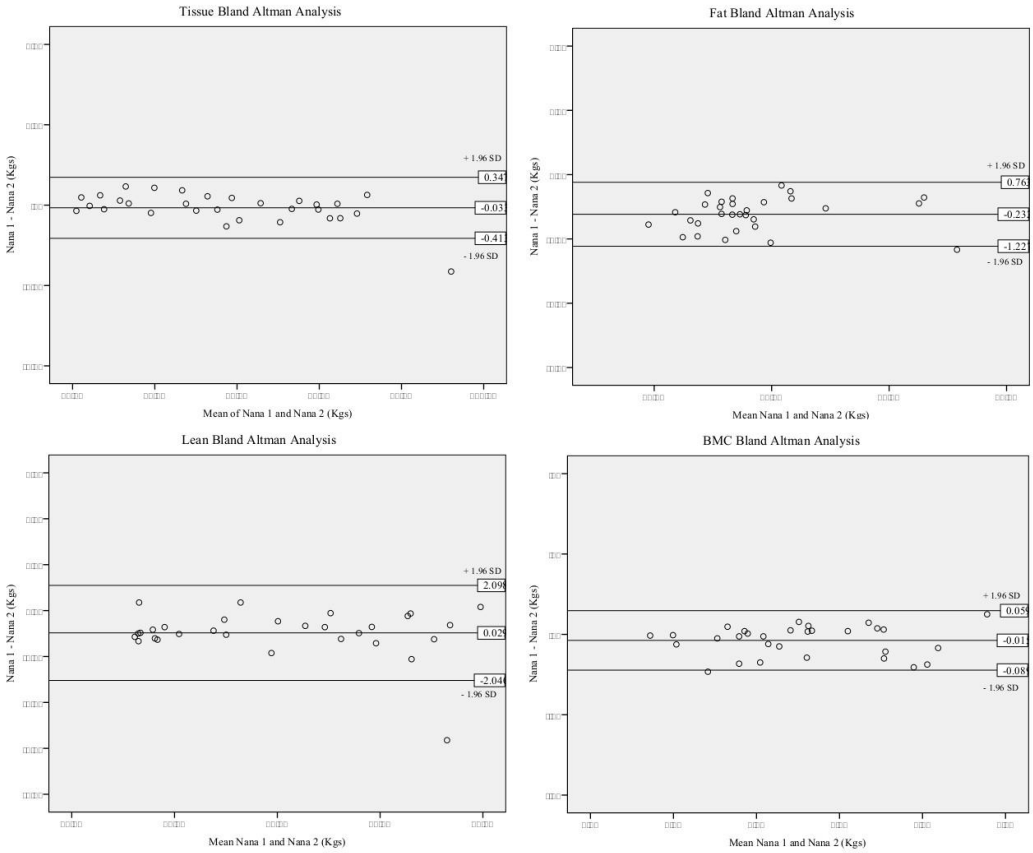

Figure 3. Bland Altman Plots for whole body Nana versus Nana positioning